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Soil tillage and crop rotation effects on *Triticum durum* (Desf.) yield and mycotoxins content in its grain

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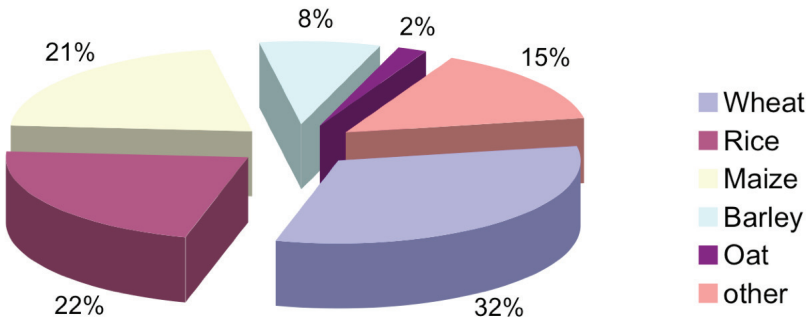
Introduction

Durum wheat [*Triticum durum* (Desf.)]

Wheat (*Triticum* spp.) is one of the first domesticated species. Grown by man since the Neolithic revolution, for 8,000 years it has been the basic staple food of the major civilizations of Europe, West Asia and North Africa. Today it is the most cultivated crop in the World (on more than 215 million ha [16% of all tilled land], 627 million t of production, FAO source), more than all other cereals (figure a).

Figure a. Main cereals in the World (% cereal cropped area in 2000-2005; FAO Datasheets)



Together with rice, wheat provides more nourishment to humans than any other food source. It is a major diet component mainly because of the ease of its dry grain storage. It is usually grounded into flour for making edible, palatable, and satisfying foods. Wheat is the most important source of carbohydrate in a majority of countries. Its starch is easily digested, as are most of its proteins. Because of the high content of minerals, vitamins and fats

(lipids), with the addition of a small amount of animal or legume proteins, wheat grain becomes highly nourishing. A predominately wheat-based diet is higher in fibre than a meat-based diet (Johnson *et al*., 1978).

Wheat has a very wide agronomic adaptability. Moreover, widely differing pedigree varieties exist, which allow its cultivation in many environmental conditions. Although successfully grown between the ranges: 30-60°N and 27-40°S of Latitude (Nuttonson, 1955), wheat is cultivated well beyond these limits, from within the Arctic Circle to the Equator, at high elevations. In the past couple of decades, researchers of the International Maize and Wheat Improvement Centre (CIMMYT, Mexico) have shown that wheat production can be technologically feasible also in much warmer areas (Saunders and Hettel, 1994). In altitude, the crop is grown from sea level to more than 3,000 m a.s.l., and in Tibet there are wheat fields up to 4,570 m a.s.l. (Percival, 1921).

The optimum growth temperature of wheat is about 25°C, with minimum and maximum of 3-4°C and 30-32°C, respectively (Briggle, 1980). It is adapted to a broad range of moisture conditions: from xerophytic to littoral. About three-fourths of the World wheat area receives an average of 375-875 mm of annual rainfall, but it grows also where precipitation ranges from 250 to 1,750 mm (Leonard and Martin, 1963). Sufficient water availability during the whole growing season is needed for optimal production. However, too much rain can lead to yield losses from disease and root asphyxia. Although in any months of the year somewhere in the world wheat is being harvested, the harvest in the main temperate zones generally occurs between April and September in the Northern Hemisphere and from October to January in the Southern Hemisphere (Percival, 1921).

The traditional classification into spring and winter wheat refers to the growing season of the crop. For winter wheat, heading is delayed until the plant experiences a period of cold (vernalization with an optimum of 0° to 5°C). So, it is usually planted in the autumn, remains in the tillering phase during winter and resume growth in early spring, to mature in early summer. This cycle has the advantage of profiting by autumn moisture for germination and spring sunshine,

warmth, and rainfalls for rapid vegetative growth. Spring wheat, on the contrary, is planted in spring and matures late in summer because of a low vernalization requirement to flower. However, in countries with mild winters, such as in South Asia, Mediterranean Basin, and Middle East, spring wheat can also be sown in the autumn.

Durum wheat in the World

Durum wheat (*Triticum durum* Desf.) is traditionally produced in only a few areas of the Planet. Low rainfalls and frequent water shortages characterize these zones. Therefore the cultivation of this cereal is heavily dependent on weather, which can substantially affect both its product quantity and quality. Adverse climatic conditions often limit the production and worsen grain characteristics, thereby pushing up market prices, which are widely variable over the years.

Durum wheat is considered a minor cereal crop, representing only the 5% of the global wheat production, but it has a great relevance in the Mediterranean countries, where it is largely used for human consumption, as pasta, couscous, and bread. In Southern Europe it is mainly used for pasta production (85%). The Italian, French and Greek rules authorize only semolina and water in pasta making. Therefore, the quality of this food highly depends on the characteristics of the raw material (wheat grain) and on the industrial technology.

Today durum wheat in the World is grown on 14 million ha, approximately, that are concentrated in the Mediterranean Basin and North America (table a). On a global basis, its surface is spreading, due to a continuous increase in pasta consumption. In the last four years World production reached about 28 millions tons. Together, Italy and Canada gave the 15% of this amount (table b).

The yield of this cereal widely varies in the different countries. According to FAO statistics, the last few years the highest average yields (about 5 t ha^{-1}) have been obtained in UK and Germany. In France, Austria, and Mexico the

mean yields have varied between 3 and 5 t ha^{-1}, while 2-3 t ha^{-1} have been the means in Argentina, India, Syria, Italy, USA, Spain, Turkey, Greece, and Australia. The lowest productions were recorded in Russia (less than 1 t ha^{-1}). In 2006 the UE average yield was 3.4 t ha^{-1}; 32% higher than that of the World (Eurostat Database).

Table a. Durum wheat area in the World (2002-2006 avg.; Source: FAO Datsheets)

Country	*1,000 ha*	*Country*	*1,000 ha*	*Country*	*1,000 ha*
Canada	2,216	Spain	895	Australia	178
Italy	1,681	Syria	851	Portugal	146
Algeria	1,230	Tunisia	702	Kazakhstan	80
Russia	1,220	Greece	450	Argentina	48
USA	1,067	India	440	Austria	14
Turkey	1,060	France	363	Germany	6
Morocco	1,022	Mexico	232	UK	1

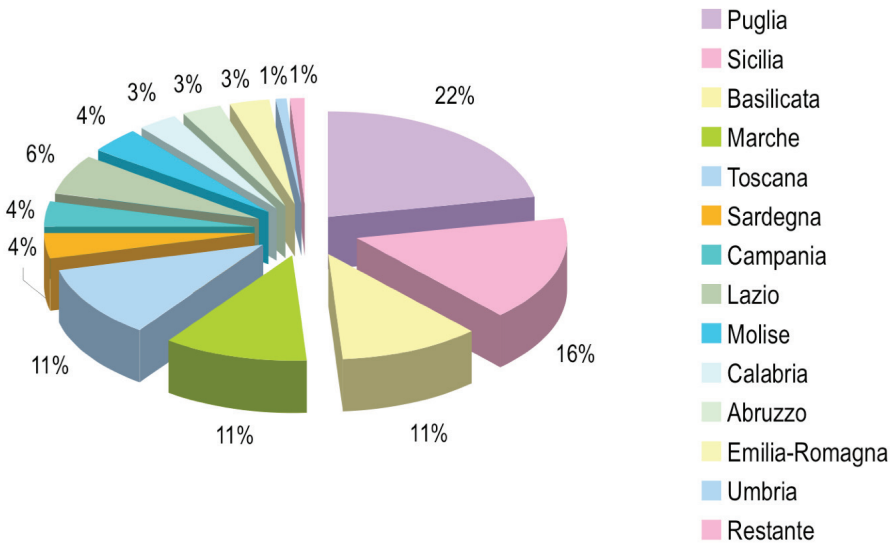
Table b. Durum wheat production in the World (2002-2006 avg.; Source: FAO Datasheets)

Country	*1,000 t*	*Country*	*1,000 t*	*Country*	*1,000 t*
Canada	4,171	Spain	1,359	Australia	350
Italy	4,166	Syria	1,322	Portugal	168
Algeria	2,418	Tunisia	1,240	Kazakhstan	144
Russia	2,240	Greece	1,160	Argentina	90
USA	2,180	India	1,160	Austria	55
Turkey	1,980	France	1,031	Germany	33
Morocco	1,616	Mexico	903	UK	6

Durum wheat in Italy

In Italy durum wheat represents the major crop, being grown on 1.5 million ha. Its annual production is 5 million t, approximately, against a need of 6.0-6.5 million t that are mainly used by the pasta industry (table c). The crop is concentrated in Southern Italy and in the islands, where the 75% of the Italian durum wheat surface and the 66% of the country production are located (figure b). In Italy the quantity and quality of grain production is quite unsteady due to variable weather and differentiated environmental conditions, agronomic management, and genetic background. Recently the Italian durum wheat yields steeply increased, thanks to the intensive breeding and to an expansion in the Northern regions, where there are more fertile soils (Boggini *et al.,* 1992). In 2006, however, because of the C.A.P. reform, the Italian durum wheat surface drastically reduced: 14% area loss and 19% less production were recorded than in the previous year.

Figure b. Regional production of durum wheat in Italy (2004-05 avg., ISTAT)



Most of the durum wheat that is produced and imported in Italy is used by the industry to make semolina.

Table c. Evolution of durum wheat balance in Italy (Source: Italmopa)

Years	Production 1,000 t	Import 1,000 t	Needs 1,000 t	Import/Needs %
1966-67	1,685	631	2,316	27.2
1976-77	3,230	396	3,626	10.9
1986-87	4,493	908	5,400	16.8
1996-97	4,419	1,311	5,730	22.9
2004-05	5,666	1,510	7,176	21.0
2005-06	3,605	1,500	5,104	29.4

This amount is mainly transformed to pasta (48%), bread (6%) and other bakery products. The transformed foods are almost equally addressed to the internal market (53%) and to the exportation (47%) (Source: Barilla).

Durum wheat yield limiting factors

Durum wheat can be damaged by several biotic and abiotic factors that limit its yield and worsen the quality of its production.

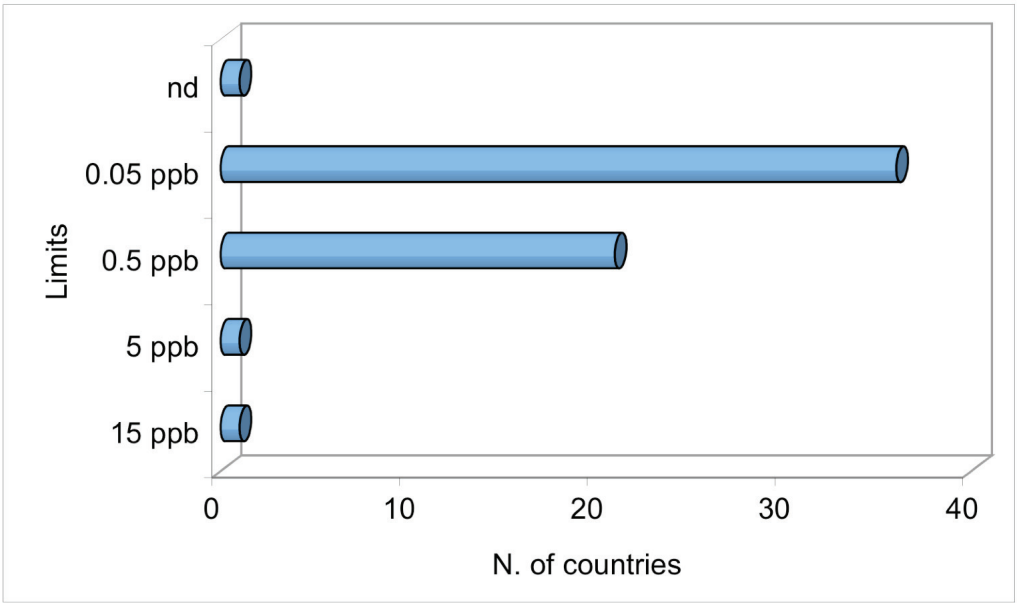
The major biotic adversities are represented by pathogenic fungi, which can infect various parts of the plant at different stages of growth. It is calculated that each year these pathogens cause million of tons grain losses (10-20% of the global production). Under particular circumstances and sites they can reduce yields of more than 80%. Many pathogens can be highly detrimental to the production quality as well. Wheat grain from diseased plants can lose the capacity to satisfy the demands of industrial procedures and health safety. In the last decade this latter problem has become increasingly important as the final consumer expects more rigorous controls on food healthiness. For this scope a series of new methodologies have been set up that can rapidly analyze great amounts of cereal grains. Today one of the most troublesome problems from this point of view remains the possible contamination of wheat kernels with mycotoxins that are difficult and costly to determinate and that are particularly

toxic in the human and animal diets, even at very low dosages (few ppm or ppb).

The mycotoxins risk

The study of mycotoxins is only recent but these molecules have always threatened man and animals health. Their diffusion is global, but the intensity of food contamination, whose detection is quite difficult, and its consequences vary among the different localities on the basis of the type of agriculture, dietary habit and health safety sensibility of the different people in the World.

Figure c. Law limits concerning the milk contamination with aflatoxin M1 in 60 countries all over the World (Van Egmond and Jonker, 2004). In Italy the limit is 0.05 ppb; in the USA it is 0.50 ppb (10 times higher).



This variability makes any comparison meaningless and partially explains why among the countries there are wide differences in the regulation limits about these substances in human food (figure c). Recently, the CEE has ruled the content of mycotoxins in food and feedstuffs, imposing precise limits for the different products (2174/03, 683/04 and 472/02 UE Reg.).

Mycotoxins are molecules that are produced by the secondary metabolism of various fungi, particularly those belonging to the genera: *Aspergillus*, *Penicillium* and *Fusarium*.

The fusari mycotoxins are considered the most troublesome for wheat all over the World because of the wide diffusion of these fungi. A recent investigation of a joined commission of food additives experts (JECFA) and of the World Health Organisation (WHO) found a significant contamination, particularly by DON (a mycotoxin produced by fusari), in the grain of wheat grown everywhere in the World (57% of 11,022 samples contained up to 30 ppm of the toxin). The European SCOOP project aimed at the evaluation of mycotoxin risk to man, found contaminations in 61% of 6,358 European wheat samples (FAO/WHO JECFA, 2001; Schothorst and Van Egmond, 2004). In Italy as well the mycotoxins more frequently found in wheat kernels are those produced by *Fusarium* spp. Their occurrence in the Italian production was first investigated in 1995 (Lops *et al.,* 1998). The analyses revealed a high contamination frequency in the Emilia Romagna grains, sometimes with more than 1ppm DON, while the Southern samples resulted uncontaminated *Triticum durum* Desf. was more prone to contamination than *Triticum aestivum* L.

Since then mycotoxins in wheat grain were thoroughly studied also in Italy. Some researches confirmed a strong association between *Fusarium* spp. ear diseases and DON in the kernels (Campagna *et al*., 2005). Pascale *et al*. (2000, 2001 and 2002) studied the effects of weather on ear syndrome and consequent DON contamination. In particular, they found that the disease diffusion and intensity, together with DON frequency and concentration levels, greatly depend on the amount of propagules that are present in crop residues and on the occurrence of high temperatures, humidity and rainfalls during the period: ear emergence (10.1 Feekes' scale) to grain milk-dough maturation stage (11.3 Feekes' scale). These requirements explain why, in Italy, the Northern productions, where these conditions are commoner, are more frequently contaminated than the Southern ones.

The fusari are the main causal agents of the “head scab” blight, a common syndrome affecting the spike of many cereals. The ear partially or totally dries down, with consequent heavy yield reductions. The caryopses of infected ears have a high probability to be contaminated by mycotoxins that, under favourable conditions, are rapidly and abundantly produced by the fungi. Mycotoxin production generally begins in the field but it can continue throughout the storage period, encouraged by a scarce cleanliness of the storage facilities.

The optimal conditions for mycotoxin production vary with the substrate, the fungus species and the isolate. Their production, however, depends also on well-defined ranges of both temperature and humidity.

The commoner mycotoxins produced by fusari are trichothecenes, zearalenone, and fumonisins. In addition, moniliformin, beauvericin, and fusaproliferin have been occasionally reported as problematic (Logrieco *et al*., 1990; Wiese, 1987).

Trichothecenes

They can be divided into two types: A and B.

A-Type trichothecenes include: T-2 toxin (T2) and its derivatives (HT-2 toxin, T-2 triol, T-2 tetraol), that are produced by strains of *F. sporotrichioides*, *F. acuminatum*, and *F. poae*; diacetoxyscirpenol (DAS) and monoacetoxyscirpenol (MAS), produced by strains of *F. poae*, *F. equiseti*, *F. sambucinum,* and *F. sporotrichioides*; and neosolaniol (NEO), produced by strains of *F. sporotrichioides*, *F. poae* and *F. acuminatum*.

B-Type trichothecenes include: deoxinivalenole a.k.a. vomitoxin (DON) and its mono-acetylated (3-AcDON, 15-AcDON) and di-acetylated derivatives (3,15-AcDON), which are produced by strains of *F. graminearum* and *F. culmorum*; nivalenol (NIV) and its monoacetylated derivative (fusarenone X, FUS) and the di-acetylated derivative (4,15-AcNIV), produced by strains of *F. cerealis*, *F. poae*, *F. graminearum* and *F. culmorum*.

Trichothecenes have shown to cause a variety of toxic effects in laboratory animals, including skin inflammation, digestive disorders,

haemorrhagic syndrome in internal organs, blood disorders, haemolytic imbalance and depletion of the bone marrow, immuno-suppression (leukopenia) and nervous system disturbances (IARC, 1993). In farm livestock they are held responsible for several mycotoxicoses, including haemorrhagic syndrome caused by A-type; emetic and feed refusal syndromes, associated with B-type (Rotten *et al.*, 1996). T2 and DON have also been implicated in human toxicoses (ATA); but they have not yet proved to be genotoxic. Indeed, no trichothecene is classified as carcinogenicity compound to animals or humans by IARC (1993).

Zearalenone

Zearalenone (ZEA) is produced by *F. graminearum*, *F. culmorum*, *F. cerealis*, *F. equiseti* and *F. semitectum*. It is among the most widely distributed *Fusarium* mycotoxins in agricultural commodities and it is often found at relatively high concentrations, especially in maize grain. ZEA is both uterotrophic and estrogenic, and may cause reproductive disorders in domestic animals, particularly swine. It is responsible for recurring toxicoses in livestock, characterised by hyperestrogenism in swine, infertility and poor performance in cattle and poultry, and there is a possible impact on human health. The preliminary scanty evidence of ZEA genotoxicity is limited to mice and cultured mammalian and human cells. Thus it is not classified as human carcinogen (IARC, 1993).

Fumonisins

Fumonisins were first isolated from *F. verticillioides*, then they were found in cultures of *F. proliferatum* and in a few other *Fusarium* species, with unclear ecological distribution. Amongst them, fumonisin B1 (FB1) and fumonisin B2 (FB2) represent the greatest toxicological concern. Feed contaminated by FB1 cause leukoencephalomalacia in horses, pulmonary oedema in swine, poor performance in poultry, and altered hepatic and immune function in cattle. Moreover, home grown maize contaminated by FB1 has been associated with

oesophageal cancer of humans in Africa, China, and in the United States. The structural similarity with sphingosines suggests a role for fumonisins as depletion agents of the complex sphingolipids from biological membranes. This could account for their toxicity and, perhaps, their carcinogenicity. However, the evidence that *F. verticillioides* cultures and FB1 samples can promote liver cancer in rats, led to the classification of fumonisins as carcinogenic to animals and possibly to humans (Group 2B) (IARC, 1993).

Wheat root and crown disease

Wheat pathogens can be schematically classified according to the plant organ that they infect. Generally they are grouped into pathogens causing blights on leaves and ears and those rotting the root and the basal part of the culm (the so-called crown).

In Italy these latter diseases are frequently referred to as “mal del piede”. This is a vague, all including terminology. It comprehends a complex syndrome that can be attributed to many soil-borne fungi, with different morph-physiological traits. In other languages the disease is more precisely distinguished, according to the pathogen. For example, in French the terminology “piétin echaudage” refers only to the disease caused by *Gaeumannomices graminis* var. *tritici* (Ggt), while “piétin verse” indicates the root rots due to *Fusarium* spp., *Bipolaris sorokiniana*, *Rhizoctonia cerealis* and *Ramulispora herpotrichoides*. In English there are five terms: “take-all” defines the pathology caused by Ggt, “eyespot” that caused by *Ramulispora herpotrichoides*, “sharp eyespot” by *Rhizoctonia cerealis*, “rhizoctonia root rot” by *Rhizoctonia solani*, and “common (dryland) root and foot rot” the disease due to *Fusarium* spp. and *Bipolaris sorokiniana.*

All the reported pathogens produce a certain amount of inoculum, whose modality of action vary according to:

a) Geographic site
b) Edaphic condition (soil conditions)
c) Climate and weather courses
d) Agronomic practices

To better identify the disease causal agents, the influence of these variables has been studied for most single pathogens. However, the rot is frequently provoked by the infection of several species that can simultaneously or subsequently proliferate on the same plants and show synergic effects during the whole cultural cycle (Corazza *et al*., 1993 a and b).

A common feature of all root and crown disease pathogens is that they spend most of their vital cycle in the soil, where they use crop residues as survival and diffusion means. They live on them as active mycelium or as dormant propagules. Their saprophytic ability is not only a specific trait but often depends on the amount and distribution of plant debris along the soil profile (Bateman *et al*., 1998, Innocenti, 1993).

The competitive phenomena between the pathogens and the microflora living in the soil influence the primary infection potential in the autumn, at the beginning of the vegetative season (Garrett, 1970). A secondary contamination by low pathogenic species largely depends on the intensity of that primary contagion. Later infections, caused by species with long living soil-borne propagules, can also be important for the quantity and quality of produced grain.

The simptomatology of the root and crown syndrome can vary according to:

a) Main causal agent
b) Weather conditions
c) Edaphic conditions
d) Phenological stage of the host plant

A typical symptom of the root and crown disease is a more or less wide necrosis on the roots and/or on the basal portion of the culm. This causes an insufficient water and nutrient supply to plants because the culm mechanical resistance and its conductivity potential are reduced. In both instances hot and dry springs, provoking water stress, cause the appearance of the so-called "white ears", which give typically scanty caryopses. The mechanical damage to the culm favours lodging, which is worsened by strong wind and heavy rains. The ears, contacting the soil, can easily be infected by opportunistic pathogens that cause rot. Moreover, lodging increases the grain losses at harvest. Plant can die under heavy infections; this often happens soon after crop emergence (1.3-2 Feekes' scale), when autumn is moist and mild, and with large amounts of inoculum in the soil (Wiese, 1987). The plant physiological status represents a major factor in the starting and development of the pathology. For example, a nutrition deficiency favours the attacks of *G. graminis* by slowing down the root growth (Hornby, 1998). Instead excessive N supply favours lodging of the diseased plants. It can also augment the "white ears" density due to water stress because N increases transpiration (Wiese, 1987).

There exists a specific difference in susceptibility to this disease among the cereals. Wheat (both *T. aestivum* and *T. durum*) and triticale proved the most susceptible crops. Barley, thanks to its stronger root system, resulted less damaged by a same amount of inoculum in the soil. Oat revealed a higher resistance, particularly against *G. graminis*. Maize, rice, sorghums, and several wild *Poaceae* have a great importance in the pathogens survival and spreading in the absence of wheat (Domsh *et al.,* 1993; Wiese, 1987, Matta, 1996).

The major pathogens of wheat root and crown disease will be described more in detail in the following chapters.

Wheat root and crown disease main causal agents

Fusarium spp.

Taxonomy

The genus *Fusarium* includes several species that are considered causal agents of the root and crown disease of cereals. Many *Fusarium* spp. are metagenetically linked to the teleomorph genera *Nectria* and *Gibberella* that belong to *Hypocreaceae* family and *Ascomycetes* class. Other species (e.g. *F. culmorum*) exist only as anamorphs and belong to the *Hypomycetes* class (*Deuteromycotina*). Throughout this paper all species will be called fusari, thus referring only to their asexual state.

The main fusari causing the cereal root and crown syndrome are *F. culmorum* (W.G. Smith) Sacc., *F. avenaceum* (Fr.) Sacc., and *F. graminearum* Schwabe. Among them traditionally was included also *Microdochium nivale* (Fr.) Samuels & I.C. Hallet (in metagenetic linkage with *Monographella nivalis* (Schaffnit) E. Müller) that was classified as *F. nivale* (Fr.) Ces., until recently. The role of other *Fusarium* spp. as causal agents of this disease is still uncertain. Often they are considered scarcely pathogenic or not pathogenic at all: simple saprophytic fungi thriving in the rhizosphere, but their pathogenic capacity was reported variable (Balmas *et al.*, 2000). Among them there are: *F. sporotrichioides* Sherbakoff, *F. equiseti* (Corda) Sacc., *F. oxysporum (*Schl.) Snyder & Hansen, *F. solani* (Mats.) Nirenberg*, F. proliferatum* (Mats.) Nirenberg, and *F. tricinctum* (Corda) Sacc. (Specht *et al.*, 1988; Burgess *et al.*, 1988).

Symptomatology

The *Fusarium* disease on cereal plants is mainly characterized by brown spots on the roots, at the first or second node or flame-like necrosis spreading along the first internodes of the culm. However, many fusari can also cause seedling and ear diseases. Climatic conditions and, partially, the inoculum soil content can greatly change the symptoms (Cook, 1968; Parry, 1990). Seedling

withering can occur when infected seeds are used and with hot and dry spells at seeding time (Wiese, 1987). Root and crown rot appears later, and, again, it is favoured by hot and dry weather. Diseased plants, particularly when water stressed, suddenly dry down becoming easily recognizable (the so-called “white ears”) from the still completely green, healthy, plants. They mature earlier and produce few, scanty caryopses. All these effects can be ascribed to the injured parenchyma tissue at the culm base. The major interferences to the plant vascular system due to heavy infections can even kill the plants before flowering (Wiese, 1987, Matta, 1996).

Under wet springs, many fusari can reach the ear. There they cause spike sterility and withered kernels. Beside, the caryopses can be contaminated by the mycotoxins produced by the fungi.

Host range

Fusarium spp. are generally polyphagous fungi but some of them can show a narrow specialization on single hosts, as to present “*formae specialis*”. Moreover, many fusari can saprophytically live for long periods on alternative hosts, such as wild and cultivated *Poaceae*, or even broad-leaf weeds (Matta, 1996). Their pathogenicity varies according to the geographic site. For example, *F. graminearum* is considered the main responsible of fusari diseases on cereals in Australia and North America (Colhoun *et al*., 1964; Wong, 1985) but its importance is negligible in Europe (Corazza *et al*., 1987; Bateman *et al*., 1995; Rossi *et al*., 1995) where, on the contrary, *F. culmorum* is more widespread and dangerous. The pathogenic activity is variable also in different isolates. Some isolates of *F. equiseti* and *F. tricinctum*, which are common species not only in agricultural land, have shown a high pathogenicity towards cereals both in controlled environment and in the field (Balmas *et al*., 2000), while innocuous *F. culmorum* isolates are largely reported in the literature (Salt, 1978). Moreover, certain conditions of the host plant and weather can suddenly turn the fungus from a saprophytic to a pathogenic state (Matta, 1996).

Life cycle

Although the transmission through infected seed can be frequent, the contaminated residues of the preceding crops represent the main inoculum source. The primary infection starting from the organic debris on the soil can last throughout the vegetative season. Notwithstanding a high tolerance to various climatic conditions, the infections by several fusari (e.g. *F. culmorum* and *F. graminearum*) prevalently occur under hot and dry weather, while others (e.g. *F. avenaceum* and *M. nivale*) are favoured by high moisture and relatively low temperatures. At crop emergence, the primary infection starts on the coleoptile and on the seedling roots, later it can infect the crown, the developed roots and the culm internodes. The upward progress of the disease is accomplished by secondary infections, which, however, still depend on the success of the first entrance (Wiese, 1987). The inoculum content in the soil before seeding, although can affect seedling emergence, is not always correlated to the disease intensity on adult plants, which appeared more influenced by weather conditions. Under rainy and humid springs (Pancaldi *et al*., 1994), the inoculum originated from the sporodochia at the stem base can infect all aerial plant organs through rain spattering (Jenkinson *et al*., 1994) or even with the insect aid (Sturz *et al*., 1983).

On the crop residues the fungus can survive as active mycelium or as chlamydospores and perithecia, in the instance of pathogens with the sexual state. In the soil the survival is achieved only by chlamydospores, which, in some species, remain viable longer than two years.

Ecology

Fusarium spp. are common on the cereal crops all over the world. Species are not differentiated on the basis of their diffusion, but mainly on their pathogenic capacity. For example, in Australia the *Fusarium* disease is mainly ascribed to pathogenic strains of *F. graminearum*, while the isolates of this fungus were found scarcely damaging in Europe (Colhoun *et al*., 1964). In particular habitats Balmas *et al*. (2000) and Domsh *et al*. (1993) reported

virulent strains also of *F. equiseti*, which is a typical saprophytic, ubiquitous fungus that is quite common everywhere, in untilled soils. The various species show different climatic requirements. *M. nivale* is particularly abundant and harmful in Northern Europe (Colhoun *et al*., 1964) because it is mainly active in cold and wet periods, which are too short in warmer climates. However, a high presence of this fungus has been reported also in some parts of Southern Italy (Piglionica *et al*., 1975) under particularly wet winters. *F. avenaceum* as well results more harmful in colder climates, where it mainly damages emerging seedlings (Domsh *et al*., 1993). This marked behavioural variability of the different *Fusarium* strains as a function of edaphic and climatic condition, make their control extremely difficult and site-specific.

A high N fertilization generally favours *Fusarium* spp., while they show a great adaptability to other soil factors, like pH, P, K, and organic matter soil content. Their survival ability endures a quite wide range of soil temperatures and moistures, with different optima for the single species. For example: *F. culmorum* and *F. graminearum* prefer hot and dry soil, while *F. avenaceum* and *M. nivale* colder and wetter conditions.

Diagnostic techniques

Visual diagnosis: Typical disease symptoms are spots on the first 2-3 basal internodes, slightly narrowing at the nodes. At the crown a flame-shaped necrosis can be often noted, that can extend over a large portion of the first internode. Inside the culm, mainly at the node level, a whitish or rose mycelium can often be observed.

The fusarioses identification is not easy for it is quite impossible to distinguish them from *B. sorokiniana* disease. In case of serious infections, the typical brown elliptical spots of this latter fungus join together and give a diffuse browning that resembles that of *Fusarium* spp. The symptoms of *G. graminis* disease are alike as well, mainly in heavy infections. Moreover, all three pathogens, besides causing similar symptoms, can be simultaneously found on a same host plant.

Photo 1. Darkening at the crown up to the 1st-2nd node of the culm. The symptoms can be ascribed either to *Fusarium* spp. or to *B. sorokiniana*.

Conventional isolation methods: *Fusarium* spp. can be isolated in the soil either directly, from the solid matrix, or through a serial dilution technique. The latter method is mainly used for the species that survive only by chlamydospores (Windel, 1992). The isolation from diseased tissues is also possible. They can be grown on many agar media (e.g. PDA, MPA, etc.). For species identification it is advisable to induce the sporulation and especially the macroconidia production. For this scope PDA nor MPA are not always adequate. Many species do not sporulate on them and, even if they do, their spores are misshapen. For the sporulation, many researchers use sterile dianthus leaf water agar (Nelson *et al*., 1983) or SNA (Spezieller Nahrstoffarmer agar), with a previous exposure to near ultraviolet light (Nirenberg, 1981). The species diagnosis is based on several parameters, such as the morphology, the colour, the growth speed of mycelium, the presence of chlamydospores, the sporodochia colour, the conidiophora type, the presence of micro- or macroconidia, etc. (Nelson *et al*., 1983).

Bipolaris sorokiniana (Sacc.) Shoemaker

Taxonomy

Bipolaris sorokiniana is the anamorph of *Cochliobolus sativus* (Ito & Kurib.) Drechs., an *Ascomycete* of the *Plesporaceae* family, *Dothideales* subdivision. Once it was included in the genus *Helminthosporium*; for this reason the disease that it causes on many *Poaceae* is still frequently called Helminthosporiosis (Matta, 1996).

Symptomatology

The symptoms are similar to those reported for *Fusarium* spp. at the crown zone and on the first internodes scorches that are slightly darker than those that fusari develop. The disease can also reach the root system, but generally with little damage. Under wet weather, the lower leaves can be infected from the diseased crown, developing dark spots, particularly evident after flowering. *B. sorokiniana* disease is more manifest under hot and dry weather, which favours water stress in the host plant. However, differently from Fusarium spp., its infection never reaches the spikes (Wiese, 1987).

Host range

It is a pathogen of a wide range of *Poaceae*. However, it can be isolated also from several dicotyledonous crops, on which it causes only slight damages (Stack, 1992; Matta, 1996).

Life cycle

It is similar to that of *Fusarium* spp. The mycelium mainly survives on infected plant debris as a saprophyte. It contaminates the kernels with which gets in touch. Its dormant conidia can survive in the soil for many months, waiting for a host. Therefore primary infections can start from the seed, from crop residues or from free-living in the soil conidiospores. Not too low

temperatures are required. Secondary infections develop from the culm lesions, from which conidia are produced that infect leaves in wet periods. However, the most serious damages occur in hot and dry early summers, when the injured vascular system causes water stress. In the open field the disease often manifests with irregularly spread patches of dwarfed and chlorotic plants (Wiese, 1987)

Ecology

It is widespread all over the World, wherever cereals are grown. This fungus is well suited to various climatic situations. Its ecological requirements are similar to those of *Fusarium* spp., therefore the two pathogens are often isolated together. Like *Fusarium* spp., it benefits from high N concentrations in the media. In general, it tolerates wide variations of soil pH and humidity (Domsh *et al*., 1993).

Diagnostic techniques

Visual diagnosis: As already mentioned, it is hard to visually identify the pathogen because of the resemblance of its symptom with that of fusariosis. The spots on the internode just below the crown are perhaps the most distinctive symptom.

Conventional diagnostic methods: They consist of the isolation of the pathogen from soil or from diseased tissue samples kept on culture media. Its mycelium is green-black and originates several erect and septate conidiophores. They stand alone or clustered so to give a velvet aspect to the colony. The conidia are slightly curved with 2-3 septa, where the cell wall is thicker. They are well identifiable with an optical microscope.

Gaeumannomyces graminis (Sacc.) Von Arx & Olivier var. tritici Walker

Taxonomy

This *Ascomycete* was previously classified as belonging to the *Diaphotales* order. Recently it was linked to *Magnaporthe* spp. and now it is included in the *Magnaporthaceae* family, which hasn't yet been assigned to any specific order (Cannon, 1994). The anamorph of *G. graminis* var. *tritici* is *Phialophora radicicola* Walker and both states are collectively referred to as the "*Gaeumannomyces-Phialophora* complex".

Symptomatology

The primary infection starts on the seminal roots of autumn sown wheat on whose surface dark runner hyphae develop. The hyphae enter the root through the cortex, colonizing and destroying the vascular tissue. Diseased roots and stem darken to nearly black and the lower leaves typically become chlorotic. If the plant doesn't die, it produces few or no tillers. With time, the black lesions can spread toward the root tips and extend up into the crown tissue. If the soil remains moist for long periods, the disease may patchily spread in the field. Eventually, the infected plants develop "white ears" and die prematurely.

Host range

G. graminis var. *tritici* is a pathogen of wheat but can also be found on many other cereal crops wild grasses and volunteer cereal weeds.

Life cycle

During the intercrop period, *G. tritici* saprophytically survives on crop residues, which represent the major inoculum source. The amount of viable inoculum that remains in the soil sharply drops in the absence of susceptible

crop. This is usually sufficient to drastically reduce the disease incidence after a break year in cereal successions. Perithecia, which subsequently release ascospores, are sometimes produced at the crow of cereal plants or on stubbles. However, this propagation is considered negligible in the field.

Ecology

G. tritici distribution is worldwide, wherever wheat is grown. It is more frequent in temperate regions or at high elevation in the tropics. It prefers neutral or alkaline soils and thrives on water soaked and NP deficient soils. Nitric-N is more favourable to the fungus than ammonium-N.

Photo 2. *G. graminis*. Black lesions develop on the root and extend up into the crown tissue

Diagnostic techniques

Visual diagnosis: As above mentioned, the identification of the fungus on the basis of a simple visual diagnosis is not easy because of the resemblance

of its symptoms with those of *Fusarium* spp. Perhaps the darkening of the first internode below the crown is the more typical feature.

Conventional diagnostic methods: They consist in the isolation from infected tissue or soil samples kept on cultural media. The main fungus feature is a black-green mycelium, which produces several erect and septate conidiophores. They develop alone or clustered conferring a velvet aspect to the colony. The conidia are slightly curved with 2-3 septa. They are easily recognizable with an optical microscope.

Ramulispora herpotrichoides (Fron) Arx

Taxonomy

It is the anamorph of *Tapesia yallundae* Wallwork & Spooner, which is an *Ascomycete* of *Helotiales* subdivision (Matta, 1996). *R. herpotrichoides* has two varieties that differ according to their conidia morphology: vaR. herpotrichoides, with 4-septate conidia (35-80 µm length), and var. *acuformis,* with 4-6 septate conidia (43-120 µm length). Both of them are cereal pathogens (Murray, 1992).

Simptomatology

The initial disease symptoms are dark, irregular spots that become more evident at the ear emergence stage. The symptoms at the plant base are typically long strikes running along the culm. They are grey to yellowish-brown in colour, with darker centre and margins, and are called “eyespot” lesions.

Host range

R. herpothrichoides parasites all herbaceous plants; but mainly *Poaceae*.

Life cycle

It is a polycyclic pathogen. Its life starts with a primary infection that originates from the crop residues, where the fungus survives as active mycelium. Then the disease progress with secondary infections brought about by conidia that are produced by the mycelium from the crown lesions. The primary infection begins whenever the autumn, winter, or spring temperatures fall below 16°C. Epidemiologic researches have shown that the amount of inoculum left in the soil until the next crop depends on the secondary infections (Wiese, 1987). *R. herpotrichoides* mycelium can survive also on wild *Poaceae,* which represent another major factor of inoculum diffusion and survival. The sporulation has a maximum at 10°C, but can occur within 0-20°C range.

The fungus can survive as a saprophyte on infected residues up to three years. However, adverse edaphic conditions and many agricultural practices (e.g. soil ploughing) can markedly reduce its survival (Garrett, 1970; Wiese, 1987).

Ecology

In Europe *R. herpotrichoides* has been frequently reported in France, where it is considered a major problem in all cereal crops (Colbach et al., 1995), and recently also in Central Europe (Cavelier et al., 1997). It has also been found in South and North America, in New Zealand, Australia, and Africa. In Italy it appeared less common and less damaging (Innocenti, 2000 b).

Mild winters and cool, rainy spring favour the fungus. However, also dry and hot spring can be dangerous on the plants infected in winter. The fungus thrives in high N fertilized soils, particularly when their moisture remains high throughout plant life.

Diagnostic techniques

Visual diagnosis: The disease shows clear symptoms on adult plants, but only when the lesions are not too extended. They consist of eyespots that are grey to yellowish brown, darker in the central zone than in the margins. The lesions can look like those of *R. cerealis*, but they differ having more blurred margins and a darker centre. The grey mycelium inside the culm can be seen trough the lesions, while externally, on the infected tissue, dark hyphal stroma develop and produce conidia. Sometimes, in heavy infections, the culm twists and breaks down, causing lodging. As mentioned above, many lesions can merge together and determine a diffuse darkening of the culm that is not easily distinguishable from the symptoms of *Fusarium* spp. or *B. sorokiniana*. All three species can live together on the same culm part. This coexistence has been shown both with conventional isolates (Bateman, 1993) and with PCR techniques (Turner *et al.*, 1999). It often causes an overvaluation of *Fusarium* spp. dangers with respect to *R. herpotrichoides*.

Conventional diagnostic methods: The isolation from diseased tissues is not simple because of the scarce competitiveness of the fungus in cultural media, mainly in the rich ones, like PDA. The isolation is easier when the tissues are still green and with less presence of bacteria and other fungi. After having superficially sterilized the tissue, it is better to use poor agar media (e.g. water agar), enriched with antibiotics, in particular with rifampicin (Murray, 1992). Mycelium needs 18-20°C to grow and produce conidia when stimulated with NUV light. A typical mycelium growth progress slowly, regularly, and in a concentric way around the inoculum site. The mycelium appears compact and hyaline. The isolation from the soil is very hard. It can be done from infected residues, after an incubation period in a humid chamber. The conidia directly originate from the tissue and can thus be collected with a small spatula (Murray, 1992).

Photo 3. *Ramulispora herpotricoides*. The symptoms at the plant crown are grey to yellowish-brown longitudinal spots with darker centre and margins which are called eyespot lesions

Rhizoctonia spp.

Taxonomy

Both *Rhizoctonia solani* Kühn and *Rhizoctonia cerealis* Van der Hoeven can be cereal pathogens. The first one is the anamorph of the *Basidiomycete Thanatephorus cucumeris* (Frank.) Donk; the second of *Ceretobasidium cereale* D. Murray & L.L. Burpee. In anamorph state they spread only by mycelium. Both species can be divided into anastomosis groups, i.e. in accordance with the possible hyphal fusion between compatible strains. AG-8 is the commoner *R. solani* pathogen of the cereal root system. Also *R. cerealis* was divided into several anastomosis groups (CAG), but their incidence is still unclear (Carling *et al*., 1992).

Simptomatology

R. solani can be a very noxious pathogen of the cereal root systems, where it causes rot. Its visual diagnosis is difficult because at sampling the infected root tear off, remaining in the soil. The extracted plants show only slight spots on short pieces of roots. In some circumstances the infection is so heavy that all roots almost disappear. The root injury causes plant dwarfing, lodging and “white ears” in adult crops. Sometimes the fungus directly kills the seedling. The pathogen infection can facilitate the entrance of other, secondary invaders (e.g. *Acremonium* spp., *Epicoccum* spp., etc.). These fungi can prolong the radical disease even during the hotter months, when *R. solani* normally regresses. On the contrary, *R. cerealis* is a pathogen of the culm. Its symptoms are often mistaken with those of *R. herpotrichoides*. They appear as very sharp eyespots, with dark margins and white centre, generally located on the first basal internodes. These spots are some cm of width but they can merge so as to enclose the whole culm circumference. When this happens the plant is particularly susceptible to lodging. Also the vascular system is damaged, but

usually less intensively than by other pathogens. However, patches of “white ears” plants can be observed in the field.

Host range

The hosts vary with the anastomosis groups. The cereal root rots is usually ascribed to AG-8 *R. solani*. The other groups are either saprophytic or parasite other hosts, many of which are cultivated plants, like potato, sugarbeet, *Fabaceae* and *Brassicacae* species (Carling et al., 1992). *R. cerealis* is a polyphagous fungus on the *Poaceae* species, with a high saprophytic capacity. Probably for this fungus as well there are specializations of various anastomosis groups, but they are still scarcely known.

Life cycle

The mycelium of both species can easily saprophytically survive on plant residues or free in the soil, where it is very resistant to microbial attack. The infection starts soon as it gets into contact with the host, generally soon after emergence. Usually *R. solani* enters the root, while *R. cerealis* infects the lowest leaves sheaths. At first it spreads superficially towards the apex, then inside the foliar parenchyma. When it reaches the culm it determines the typical eyespots. For this fungus primary or secondary infection cannot be differentiated because the mycelium is always active. Its constant growth does not allow any conidial stage. Therefore the teleomorph role in the disease seems negligible (Wiese, 1987).

Ecology:

R. solani is widespread in all temperate regions, but not always behaves as a pathogen. For example, heavy damages have been reported in Australia, USA, Europe, but not in Italy. *R. cerealis* is common in North America, Europe and also in Italy (Innocenti *et al*., 2000 a; Rossi *et al*., 1995). The first fungus is prevalent in wet and cool environments, while *R. cerealis* thrives in hot and dry

climate. *R. solani* prefers humid soils while sandy and slightly acid soils favour *R. cerealis*.

Photo 4. *Rhizoctonia* spp. Symptoms located on the first basal internode. They look like sharp eyespots, with marked dark margins and white centre.

Diagnostic techniques

Visual diagnosis: The determination of *R. solani* through the examination of its symptoms is particularly difficult because the infected roots easily tear off from the plant and remain in the soil. On the contrary, *R. cerealis* is easier to recognize for the typical eyespots shape of its disease. Moreover, pseudosclerotia, which are characteristic survival propagules of the fungus, are often found on the lesions.

Conventional diagnostic methods: The mycelium isolation is quite easy when infected tissues are grown on agar media. Instead the serial dilution method to isolate *Rhizoctonia* spp. from the soil cannot be used for the absence of conidia production. However, the fungus sclerotia can be directly extracted by

sieving the dried soil particles. In the agar media rhizoctonia colonies are typically yellow-brown in colour and grow rapidly. They develop little aerial mycelium and concentrically produce sclerotia that become dark brown with time. Microscopically, the fungus is characterized by narrow hyphae at the lateral branches that depart at right angle from the main hypha. *R. cerealis* differs from *R. solani* for the darker colour of its mycelium, for larger hyphal diameter and, particularly, for binucleate cells instead of polynucleate. The anastomosis groups can be determined by growing mycelia on water agar and letting intercrossing in a same plate the unknown colonies with tester strains (Parameter *et al*., 1969; Carling *et al*., 1988).

Wheat root and crown disease in Italy

Notwithstanding the wide diffusion of root and crown disease in the Italian wheat, the papers dealing with this pathology are relatively few, particularly those treating the epidemiologic aspects. This is mainly true in the Northern regions, where only Picco (1985), Innocenti *et al.* (1985, 1986 and 1992), Rossi *et al*. (1995), and Toderi *et al*. (1976 and 1978) have studied the pathology in the last 20 years. In the South, where durum wheat is concentrated, the researches have been more numerous (Frisullo *et al*., 1991; Piglionica *et al*., 1975 and 1976; Corazza *et al*., 1987, 1993 a; Balmas *et al*., 1992; Cappelli *et al*., 1977; Covarelli *et al*., 2000).

Many authors studied the disease as influenced by various factors, such as: botanical species (Corazza *et al*., 1998 and 1999), crop variety (Corazza *et al*., 1987; Rossi *et al*., 1995), fungicide efficacy (Frisullo *et al*., 1978; Covarelli *et al*., 2000), N fertilization (Cappelli *et al*., 1977), crop sowing date (Covarelli *et al*., 2000; Cariddi *et al*., 1985), crop residue management (Corazza *et al*., 1993 a) soil tillage, and crop rotation (Innocenti, 1993 and 1996; Innocenti *et al*., 2000 a).

Amongst the fungi responsible for the “mal del piede” syndrome, *F. culmorum* resulted the most frequent and dangerous pathogen all over Italy, particularly on durum wheat grown in the South (Innocenti, 1996, Innocenti *et al*., 2000 a; Corazza *et al*., 1987, 1998 and 1999; Piglionica *et al*., 1976; Frisullo *et al*., 1991). Beside this species, *M. nivale* was found extensively spread (Rossi *et al*., 1994; Piglionica *et al*., 1975 and 1976), particularly when investigations were made in spring, with weather more suitable to the fungus. All other species have been reported only sporadically, though sometimes they showed really heavy infestations. This happened, for example, for *G. graminis* (Innocenti, 1992), *R. herpotrichoides* (Innocenti *et al*., 2000 b; Covarelli *et al*., 2000), *B. sorokiniana* (Rossi *et al*., 1995; Corazza *et al*., 1999), *R. cerealis* (Rossi *et al*., 1995; Innocenti *et al*., 2000 a) and *R. solani* (Corazza *et al*., 1998).

Sporadic resulted the frequency of other *Fusarium* species that are potentially pathogenic to durum wheat.

The influence of several factors on the development of the disease was found extremely variable, probably as a function of the different environmental conditions, where the researches took place. Thus the experimental results are not easy to generalize.

Control methods of wheat root and crown disease

The control of root and crown disease in the Italian wheat is extremely difficult for several reasons, among which: a) the number of possible causal agents, b) the weather fundamental role on the infection frequency and severity and c) the necessity to obtain satisfactory crop yields with a minimum environmental impact. Being a complex syndrome, its control requires multiple managements. Indeed, the techniques that are aimed at the control of a single pathogen are usually unsuccessful. Sometimes good results are obtained, but they are sporadic and generally last for short periods of time. The eradication of a causal agent often favours another one, which can be even more noxious than the target organism. The large diffusion in French wheat fields of *R. cerealis*, was attributed to the successful, widespread chemical control of *R. herpotrichoides*. According to Bateman *et al*. (1999), a complete control of *G. tritici* can increase the *F. culmorum* infections. This agrees with what Cavazza (pers. comm.) observed on durum wheat grown in the Ozzano experimental farm of Bologna University, where the control of *G. graminis* with agronomic practices favoured the fusariosis. Chemical control and the development of genetic resistances in crops have received great importance in fungi management within the intensive agriculture of the developed countries. Unfortunately, for most of the wheat pathogens both methods are often not so effective or cheap enough to be extensively employed. This is particularly true for *Rhizoctonia* spp. and *G. graminis*. The introduction of genetically resistant varieties has been sometimes successful, but not thanks to a direct action against the pathogen, but for a better resistance to the consequences of its

infection. This is the instance of short wheat varieties, which can better resist to lodging, or varieties with stronger and branched root system that can replace rotten portions (Hornby, 1988). However, it should be stressed that the new cultivars do not reduce the amount of inoculum in the soil. Therefore from a phyto-pathological standpoint their use is not so useful. In the past the seed treatment with systemic benzimidazoles resulted quite successful against *Fusarium* spp. (Frisullo *et al*., 1978; Roberti *et al*., 1992). However, the currently increasing distrust of the public opinion regarding pesticides makes their use less recommended also in intensive agriculture.

Within a sustainable agroecosystem the biological control should receive a major importance because it exploits the natural cycles of biotic and abiotic elements, with a reduced environmental impact. This term comprehends all measures that negatively affect the pathogens. The methods can be direct or indirect: organisms that are not normally involved in the Host-Parasite complex can be used (Matta, 1996), or we can physically modify the environment to disfavour the parasites. The direct biological control consists in introducing antagonists with the target pathogen in the soil or on the host plant. They can act through the following mechanisms: a) predation, 2) competition, 3) hyperparasitism, 4) antibiosis and 5) plant resistance induction. The conventional techniques for using mycoparasites to control fungi are the treatment of seed or propagation material and the spreading on tilled soil of their propagules in liquid or semi solid formulations (Chet, 1990).

The most utilized organisms against the mycopathogens belong to the genera *Trichoderma* e *Gliocladium*. In controlled environments (laboratory and glasshouse) *Trichoderma* strain on wheat proved successful in controlling root and crown disease pathogens, such as *Fusarium culmorum* (Roberti *et al*., 2000), *G. tritici* (Almassi *et al*., 1991; Dunlop *et al*., 1989; Simon *et al*., 1988) and *R. cerealis* (Innocenti, 1989). Unfortunately their application in the open field seldom gave satisfactory results and appeared not yet economically recommended. The use of low virulence strains of *G. graminis* or *Phialophora* spp. to check *G. graminis* gave some positive results (Tivoli *et al*., 1974), but

the action of less virulent strains is still uncertain. The use of not-pathogenic or scarcely pathogenic fusari (e. g. *F. equiseti*) can be interesting. In the glasshouse they showed the ability to reduce the occurrence of *F. culmorum* disease, probably through competition for infection sites (Balmas *et al*., 2000), but further confirmations in the open field are needed. The successes obtained with *Pseudomonas* spp. strains applied in seed treatments to contrast *G. tritici* appear more promising (Cook, 2000). These organisms produce antibiotics with a powerful inhibitory activity towards *G. graminis* (Thomashow *et al*., 1988). However, their scarce action against other root pathogens, like *R. solani*, represents a limiting factor in their widespread agricultural use (Cook, 2000). Biological control has other drawbacks. For example, the current methods of antagonist applications in the field do not allow it to develop sufficient biomass and persistence in the soil. Indeed the newly introduced organism suffers both from the competition of soil microflora and from sub-optimal soil conditions. Therefore repeated applications would be required, intolerably rising the costs of control. Moreover, many of the purposed antagonists are site-specific and require precise environmental, edaphic conditions that hinder their widespread commercialization. Recently organic matrixes as means to carry the useful organism into the soil have been tested with the aim of developing an active and stable biomass in the soil. Today the most promising matrix appears the compost originated from the differentiated organic waste disposal. Several researches have found that some physical, biological and chemical factors in the compost enhance the efficacy of the antagonist, prolonging and stimulating its activity (Postma *et al.* 2000). If this use will spread we could simultaneously obtain numerous benefits: the amendment of soils, their protection from runoff and erosion (Giardini, 1982), together with a reduction of waste disposal problems.

The indirect biological control is essentially based on the use of one or more agronomic practices aimed at limiting the pathogens spread by: a) eradication of the inoculum from the soil, b) enhancement of the competition

and inhibitory activity of naturally occurring soil microflora and c) optimization of the soil conditions to create a more favourable environment to cropped plants.

Amongst the agronomic practices, soil tillage can directly destroy the inoculum in the soil. Ploughing, by overturning sods, carries the pathogen inoculum present in crop residues in deeper soil layers, where there are sub-optimal edaphic and environmental conditions (Giardini, 1982). Moreover, it determines a dilution along the soil profile of the inoculum. This reduces its infective potential by removing it from the first cm below the soil surface, where the crop seed will be placed. In that way a seedling infection soon after germination is less probable (Innocenti *et al*., 2000 a). Also the soil ripping (without the sod turning) can destroy some fungi by fragmenting their hyphal chains, particularly those, like *Rhizoctonia* spp., which develop large mycelium nets in the soil (Wardle, 1995).

Some other agronomic practices can reduce the soil inoculum. Rotations can be particularly successful against highly specialized pathogens, like *G. graminis* or *R. herpotrichoides*. The best results can be obtained by alternating wheat with not-host crops (Innocenti *et al*., 2000 a; Rovira, 1985), with set aside (Cook, 2000), with host crops sown in spring (Cook, 2000), or late in the autumn (Covarelli *et al*., 2000; Hornby, 1998). The fungi with a low saprophytic capacity can't survive on plant debris for a long period without the host plant. Therefore they will be rapidly replaced by the resident saprophytic microflora (Cook, 2000). The rotation of wheat or other winter cereals with not-host crops enriches the soil with an alternative biotic community that replaces the previous population by competitive effects (Innocenti *et al.,* 2000 a; Kollmorgen, 1985). The positive effect of not-host crops can be due not only to the selection of a different microflora but also to the production of root essudates with antibiotic actions (Baker *et al*., 1982). According to some authors (Cook, 2000; Yarham *et al*., 1981), soil tillage that less disturb or do not disturb the soil at all (like minimum or no-tillage), can favour the settlement of the antagonist fungi, which can help control the diseases.

Many agronomic practices are aimed at the improvement of crop plant physiology. The same ploughing, for example, by creating large macroporosity in the soil: a) increases the water availability for the crop by enlarging the water reserves, b) prevents water stagnation risks, reducing the problems of root asphyxia and root rot and c) increases the volume of soil available for root growth. However, also no-tillage can improve soil structure and nutrient availability by favouring the organic matter build-up in the first layers of soil (Giardini, 1982). A uniform and adequate sowing density can reduce the wheat susceptibility to lodging (Wiese, 1987). Nitrogen fertilization as well can greatly influence the physiological status of the crop. It can improve the growth and strength of host plants that thus can better resist to the infections. But excessive N rates favour lodging and increase crop transpiration, thus worsening the water stress of infected plants (Wiese, 1987). The same type of nitrogen compound used in the fertilization can influence the interaction host-parasite. It was shown that ammonium is less favourable to *G. graminis.* By lowering soil pH, it increases manganese availability that stimulates antagonist bacteria in the rhizosphere (Sarniguet, 1990).

Effects of soil tillage on wheat root and crown disease

Schematically soil tillage today has three variants:

a) Ploughing (to various depths, generally from 20 down to 50 cm)
b) Minimum tillage (with various tools, without the sod turning effect of ploughing)
c) No tillage (direct sowing, with crop residues on the soil surface)

The three systems have a great impact on soil habitat. Soil tillage directly influence the physical and chemical soil properties, such as the moisture content, the aeration, the temperature, etc., all of which determine the root growth and nutrient assimilation. The physical impact on soil microhabitat and the dislocation along the soil profile of soil pathogen and of their antagonists

can directly affect the incidence and severity of root and crown disease (Sumner *et al*., 1985). The sod overturning operated by ploughing has numerous advantages, like the reduction of weed seedbank, the burial of crop residues and propagules, the placement of fertilizers at an optimal depths, the reduction of water stagnation risks, etc. However, in the last years this practice has been abandoned in many developed countries due to its high costs both economic and energetic. Besides, its ecological impact is considered too heavy. Ploughing, by a better soil aeration, improves organic matter mineralization, thus depleting the soil of this important component. Soils with less organic matter, particularly in the shallower layers, are more prone to erosion, superficial runoff and pollution risks due to applied chemicals. Moreover they show a reduced nutrient availability to plants (Giardini, 1982). Therefore nowadays ploughing is considered beneficial only in heavy soils and in localities with high precipitations in the autumn-winter period, where soils can get water saturated. The economic benefits of reduced tillage greatly depend on the crop requirement of soil structure. Some plants, like sugarbeet, sunflower, etc., are particularly favoured by aeration deep along the soil profile; others, like many shallow rooted cereals (e.g. wheat, barley, etc.) are less demanding. According to Giardini (1982), also in the heaviest soils a ploughing depth to 20 cm should be sufficient to obtain a satisfactory economic return from these latter crops. For them minimum or no tillage is often preferable.

No tillage (sin. zero-tillage, sod seeding, direct drilling, etc.) is one of the most revolutionary agronomic practices of the last century. It consists in the sowing of a crop without disturbing the soil. That means with the soil surface still covered by the residues of the preceding crops. It demonstrated successful all over the World, particularly on lighter soils, on sloping land prone to erosion, or on low in organic matter soils. With respect to ploughing no tillage has the advantage to: reduce cultivation costs to a minimum, limit the erosion risks and favour the humification processes of soil organic matter. A stable high organic matter reserve is particularly beneficial also from an ecological point of view. It arises the soil cation exchange capacity and absorption power, which are

important prevention means of water table pollution by agricultural chemicals. A drawback of no tillage is a major presence of weeds whose seeds are less disturbed. This contributes to a seedbank build up, mainly on the soil surface, with an increased emergence of weed seedlings. The recent progress in the weed control with the availability of safer and economic herbicides (e.g. glyphosate) has markedly reduced this problem in the last years. One of the disadvantages of no tillage not so easily resolvable remains the risk of water stagnation in heavy, clayey soils, which are typical, for example, in the Italian eastern Po Valley, particularly with high precipitations in the autumn-winter period.

Regarding the no-tillage effects on the root and crown syndrome in wheat, the researches have been few and their results often contrasting. Direct sowing showed little influence on the mobility and diffusion of *R. herpotrichoides* (Herman *et al*., 1985; Vez, 1979; Yarham *et al*., 1979; 1981), and Cook (1977) did not find any significant difference between no tillage and ploughing. *G. graminis* demonstrated a variable response: in an American research take-all was more aggressive on direct tilled crops (Moore *et al*., 1984), while a study in the Czech Republic gave the opposite results (Novotny *et al*., 1981). Other experiments showed no significant differences on this fungus between ploughing and no tillage (Yarham *et al*., 1981). When no tillage was found more effective against the pathogen this was attributed to the more compact soil at the shallower layers that limits the movements of the fungus propagules or mycelium.

Minimum tillage has been studied on heavy Italian soils (Triberti *et al*., 2000). This practices is aimed at the same beneficial effects of not ploughing: a decrease of cultivation costs, a reduction of erosion risks, and slower soil organic matter degradation, which makes the soil management more sustainable. In one of its simpler variants minimum tillage is obtained by a unique disc harrow passage before the seeding, comporting a shallow soil disturbance (15-20 cm, on average). In more intense minimum tillage a ripper passage and one or two harrow passages are added (Giardini, 1982). With

respect to no tillage, minimum tillage has the advantage to form a looser soil, with consequent soil warming, especially in spring, and minor water stagnation risks, and to favour a slightly better burial of cultural residue into the soil, but never so good as that obtained by ploughing. Thus it can be considered a middle course between ploughing and no tilling. As ploughing it superficially disturbs the soil and thus bring about a direct physical eradication of many fungal inoculums, but, like no tillage, it leaves most crop residues, which are the major infection sources, in the first cm of soil, where the crop seed will be placed. From the literature it seems that minimum tillage favours the fusariosis and *R. herpotrichoides* infections (Innocenti *et al.,* 2000 a; Innocenti, 2000 b). Instead its effects on *G. graminis* are still uncertain (Yarham et al., 1981). However, high disease damages have been reported in the instances of heavy infestations of this fungus on minimum tilled plots (Innocenti, 1992). Minimum tillage can affect soil mycopathogens also indirectly. For example a repeated minimum soil disturbance over the years usually lowers soil pH and this acidification, in the long term, should reduce the pathogenic activity of some fungi, such as the same *G. graminis* (Triberti *et al*., 2000).

Crop rotation effects on wheat root and crown disease

Since the Roman times, and even before, the crop rotation has always been considered an important mean to prevent the soil fertility loss that is frequently observed when a same crop is repeated on a field. Today the monosucession of some crops (e.g. maize, rice, cotton, etc.) is widespread in some countries with a highly specialized and mechanized agriculture, but it is more often criticized. The monosuccession cannot be included in a sustainable agriculture (particularly in organic farming) mainly because it greatly relies upon an effective chemical control against pathogens and weeds. The crop succession beneficial effects on the agro-system, however, are highly variable according to the adopted rotational design and to the chosen crops. From this point of view a rough classification divides crops into these groups:

Improving crops (e.g. leguminous leys) that improve the soil structure and the nutrient (mainly N) availability.

Impoverishing crops (e.g. winter cereals, sorghums, colza, etc.) that leave the soil after being harvested in worse condition than before their sowing.

Preparatory (or renewal) crops (e.g. sugarbeet, sunflower or peas) that improve soil structure for the intensive tillage practices they need. Set aside (fallow) can be included among them because it can contribute to a better water and nutrient availability to the subsequent crops and to a drastic reduction of the weed seedbank in the soil, particularly when it is covered with *Leguminous* plants to be buried under (N enrichment) or when it is well managed with the aid of chemical or cultural means (weed suppression) (Giardini, 1982).

The effects of crop successions on the mycopathies are extremely variables. The first factor is whether the alternating crops consist of plants that are hosts, not-hosts or alternative-host of a single pathogen. In the latter instance the crop can be or not damaged by the pathogen. If the crop is not particularly injured, its presence can even augment the inoculum content in the soil, thus resulting in heavier infections in the subsequent cultivation. This represents a so-called “bridge crop”. Oat is an example of this phenomenon. Without revealing any fusariosis symptoms it caused a greater infection on the successive wheat crop in many researches in different parts of the World (Innocenti *et al*., 2000 a; Cook, 1981; Corazza *et al*., 1993 b). Maize resulted a bridge crop for *G. graminis* and *R. cerealis* (Colbach *et al*. 1997). The same authors (Colbach et al., 1995) found that an alfalfa ley of three years has a bridge effect for *R. herpotrichoides*, not because the fungus directly infects leguminous plants but because the ley is frequently infested by *Lolium* spp., which are particularly susceptible to its disease (Ponchet, 1959 and Maenhout, 1975). Even barley, which is usually tolerant to *G. graminis* resulted in heavier pathogen damages on the subsequent wheat (Innocenti *et al*. 2000 a,

Proceedings of the First International Workshop on Take-all of Cereals, 1983, in Ecology and Management, 1985). The more a not-host crop is grown in a field less pathogen agents in the soil can be found. The pathogen decline, that can be more or less rapid, depends on a reduction in the soil of its required specific nutrients, or on the selection of alternative microflora that competitively invades all the pathogen living space. This particularly happens for those species that are highly host specific and have a reduced capacity of saprophytic life. For example, *G. graminis* and *R. herpotrichoides* infestations in wheat are drastically controlled even by one year's break of winter cereal monosuccession with a not-host crop (Innocenti *et al*., 2000 a; Colbach *et al*., 1995; Wiese, 1987). These rotational benefits are less evident against polyphagous fungi (e.g. *Fusarium* spp. and, particularly, *Rhizoctonia* spp.). Indeed, as already mentioned, many *Fusarium* spp. can infect a wide range of plants and even with no host they can survive in the soil at least for a couple of years as dormant chlamydospores. This phenomenon partially explains why alternating wheat with other crops in biennial rotations couldn't significantly reduce the gravity of this fungus (Innocenti *et al*., 2000 a). Even scarcer resulted the rotation effect on *R. cerealis* spreading. Indeed the fungus is endowed with a high capacity of saprophytic survival. Moreover, it can actively live on many other wild *Poaceae*, on which it encounters a slight competition from other specific pathogens, like *R. herpotrichoides* (Colbach *et al*., 1997). In Australia the precession of not-host crops to wheat even worsened the infections of *R. solani* on the cereal (Anon, 1994).

The influence of fallow on the pathogens of the subsequent wheat is actively studied today because of the contributions that UE gives for this kind of set aside. However, up to now its effects on mycopathologies is not yet well defined. It appears highly variable according to many factors; mainly as a function of the edaphic conditions and of the type and amount of plant cover during set aside. The benefits against soil borne diseases could come from the development of a stable antagonistic microflora and the drawbacks from the occurrence of bridge phenomena due to the presence of certain wild plants.

Notwithstanding that wheat monosuccession has always been considered a highly negative practice for several soil borne phytopathogen organisms, in some instances it was shown that it can confer the soil a repression potential against some parasites. The phenomenon, which has been discovered for *G. graminis* wheat infestations, was named TAD (Gerlagh, 1968).

TAD (Take All Decline)

TAD is a classical example of how an agronomic practice can enhance the soil repression against a fungus. It was a well-known phenomenon in England and the USA since the 30's years, but it had been scientifically proved only at the end of the 60's. It consists of a drastic decline in the *G. graminis* infections after 3-4 years of continuous wheat growing. The repression agents are purely biological. Indeed this capacity is temperature sensitive (it disappears with high moisture and at temperatures above 55-60°C) (Grelagh, 1968; Shipton *et al*., 1973), it can be transferred form one soil to another and it can proliferate (Baker *et al*., 1982). Which is the causal agent of this repression is still uncertain. Very important seem the fluorescent *Pseudomonas* bacteria that grow together with *G. graminis* (Sarniguet et al., 1993). American and English researches confirmed that these bacteria, besides being highly competitive in the rhizosphere, synthesize specific antibiotics against the fungus (Thomashow *et al*., 1988; Raaijmakers *et al*., 1997 and 1998). Other studies identified other probable repression agents, such as *Trichoderma* spp. (Simon *et al*., 1988) or fungi acting through antagonistic mechanisms (Andrade *et al*., 1994). Instead, some authors have hypothesized a genetic variation inside the pathogenic population toward less virulent forms (Asher, 1980; Cunningam, 1975) or the selection of less damaging species, like *Phialopora* spp. and Ggg, that are correlated with *G. graminis* and competitive with it at the infection site level (Asher, 1981). All these results, which were obtained in various parts of the World, imply that TAD should be a ubiquitous phenomenon, but with variable intensity in the different sites.

The time distribution of TAD varies as well. Some English research showed that the repression takes place only during the pathogenic phase of the fungus, particularly on the secondary infections (Cook *et al*., 1986). On the contrary, in Australia Simon *et al*. (1989) revealed a repressive action also during the saprophytic stage.

What is confirmed in most of the literature is that TAD can be annulled by just one year of interruption of the monosuccession. This is frequently explained with the disturbance that this break causes on the TAD antagonistic microflora in the soil. This was clearly demonstrated when the break of monosuccession was made with not-host crops, but it is still to be verified in the instance of an alternation with susceptible cereals, for example in a rotation barley-wheat. Cook (2000) and Hornby (1995) have discordant views. The first author claims that barley growing can maintain the soil repressive capacity, while the second thinks that TAD will be drastically reduced due to rapid selections inside the two pathogens populations, that, in a long term, could develop distinct antagonistic microflorae (Ward *et al*., 1992; Bateman *et al*., 1997).

TAD cannot be stable over many years, even after a prolonged wheat monosuccession. Indeed sudden burst of disease were detected even after many years of TAD, particularly with climatic conditions unfavourable either to the microflora linked to TAD or to the same *G. graminis*. As a paradox, a low intensity of the disease doesn't encourage the antagonistic microflora and causes a slower decline in the following years (Hornby, 1988). This fact, together with the high yield losses in the first years of continuous wheat and with the probable increment of other wheat phytopathogens, advise against the wheat monosuccession, particularly in the Italian conditions, where *Fusarium* spp. are the prevalent causal agent of the root and crown disease.

Interactions between tillage and rotations on root and crown disease of wheat

The knowledge of the interactions on wheat soil-borne diseases between the two agronomic practices is still scarce. However it must have significant

effects. Indeed, tillage influences the position of crop residue in the soil profile. The previous crop residues have a paramount importance in determining the possibility and seriousness of primary infections, which are linked to their origin and type, that is to their ability to host the pathogen during the intercropping period. Schematically, and in theory, a great concentration at the soil surface of host-residues should bring high infestations, whilst the agronomic practices that cause a presence on the soil of not-host residues should be advantageous in favouring a potentially antagonistic microflora. Colbach *et al.* (1995) verified this hypothesis, at least regarding *R. herpotrichoides*, in an experiment where various biennial rotations (host/host, host/not-host and host/bridge crops) where compared with or without ploughing. No tillage gave the highest infections in the succession host/host or host/bridge, while the opposite happened with the host/not-host succession. In this latter instance ploughing, by turning sod, brought again at the soil surface the infected residues of the host crop grown two years before. The plant debris still showed an infective ability after two years due to the content of inoculum that had survived in the deep layers of the soil. On the contrary, no tillage favoured the not-host crop residue presence at the soil surface, thus an antagonistic microflora that can limit the pathogen development. Other experiments showed that the increment of root and crown disease, which is usually brought about by minimum tillage, is less marked when wheat alternates with renovation crops than in cereal succession (Innocenti *et al*., 2000 b).

Fusarium Head Blight (FHB) of small grains

The Fusarium head blight of small grains (a.k.a. ear scab or ear blight), that infects wheat and other cereals, is an important disease in many parts of the World, especially where humid or moist conditions prevail in the period from ear emergence (10.1 Feekes scale) to kernel maturity (11.4 Feekes scale). Poland, the Netherlands, United Kingdom, Russia and Austria are countries where ear scab most frequently occurs. There it can reach high intensities.

During 1979-85 its frequency in Nederland fields was about 67%, with 2% severity on infected spikelets. Therefore this pathology can cause important production losses : for example, it halved the yield of Chinese and Japanese wheat in certain year. Not only ear scab can drastically reduce grain yields; the mycotoxins that can be produced by fusari can contaminate grain and are now regarded as a major problem in the diet of both animal and humans.

Table d. *Fusarium* spp. isolated and identified as causal agents of FHB (Wang 1988)

F. graminearum Schwabe
F. culmorum (W.G. Smith) Sacc.
F. campoceras W&R
F. moniliforme Sheld *var. subglutinans* (W&R) Nelson, Tousson &Marasas
F. longipes W &R
F. equiseti (Corda) Sacc.
F. compactum Gordon
F. sambucinum Fuckel (W&R) W&R
F. graminum Corda (W&R)
F. avenaceum (Fr.) Sacc.
F. tricinctum Corda W&R
F. acuminatum Ell et Ev.
F. nivale (Fr.) Ces
F. sporotrichoides Sherb
F. chlamydosporum (W&R)
F. semitectum Berk &Rav.
F. oxysporum (Schlecht.) Snyd & Hans.
F. solani (Mart.) Appel & Wollenw

Photo 5. FHB symptoms – Above: red margins on the wheat spikelets - Below: infected kernels, on the left, are compared to healthy ones (right).

The fungus *Fusarium graminearum* was more frequently found as the ear blight causal agent. Some investigations in the Netherlands and other areas of Central Europe, however, have detected similar virulence levels (from severe to acute) also for *F. culmorum* and *M. nivale*, while *F. avenaceum* was reported as mildly to moderately virulent. In several other studies on FHB as many as 18

Fusarium spp. were isolated and identified (table e). In particular, on wheat infected ears Wang (1988) isolated the following *Fusarium* spp. (in order of frequency): 98% *Fusarium graminearum*, 8,2% *F. poae*, 2,4% *F. acuminatum*, 1,8% *F. moniliforme* var. *subglutinans*, 1,6% *F. equiseti,* and 0,1% *F. culmorum*, *F. avenaceum* and *M. nivale,* cumulatively.

F. graminearum is teleomorph of *Giberella zeae* (Schw.) and produces perithecia also in the field. On wheat it grows on the glumes, protruding from them. Under favourable circumstances it releases ascospores, which constitute the initial inoculum source for head scab. The intercrop survival of *F. graminearum* is allowed by mycelia or immature perithecia remaining on infected spikelets and grain left on the soil surface at harvest. Thus cultural practices play an important role in its survival. If crop residues are ploughed under, perithecia mainly die and the primary inoculum source drastically decreases. High moisture and warm weather are the main climatic factors favouring the inoculum production The required temperature for macroconidia formation are 16-36 °C, with an optimum at 32 °C. Rain and wind are the main means of inoculum dispersal. Besides wheat, barley, oats, rye, maize, alfalfa, and triticale represent the commoner hosts of *F. graminearum*. Some wild grasses are either secondary hosts or saprophytic substrata. The infection site of *F. graminearum* is the wheat spike, where it invades all the floral organs. This affects both wheat pollination and grain filling. Macroconidia or ascospores represent the principal inoculum that is dispersed by wind. Infected spikelets quickly fade losing chlorophyll. Later they turn pink or peach colour, especially at the base and at the glumes margins. If wet weather continues, disease spikelets are invaded by saprophytic fungi and turn dark or black. For this reason, scab is sometimes mistakenly called “head smut”.

Primary inoculum comes from infected plant debris on which the fungus overwinters as saprophytic mycelium. In spring, warm and humid weather favours the growth and maturation of conidia and perithecia that produce ascospores simultaneously to wheat flowering. The contact of spores with spike tissues soon starts the infection process. Thus wheat head are most infected

during anthesis (Sutton, 1982). The fungus spread in wheat from floret to floret inside a spikelets and the movement from a spikelet to another occur through the rachis and rachilla vascular bundles (Ribichich *et al*., 2000). Under wet condition mycelia can spread over the external surface of the glumes (Bushnell *et al*., 2003). The fungus has a brief biotrophic relationship with its host before switching to the necrotrophic phase. This stage is associated with enhanced fungus colonization. Eventually, plant death leads to a complete colonization of the host substrate. Asymptomatic *F. graminearum* can be found in various grass hosts (Farr *et al*., 1989; Inch and Gilbert, 2003) or colonizing different plant organs, such as corn stalks (Bushnell *et al*., 2003).

Fusarium is one of the most prolific mycotoxins-producing genera, especially on such cereals as maize, wheat, rice and sorghum. It is also one of the most dangerous pathogen because the many produced toxins have diverse metabolism origin and mode of action on human and animal health. (D'Mello *et al*., 1999)

Agronomic practices and soil biodiversity

The soil biotic community consists of several trophic levels, starting from that of bacteria and fungi, mainly decomposers, through that of primary producers up to the primary and secondary consumers, which are composed by micro-, meso- and mega fauna. The knowledge of the interactions between all trophic levels is still fragmental and obscure, considering the great complexity of trophic nets in the soil. The confusion is increased by the great variability of the soil habitat, by a frequent overlapping of ecological niches, and by the interactions (predation, neutralism, symbiosis, etc.) between many taxonomical groups that vary during a same life cycle (Wardle, 1995). The high number of soil microrganisms that hasn't yet been identified testifies the richness of the soil biotic component. Up to now it is estimated that only the 1% of bacteria, the 3% of nematodes and the 5-10% of fungi living in the soil have been recognized (Wardle, 1995; Viaud *et al*., 2000).

Many authors claim the existence of a positive correlation between biodiversity and ecosystem productivity (Adams *et al*. 1989, Connell *et al*. 1964). Although a straight relation is partially criticized (Connell, 1978; Colinvoux, 1995), some researchers try to transfer this model to the soil system. A wide biodiversity enhances the ecosystem capacity to rapidly regain the equilibrium after a perturbation (resilience) (Pankhust, 1997). In the soil a high resilience could be favourable because it could sustain a microbial population rich in antagonists of different plant pathogens (the so-called "soil repression ability") (Caporali 1993; Pankhurst, 1997; Sivapalan *et al*. 1993; Altieri, 1991).

Many scientists studied the impact of different agronomic practices on soil population stability and on soil system functioning. For example, Wasilewska (1979) showed that the diversity of nematode population in the soil is reduced in tilled land when compared to permanent leys. Other studies on fruit trees (Houston *et al*., 1998 a and b) grown with or without chemicals didn't show any difference in the fungi number, though the abundance of single species markedly differed. Ploughing destroys some microhabitats and creates completely different ones. Thus many researches on soil biodiversity focused on this agronomic practice. As a theory, no tilled cropped soil should be the most similar to natural systems (House, 1984). This kind of agroecosystems should have the highest grade of diversity and buffering ability (Altieri, 1991). This relationship has been confirmed in many papers on macro and meso fauna (Andrén *et al*., 1983; Yeates *et al*., 1990, Bertolani *et al*. 1989), in which ploughing often reduced the biodiversity and the functional groups. However, some contradictory results were obtained (Sabatini *et al*., 1997; Hendrix *et al*., 1986), showing that the correspondence minimum impact - maximum diversity is not so straight. Less, and even more contradicting, have been the results regarding the ploughing impact on microflora diversity (Wache *et al*., 1979). Some authors reported a marked increase of the microbial content in minimum tilled soil, particularly in the shallower layers, and linked it to the positive effect of the reduced disturbance on the soil organic matter (Triberti *et al*., 2000, Saffigna *et al*., 1989; Angers *et al*., 1992; Wardle, 1992). On the contrary,

Wardle (1995) claims that the ploughing influence on fungi communities is only slight. These contradictions can be partially explained by the insufficient taxonomic resolution of the conventional isolation techniques, that doesn't allow a precise evaluation of biodiversity in the soil. Moreover, the soil decomposers can be endowed with a superior stability than most of the trophic nets in other ecosystems. But many other biotic and abiotic factors can explain the wide variability of the obtained results. For example, a reduced tillage can cause a different root growth according to the soil type and conditions. In heaviest soils this practice should cause a less extended root system. This could significantly reduce the microflora linked to the rhizosphere, which is considered the major component of soil ecosystem (Wardle, 1995).

The rotation effects on soil biological diversity were analyzed mainly regarding the quantitative variations of single species (Bateman *et al*., 1999). It's a common opinion that a greater diversity of cropped species widens the soil biotic diversity (Letourneau, 1987). Zelles *et al*. (1995) confirmed this phenomenon for the bacterial community. The fallow should have the same positive effect, thanks to the diverse microrganisms that are promoted by the wild vegetation covering the uncropped field (Hornby, 1988). As previously mentioned, Wardle (1995) thinks that our little knowledge of soil biotic diversity is mainly due to uncertainty of organism individuation and ineffectiveness of conventional isolation methods. Indeed, the major limit of conventional techniques is the hard detection of microrganisms that cannot grow on the commoner substrata. Many authors have tried to analyze the biological diversity with alternative, modern techniques, such as the analysis of the fat acid profile (Zelles *et al.,* 1995) or the soil DNA extraction (Viaud *et al*., 2000). However, besides being extremely expensive, these methods are unsuitable to handle a large number of samples and seldom give quantitative results (Pankhurst, 1997). The DNA analysis, moreover, up to now can be only approximate, due to the still scarce number of organisms, mainly fungi, that have been sequenced and whose traces are stored in available databases (Viaud *et al*., 2000).

Research aims

The main purpose of the research was to find out how to improve the production of Italian durum wheat from both a quantitative and qualitative point of view by adequately modifying some agronomic practices. With so many people still starving to death in the World the necessity of increasing such a basic staple as wheat is out of doubt. In Italy *Triticum durum* production is even more important because we are net importers of this commodity, mainly used for pasta that is exported all over the World. But an optimal grain quality is required, both on the side of the industrial performance (one of the prerequisite is a heavy, kernel, full of starch and proteins) and from the human health point of view. Today this latter aspect is becoming increasingly important. In the developed countries the consumers are well aware of the risks of food contamination and pretend strict controls on commercialized foodstuffs. In the last few years the problem of mycotoxins in cereal grains has burst out. By now these substances were confirmed to be very noxious to humans and animals and were frequently found also in the Italian durum wheat production. The control of the fungi that produce mycotoxins should be the first step to bring the risks of grain contamination below the extremely low levels that the recent European rules impose. But in a prospect of a sustainable agriculture (and even more in organic farming) the control of parasitic organisms without chemicals appears very difficult. Therefore it would be interesting to know if it is possible to reduce the spreading of the causal agents of the major wheat diseases adopting adequate agronomic strategies. First of all, however, the life and infective capacity of the pathogens must be well known. Fortunately most of the parasites of wheat have already been thoroughly studied. The take-all, eyespot, etc. at the base of the culm and the fusariosis ear blight on the ears are well known diseases and much is known about the biology and ecophysiology of their agents. A great part of the life cycle of the pathogens is spent in the soil,

particularly on plant debris. Thus to control wheat infections the management of crop residue must certainly considered. For this reason we thought important to study the interaction between the preceding crop and soil tillage on the major fungi of wheat. The study was conducted on a long-term experiment, where different crop rotations and soil tillage are being compared for many years.. Thus it is probable that in the soil of the different plots the biotic phase has reached a steady condition, in equilibrium with the repeated treatments. Indeed, because of the high resilience, the fungi population in the soil presumably changes slowly and the effects of an ecological perturbation (such as wheat monosuccession or minimum tillage) can become manifest only after a long time. For four years we observed many aspects of the fungal compartment of the long-term trial with the scope of understanding how the soil pathogen population infects wheat plants and what can be the consequences of the diseases on the grain yield quality and quantity. We also could study the influence on wheat soil-borne pathogens of the crop residue management, based on the interaction between crop precession and tillage sequences. In the research the microflora composition in the soil was assessed with recent, innovative techniques that much helped in the identification of fungi that is so difficult by conventional means. Finally we wanted to investigate the possible contamination of wheat grain by the most troublesome mycotoxins. Our intent was to verify the existence of a relation between the ear fusari disease, which is so easy to visualize in the field, and the risk of kernel poisoning. The results of our efforts should help the growers to choose a successful and economically sound agronomic strategy against the wheat diseases so that, with no risk of polluting the environment with pesticides, he can produce more wheat of good quality and absolutely safe from the human health.

Materials and methods

Field experiment

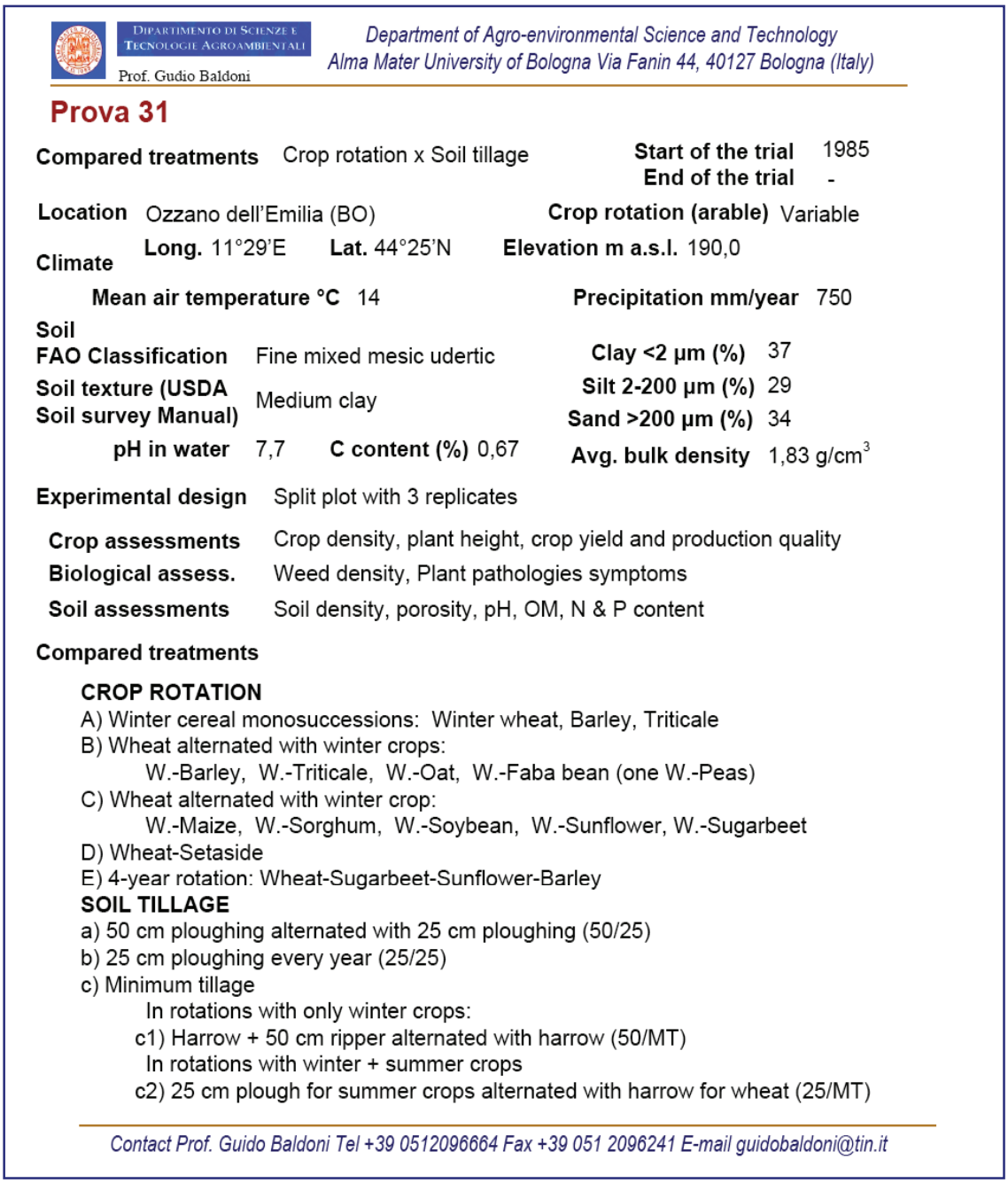
Description of the long-term experiment

All soil and plant samples that were used in this research had been collected from a long-term field experiment that started in 1985 and is still going on at the experimental and didactic farm of Bologna University, located near Ozzano dell'Emilia (Bologna), at the foot of the hills in the southeast Po Valley (Italy). In the experiment several crop successions, all including wheat, are compared under three tillage regimes consisting of sequences of conventional ploughing or minimum tillage for seedbed preparation. The treatments and some environmental characteristics of the site are reported in figure α and are more detailed described in Toderi *et al*. (2000). The rotation sub-plots are 48 m^2 of area each. The following cv. are used: 'Creso' variety of durum wheat; 'Valeria' hybrid of maize; 'Taxus' hybrid of sorghum and 'Ippolita' hybrid of sugarbeet. The experiment is not irrigated and all agronomic practices other than the compared ones are conducted according to what is normal in the zone. Wheat is seeded around mid October and harvested at the end of June. At seeding it receives a fertilization of 80 kg P_2O_5 ha^{-1} with no K. 180 kg N ha^{-1} are supplied in two fractions: 2/3 at the mid tillering stage (3 Feekes' scale, usually in the first days of March), and the rest at the beginning of stem elongation (4 Feekes' scale, in mid April). Weeds are controlled with post emergence herbicide mixtures including grass killers. No fungicide or insecticide treatment is carried out.

For this research we observed the plots reported in table α for 4 years (from 2003 to 2006) in order to study on wheat the interaction between the previous crop and the tillage sequence. The long time elapsed from the

beginning of the experiment (18 years) allows to suppose that in the assessment years a steady equilibrium was already reached in the agroecosystem, after a probable transition phase.

Figure α. Schema of the long-time trial on crop rotation x tillage sequence in Ozzano Emilia (BO)



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Prova 31

Compared treatments Crop rotation x Soil tillage

Start of the trial 1985
End of the trial -

Location Ozzano dell'Emilia (BO)

Crop rotation (arable) Variable

Climate
Long. 11°29'E **Lat.** 44°25'N **Elevation m a.s.l.** 190,0

Mean air temperature °C 14

Precipitation mm/year 750

Soil
FAO Classification Fine mixed mesic udertic
Soil texture (USDA Soil survey Manual) Medium clay
pH in water 7,7 **C content (%)** 0,67

Clay <2 µm (%) 37
Silt 2-200 µm (%) 29
Sand >200 µm (%) 34
Avg. bulk density 1,83 g/cm^3

Experimental design Split plot with 3 replicates

Crop assessments Crop density, plant height, crop yield and production quality
Biological assess. Weed density, Plant pathologies symptoms
Soil assessments Soil density, porosity, pH, OM, N & P content

Compared treatments

CROP ROTATION
A) Winter cereal monosuccessions: Winter wheat, Barley, Triticale
B) Wheat alternated with winter crops:
W.-Barley, W.-Triticale, W.-Oat, W.-Faba bean (one W.-Peas)
C) Wheat alternated with winter crop:
W.-Maize, W.-Sorghum, W.-Soybean, W.-Sunflower, W.-Sugarbeet
D) Wheat-Setaside
E) 4-year rotation: Wheat-Sugarbeet-Sunflower-Barley

SOIL TILLAGE
a) 50 cm ploughing alternated with 25 cm ploughing (50/25)
b) 25 cm ploughing every year (25/25)
c) Minimum tillage
In rotations with only winter crops:
c1) Harrow + 50 cm ripper alternated with harrow (50/MT)
In rotations with winter + summer crops
c2) 25 cm plough for summer crops alternated with harrow for wheat (25/MT)

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Table α. Treatments of the long-term experiment that were taken into consideration.

Rotations	***Year 2003***			***Year 2004***		
Wheat/ Wheat	P25 / MT	P25 / P25	P50 / P25	P25 / MT	P25 / P25	P50 / MT
Maize/ Wheat	P25 / MT	P25 / P25	P50 / P25	P25 / MT	P25 / P25	P50 / MT
Sorgum/ Wheat	P25 / MT	P25 / P25	P50 / P25	P25 / MT	P25 / P25	P50 / MT
Sugarbeet/ Wheat	P25 / MT	P25 / P25	P50 / P25	P25 / MT	P25 / P25	P50 / MT
Rotations	***Year 2005***			***Year 2006***		
Wheat/ Wheat	P25 / MT	P25 / P25	P50 / MT	Ar25 / MT	P25 / P25	P50 / MT
Sorgum/ Wheat	P25 / MT	P25 / P25	P50 / MT	P25 / MT	P25 / P25	P50 / MT
Sugarbeet/ Wheat	P25 / MT	P25 / P25	P50 / MT	P25 / MT	P25 / P25	P50 / MT
4-year rotation Sb/W/So/W	P25 / MT	P25 / P25	P50 / MT	P25 / MT	P25 / P25	P50 / MT

Tillage sequences: P25/P25 = ploughing to 25 cm depth every year; P25/MT = ploughing to 25 cm alternated with minimum tillage; P50/P25 = ploughing to 50 cm alternated with ploughing to 25 cm; P50/MT = ploughing to 50 cm alternated with minimum tillage

Photo α. View of the 25/25 cm ploughed wheat main plot in spring 2004

Weather data

Weather courses during the wheat cycles are shown in figures α and β

Figure α. Precipitation, average daily temperature and relative humidity in decades of 2002-03 and 2003-04 wheat cycles

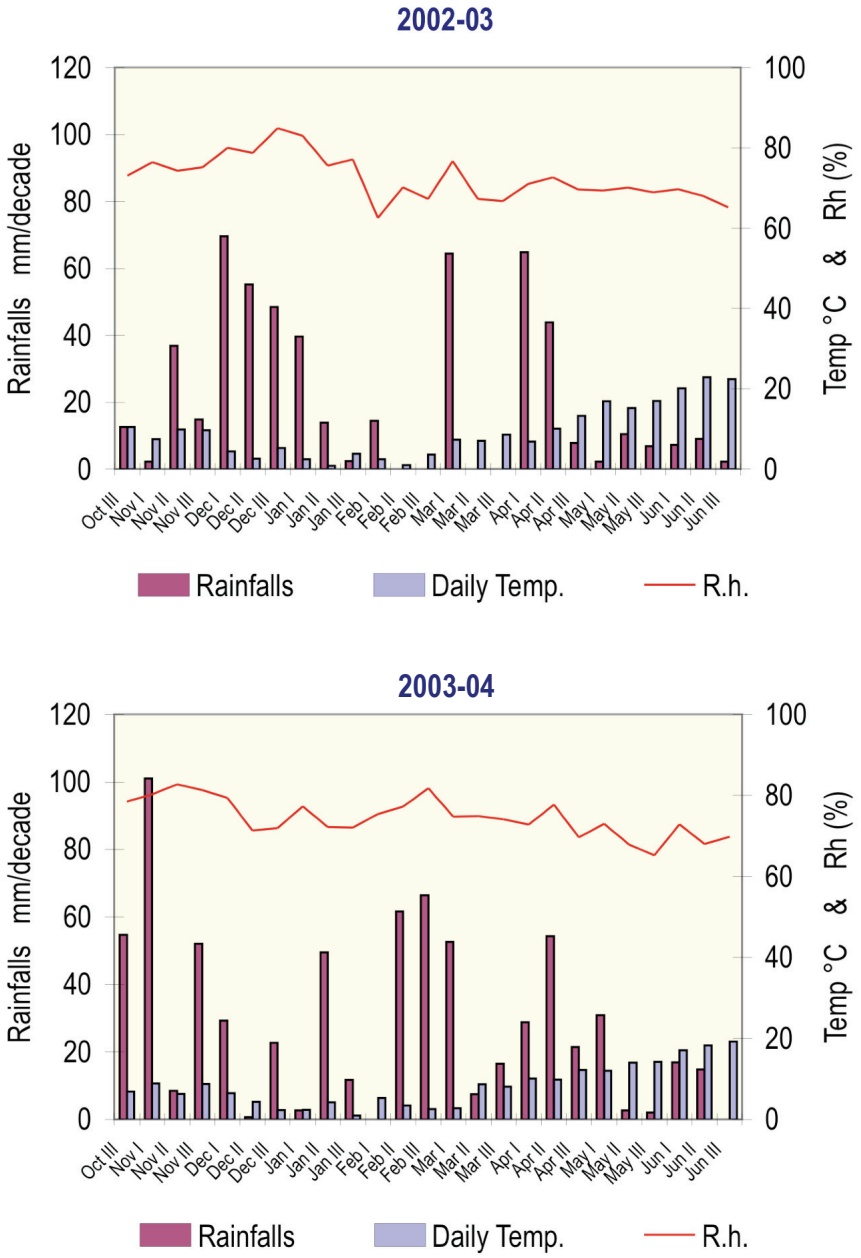
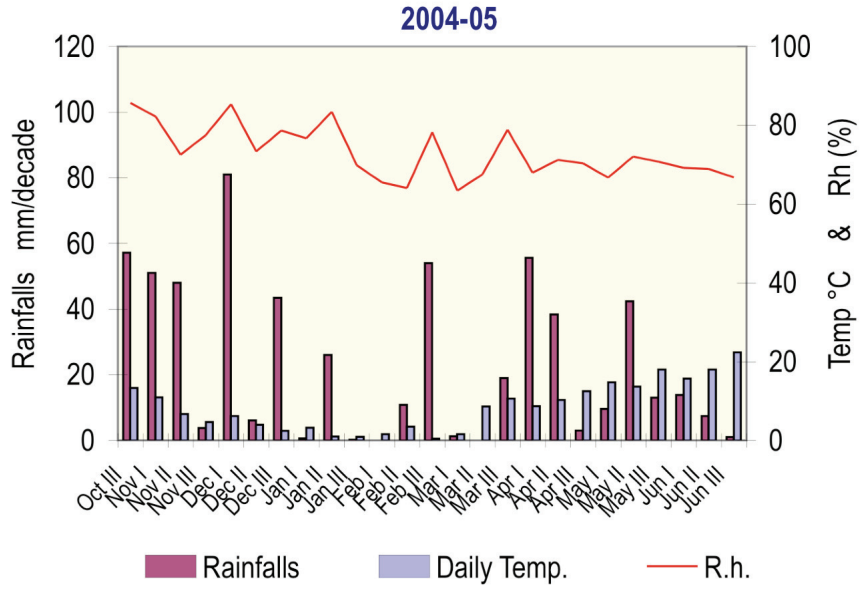
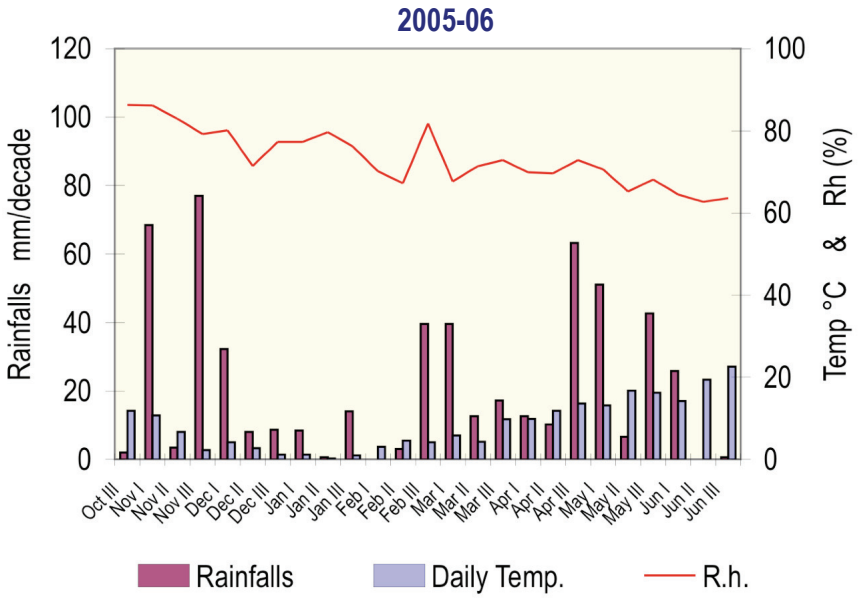


Figure β. Precipitation, average daily temperature and relative humidity in decades of 2004-05 and 2005-06 wheat cycles





In 2002 autumn-winter period was particularly rainy and wet. Winter and early spring 2003 were cold and dry. During wheat stem elongation it copiously rained and humidity remained high. Since anthesis to harvest rainfalls was scarce and days very hot. The autumn of the following year was very wet and rainy so that wheat roots suffered from asphyxia, tillering was scarce and the conditions were favourable to fungi development. During 2004 spring the rainfalls were more evenly distributed than the previous year, but temperatures kept cool (stem elongation and flowering were delayed) and humidity quite high also during the last maturity stages. In 2004/05 a mild and wet autumn-winter promoted wheat tillering. The elongation phase started early and the kernel maturation proceeded slowly, thanks to temperatures and moisture that remained optimal through harvest. The winter of the following year (2005/06) was very cold. In spring a long drought (from January to mid April) caused severe water stress to wheat, enhanced by an early and prolonged hot spell (from March to June). The hot and dry May induced early wheat maturation, and precluded a satisfactory translocation of elaborates into the kernels.

Assessments

During the 4 years the phenology and healthiness of wheat was constantly observed on the chosen plots and many parameters were measured. Schematically, they interested the soil, with core samplings, and the crop yield. Wheat grain was harvested using an experimental combine; its humidity and specific weight were measured. Regarding wheat pathologies, we assessed the incidence and severity of Fusarium blight on the spike after wheat flowering, with a visual diagnosis. At the end of the milky maturity stage we performed a visual diagnosis of the root and crown diseases, with a measurement of the damage degree. At the same time we made a collection of diseased plants to isolate pathogens in the laboratory. Samples of grain coming from the collected plants were taken to identify the causal agents. This was done in the DISTA laboratory, on the flour obtained by grinding the sampled kernels. In the same

laboratory the mycotoxin contamination was measured on the flour coming from the grain harvested in each plot. The structure of microflora population in the soil samples was analyzed in a Wageningen (NL) laboratory.

Evaluation of wheat root and crown disease

The determination took place at the wheat milky-dough maturation stage (11.1 – 11.2 Feekes' scale). From each plot 100 fertile plants were collected taking one individual each meter along two lines running parallel to the plot length. Plants and roots were freed of soil; their culm base was freed by removing all the leaf sheaths and was put into paper bags. The bags were kept into a cold chamber at 5-8°C until the assessment date, for a period never longer than 1 week. On each culm we visually determined the occurrence and the severity of the disease affecting the root and the first three internodes. When possible, the causal agent was identified on the basis of diagnostic symptoms. The visually recognized pathogens were: *Fusarium* spp. and *B. sorokiniana* (that were considered a unique group due to the difficulty in their visual differentiation), *G. graminis*, *Rhizoctonia* spp. and *R. herpotrichoides*. When there were more symptoms on a same culm only the most important was recorded. Each disease was evaluated by the following scale, based on the percentage of diseased area (Ledingham, 1981): 0 = No symptoms; 1 = slight infection (1-25% of infected surface), 2 = moderate infection (25-75%), 3 = serious infection (75-100%). A damage degree (i) of each pathogen was calculated for every plot, according to the following formula (Towsend-Heubergher, 1943):

$$\text{Damage degree (i \%)} = (\Sigma n_i v_i / N) \times 100 / M$$

where: i = Class index

n_i = Number of culms in each class

v_i = Numeric values of the class

N = Total number of samples

M = Numeric value of the highest class

To better identify the pathogens, particularly to recognize the various *Fusarium* spp., simultaneously with the visual diagnosis we performed a conventional isolation by standard laboratory methods on 120 samples taken from each plot. We started with a superficial disinfection of the samples, on the hypothesis that so we could select only the fungi living in the parenchyma or the vascular bundles of the culm or in the cortex and central cylinder of the root (Muller et al., 2000). The culm were cut at the 3rd internode and washed in tap water for at least 10 min. Then they were sterilized with 15% solution sodium hypochlorite for 2 min, rinsed three times with bi-distilled water and placed on blotting paper to dry off under sterile hood. Later the dried tissues were placed in 9 cm-diameter Petri dishes with agar and water at 14 g/l concentration added with the following antibiotics and growth inhibitors (Covarelli and Santori, 2000): 160 mg/l of Streptomycin sulphate, 60 mg/l of Tetracyclin, and 6 mg/l of dichloronitroaniline. The plates were incubated at 20-24°C for 4-5 days. From the grown colonies we took mycelium that was transferred to Petri dishes with 25 g/l of PDA (Potato Destrose Agar, Difco) for pure culture isolation. To stimulate the spore production of organisms that do not sporulate on PDA, like most *Fusarium* spp., we transferred the colonies on Sucrose Nutrient Agar (SNA) (Nirenberg, 1980) (table β) and exposed them to Near Ultraviolet light (NUV) at a constant temperature of 17°C.

Table β. SNA (Sucrose Nutrient Agar) substratum composition

Concentration		Constituent
1.0	g/l	KH_2PO_4
1.0	g/l	NaOH
0.5	g/l	$MgSO_4$
0.5	g/l	NaCl
0.2	g/l	Glucose
0.2	g/l	Saccharose
15.0	g/l	Agar – Agar

We identified the genus or species of fungi on the basis of the visual observation of the micro and macro morphologic features of the mycelium and reproductive structures, according to the identification keys proposed by Von Arx (1970), Domsh *et al*. (1993), Nelson *et al*. (1983) and Nirenberg (1980).

Analysis of the fungi community in the soil

At the end of 2006 May in the second block of the field experiment we collected soil samples to investigate the soil microflora.

Sampling procedure

From each sub-plot 3 soil samples were taken to 15 cm depth with a 3 cm diameter soil corer. They were collected along a diagonal at 1 m distance each other and from the plot border. The samples of each plot were bulked together, ground and sieved through a 1 cm mesh. They were sealed in plastic bags and kept at 3°C in the dark. Then they were sent to the Plant Research International Institute of Wageningen (NL) to investigate the fungi population of the soil.

Soil analysis

At the laboratory of the Plant Research International Institute of Wageningen (NL) the soil samples were analysed by the DGGE (Denaturing Gradient Gel Electrophoresis) method. This is a recent fingerprinting technique in which PCR-amplified DNA fragments are separated according to their sequence information. Double stranded DNA molecules of the same length, but differing in base-pair sequence can be partially separated as they migrate down a polyacrylamide gel containing a linearly increasing gradient of denaturants (Muyzer *et al*., 1996). Theoretically, each DGGE band corresponds to a single operational taxonomic unit (OTU), where the total banding pattern is reflective of a community species richness and diversity (Muyzer *et al.,* 1993).

In Wageningen each soil sample was divided into two sub-samples and each of them was twice analyzed to reduce the analytic error.

DNA extraction.

DNA was extracted from 0.5 g of soil as described by Protocol MO.BIO with Ultraclean Soil DNA isolation kit.

PCR amplification

PCR of fungal ITS sequences was performed according to Anderson *et al*. (2003). PCR amplification of bacterial 16S rDNA genes was performed according to Postma *et al*. (2000). Amplifications were performed in a PTC-100 thermal cycler (Mj Research Inc., Tilburg, NL).

Analysis of PCR products by DGGE

DGGE was performed with the phorU2 system (Ingeny, Leiden, NL). PCR products (15 to 20 ml) were directly applied onto 6% (wt/vol) polyacrylamide gels in 0.53 TAE buffer (20mM Tris-acetate [pH 7.4], 10 mM sodium acetate, 0.5 mM di-sodium EDTA) containing a linear denaturing gradient (in general, the concentration of the denaturant ranged from 35 to 65%). The gradients were formed with 6% (wt/vol) acrylamide stock solutions that contained no denaturant and 100% denaturant (the 100% denaturant solution contained 7 M urea and 40% [vol/vol] formamide deionized with AG501-X8 mixed-bed resin [Bio-Rad, Veenendaal, NL]). The gels were electrophoresed for 16-18 h at 60°C and 100V. After electrophoresis, the gels were stained for 30 min with SYBR Gold I nucleic acid gel stain (Molecular Probes Europe, Leiden, NL) and were photographed under UV light by using a SYBR Green gel stain photographic filter (Molecular Probes) and a Docugel V system apparatus (Biozym, Landgraaf, NL).

Analysis of DGGE gels and statistics

Banding pattern analysis and comparison of gels was processed by Gelcompar® II software (version 1.61; Applied Maths, Woluwe, Belgium). Correspondence of bands between different samples was performed with 1% dynamic range settings. Experimental data was exported and connected to band tables containing band positions and relative intensity. The obtained results underwent a cluster analysis to visualize the similarity between groups of populations and a RDA discriminant analysis to evaluate the main factors influencing their composition. The results where shown by a dendrogram of similarity and by a two axes graph of concentration, respectively.

Evaluation of the Fusarium head blight (FHB) of small grains

The assessment took place in 2005 and 2006, ten days after wheat flowering (10.53 Feekes' scale) approximately, in all plots of the second block. The disease visual evaluation was performed on 10 ears chosen in ten sites per plot. The sites were chosen along the perimeter and diagonal of a rectangle created at a distance of 1 m from the margins of the plot. In each site we counted the diseased spikes and obtained an incidence value (I% = frequency of infected ears). Moreover, to every spike we attributed a grade on the basis of the disease spread. Thus we obtained an indication of the seriousness of the disease (DS = Degree of Severity, expressed as percentage). The severity scale was as follow: 0 = no symptoms, 2 = some symptomatic spots; 5 = 2-3 diseased spikelets per spike, 10 = 4-5 diseased spikelets, 25 = diseased a quarter of the spike, 50 = diseased half spike; 75 = diseased three quarters of the spike, 90 = healthy only few spikelets, 100 = the whole spike was infected and completely white. By averaging all recorded data, we obtained single I (%) and DS (%) for each plot.

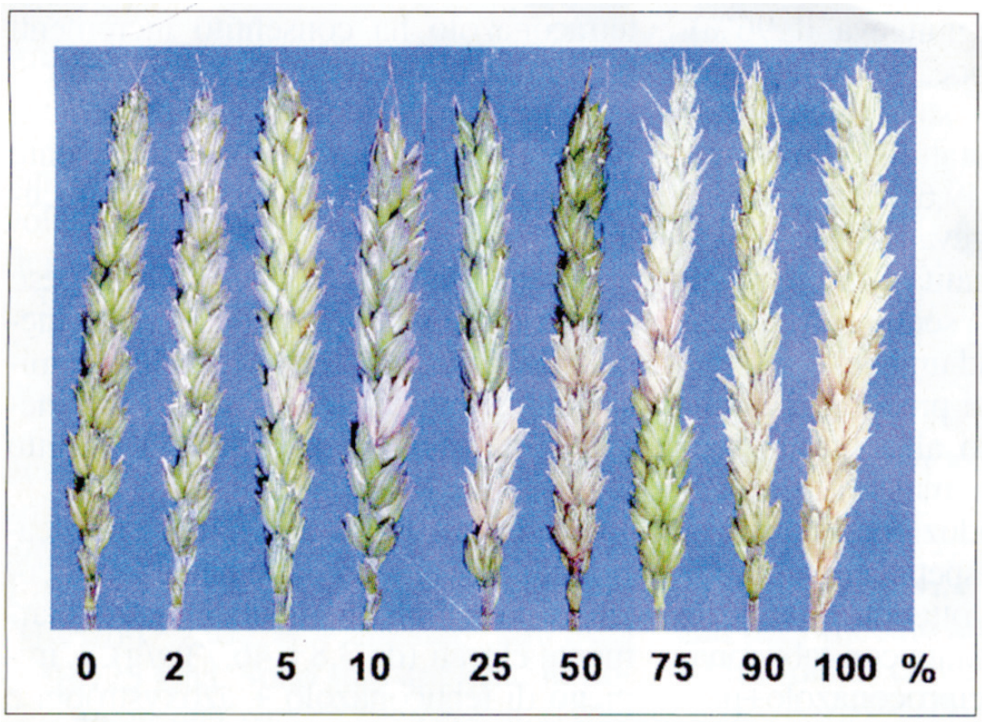


Photo β. Scale of the Fusarium Head Blight severity on wheat ears

Analysis of the Colony Forming Units (CFU) in wheat flour

At harvest, in 2004 and 2006 a sample of diseased ears were hand collected from each plot of the second block, air dried for 4-5 days and shelled. The obtained kernels were grounded with a laboratory mill to get 0.5 mm flour. Flour samples were kept in plastic bags in a refrigerated chamber at 4°C in the dark. Their analysis was performed in the laboratory of the Dept. of Agro-environmental Science and Technologies of Bologna University. Each analysis was replicated on four 0.5 g sub-samples. Through subsequent dilutions with distilled water we obtained a 1:20 = flour : water (wt/vol) solution. It was placed on 9 cm diameter Petri dishes containing 25 g/l of PDA (Difco) agar substratum and the following antibiotics: 300 mg/l Streptomycin sulphate and 150 g/l Neomycin. The plates were incubated for 4 days at 22°C and then the grown

fungi colonies were counted. They were moved to Petri dishes with PDA to obtain pure cultures and they were thus identified. For the not sporulating fungi on PDA the same above reported procedure (SNA medium and NUV exposition) was used. The already mentioned classification keys were used to identify the fungi.

Determination of mycotoxin content in wheat flour

The flour mycotoxin contamination was assessed all the four years in the laboratory of the Dept. of Agro-environmental Science and Technologies of Bologna University. The analysis concerned the plots of the second block, the same where Fusarium Head Blight had been evaluated. All the harvested grain in each plot was dried to constant weight in an oven at 60-80°C. It was mixed once then from it we took 3 sub-samples that were separately ground in <1 mm flour. The flour coming from each plot was then mixed and bulked in a unique sample. From it we took 500 g of flour to be analyzed in the laboratory. The following mycotoxins were searched:

- Aflatoxin B1, B2, G1, G2
- Fuminosin B1 and B2
- Zearalenone (ZEA)
- Deoxinivalenole (DON)

They were analyzed by high purification liquid chromatography (HPLC), after purification of extracts with the use of columns based on specific mono- and polyclonal antibodies for single mycotoxins or groups of them. The samples were extracted and purified using the immuno-affinity column methods that are reported in Vicam manuals. Mycotoxin quantity was measured by the external standard method, using calibration curves within a concentration range whose limits were those of the Italian regulation on food grains or, if absent, in the legislations of other countries. For each mycotoxin we had previously fixed the analytical determination limits, the recovery and the analytical repeatability. The

first two parameters were obtained by a simultaneous test performed on six sub units of the sample. The repeatability (r) was calculated, according to the Italian rules on aflatoxins in food, as:

r = 2.8 x standard deviation

(Gazzetta Ufficiale n. 33, 9 Febbraio 2001).

Statistical analysis of data

The wheat yield was expressed as areic grain production with 13.0% of moisture, the grain apparent specific weight in kg hl^{-1}. Each year, separately, their data were subjected to an analysis of variance (ANOVA SAS® procedure) considering the split-plot design of the experiment. When the F rate between variances of a single factor or interaction was significant at $P \leq 0.05$ the differences between the means were evaluated by a S.N.K. test at $P \leq 0.05$ (SNK SAS® procedure). To test the possible relationships between recorded parameters we used the linear correlation analysis (Excel data analysis) and obtained a Pearson's correlation-r whose significance was evaluated on the basis of the comparison degrees of freedom. A multiple regression analysis was used to evaluate the importance of diseases in determining the grain yield. For this analysis the Excel statistical package was used. The CFU data of single pathogens were related with the tillage intensity and the wheat precession to analyze their mutual influence. For this scope an analysis of correspondence was used (SSA SAS® procedure) on the basis of the relative frequency of fungi in each plot. For the analysis the previous crops were scored in the following order: Wheat/W = 1; Sorghum/W = 2; Maize/W =3 and Sugarbeet/W = 4 on the basis of an assumed decreasing presence of *Fusarium* spp. inoculum in the soil. On the same basis, the tillage sequences were ordered as a function of their increasing soil disturbance. They were graded as follow: 1 = P25/MT; 2 = P25/P25; 3 = P50/MT; 4 = P50/P25. The results of the concentration analysis were shown by a 2 axes graphs based on the two major directions of variability of the studied universe.

Results and discussion

Yield quantity: Effects of crop succession and soil tillage on wheat yield

In the field experiment the crop precession and the intensity of soil tillage significantly influenced the grain yield of durum wheat in all four years (tables 1, 2, 3, and 4).

Table 1. Crop succession and soil tillage effects on grain wheat yield (t ha^{-1} ± standard error of the mean) in 2003. (Tillage sequences: 25/Mt = 25 cm deep ploughing for preceding crop and minimum tillage for wheat; 25/25 = repeated ploughing to 25 cm; 50/25 = 50 cm deep ploughing for the previous crop and ploughing to 25 cm for wheat). The interaction: Successions x Tillage was significant at P≤0.01

	wheat/wheat	sorghum/wheat	maize/wheat	beet/wheat	Tillage means
25/Mt	2.13 ± 0.40	3.77 ± 0.25	4.45 ± 0.12	3.50 ± 0.33	3.46
25/25	3.85 ± 0.15	4.22 ± 0.21	4.64 ± 0.38	4.96 ± 0.26	4.42
50/25	3.50 ± 0.21	3.94 ± 0.18	4.56 ± 0.11	4.86 ± 0.14	4.22
Rotation means	3.16 C§	3.98 B	4.55 A	4.44 A	4.03

§ Means followed by different letters are significantly different at P≤0.05 according to S.N.K. test

Table 2. Effects of crop succession and soil tillage sequence on grain wheat yield (t ha^{-1} ± standard error of the mean) in 2004. (Tillage sequences as in table 1). The interaction: Successions x Tillage resulted significant at P≤0.01

	wheat/wheat	sorghum/wheat	maize/wheat	beet/wheat	Tillage means
25/Mt	0.49 ± 0,79	3.99 ± 0,28	3.88 ± 0.55	4.46 ± 0,33	3.21 B
25/25	3.69 ± 0,20	3.81 ± 0,61	4.56 ± 0.34	4.21 ± 0,27	4.07 A
50/Mt	3.13 ± 0,52	4.55 ± 0,19	3.87 ± 0.35	4.31 ± 0,45	3.97 A
Rotation means	2.44 B§	4.12 A	4.11 A	4.33 A	3.75

§ Means followed by different letters are significantly different at P≤0.05 according to S.N.K. test

Table 3. Effects of crop succession and soil tillage sequence on grain wheat yield (t ha^{-1} ± standard error of the mean) in 2005. (Tillage sequences as in table 1). The interaction: Successions x Tillage resulted significant at P≤0.05

	wheat/wheat	sorghum/wheat	beet/wheat*	beet/wheat	Tillage means
25/Mt	0.56 ± 0.53	5.71 ± 0.41	5.35 ± 0.63	5.13 ± 0.61	4.19
25/25	3.03 ± 0.19	5.58 ± 0.70	4.77 ± 0.30	5.68 ± 0.35	4.77
50/Mt	2.95 ± 0.51	4.96 ± 0.18	5.55 ± 0.24	5.25 ± 0.51	4.68
Rotation means	2.18 B§	5.42 A	5.22 A	5.35 A	4.55

§ Means followed by different letters are significantly different at P≤0.05 according to S.N.K. test
* Inserted in a 4-year course rotation: sugarbeet/wheat/sorghum/wheat

Table 4. Effects of crop succession and soil tillage sequence on grain wheat yield (t ha^{-1} ± standard error of the means) in 2006. (Tillage sequences as in table 1). The interaction: Successions x Tillage resulted significant at P≤0.05.

	wheat/wheat	sorghum/wheat	sorghum/wheat*	beet/wheat	Tillage means
25/Mt	1.18 ± 0.15	3.56 ± 0.36	3.88 ± 0.58	3.70 ± 0.44	3.08 B
25/25	3.33 ± 0.12	4.05 ± 0.67	3.85 ± 0.06	2.82 ± 0.27	3.51 B
50/Mt	2.96 ± 0.70	5.00 ± 0.16	4.44 ± 0.16	3.76 ± 0.49	4.04 A
Rotation means	2.49 B§	4.20 A	4.06 A	3.43 A	3.54

§ Means followed by different letters are significantly different at P≤0.05 according to S.N.K. test
* Inserted in a 4-year course rotation: sugarbeet/wheat/sorghum/wheat

On average, continuous wheat produced less than the crops following maize, sorghum, or sugarbeet. Moreover, minimum tillage for wheat generally gave lowest yields than ploughing to 25 cm depth. Tillage that was performed for the preceding crops showed only a little influence on grain yield, with the exception of 2006. However, because the interaction of crop succession with tillage was frequently significant and the variability among years was high, the effects of both factors on wheat production will be examined more in details.

Continuous wheat (W/W)

In wheat monosuccession minimum tillage gave always significantly lower yields than ploughing. The influence of depth of the ploughing that had been carried out for the preceding crop was slight. From this standpoint the straw burial appears important to mitigate the soil drop in fertility that is common in continuous wheat.

Maize-Wheat succession (M/W)

Wheat yields after maize generally showed slight but constant responses to tillage sequences in both years. The best results were always obtained with ploughing repetition to 25-cm depth. The other sequences gave lower yields, similar between them, but with a tendency of worst results given by minimum tillage to wheat. This response can be attributable to the benefits of turning the soil sod each year respectively to reducing the tillage intensity for wheat.

Sorghum-Wheat succession (So/W)

With the sorghum precession wheat yield showed significantly different responses to tillage in the four years, probably because sorghum emergence needs a good soil structure and this depends on the interaction between tillage and climate conditions. In 2003 the highest grain productions were recorded with 25-25 cm ploughings and the lowest with minimum tillage (the same pattern that was observed in continuous wheat). In 2004 and 2006 the best yields were given by the most intense tillage (deepest ploughing for sorghum), probably for the better incorporation in the soil of the residues of both crops facilitating wheat seedbed preparation that was particularly difficult in the wet autumn. On the contrary, in 2005 wheat yielded most with the shallowest tillage sequence, probably because of the drier autumn that implied a reduced wheat requirement for a good soil structure.

Sugarbeet-Wheat succession (Su/W)

In two years out of four (2003-2005), wheat yield was higher when 25 cm ploughing for wheat had followed 25 ploughing for sugarbeet. They were the years with the driest autumns. Thus it seems that minimum tillage for wheat is not particularly favourable for this cereal in dry conditions. In 2004 and 2006, with more rainy Novembers, the best productions were obtained with minimum tillage, independently from the tillage performed for the previous crop. The importance of a good structure for the rotations including sugarbeet is thus not confirmed. Again, as in sorghum precession, the interaction between tillage and climate during crop emergence confirmed very important in determining the wheat yield. Perhaps, the break in the cereal succession could have been favourable form a pathological standpoint, with the introduction in the soil of microrganisms that are antagonists to cereal pathogens.

Among the many factors that can explain the above reported yield responses, indeed, certainly there is the occurrence of plant pathologies, particularly those present in the crop early, at the stem base (wheat root and crown disease) and later, at the ear level (Fusarium Head Blight a.k.a. FHB). In the four research years all these diseases were recorded and their occurrence significantly affected wheat yield (table 5).

Table 5. Correlations between the severity of plant diseases and wheat grain yield (t ha^{-1}) based on the data of four years (2003-06) and all rotations, with repeated ploughing to 25 cm depth (25/25).

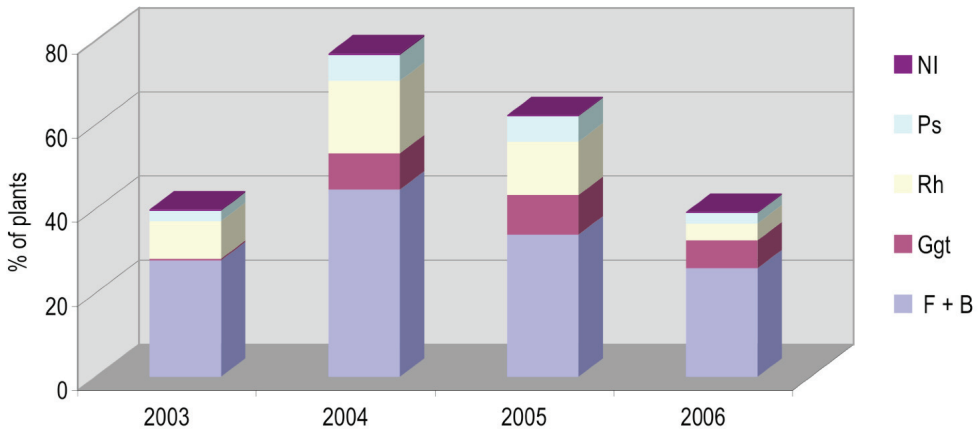
Plant pathology	Recorded parameter	Pearsons' r of their correlation with wheat yield §
Wheat root and crown disease	Damage degree [i (%)]	- 0.42**
Fusarium Head Blight (FHB)	Incidence (I %)	- 0.57***
Fusarium Head Blight (FHB)	Disease Severity (DS %)	- 0.33**

§ **,*** Correlation significant at P≤0,01 and P≤0.001, respectively (with 46 d.f.)

Effects of wheat root and crown disease on grain yield

At the end of wheat milky maturation phase, on average, the most frequent disease that we found on the roots and at the base of wheat culm was the common stem rot, which was mainly caused by *Fusarium* spp. (47.1%, on average over the four years; F) and *Bipolaris sorokiniana* (Sacc.) Shoemaker (21.5%; B). To a lesser extent we also found *Rhizoctonia* spp. sharp eyespot; (18.1%; Rh), take-all by *Gaeumannomyces graminis* (Sacc.) Von Arx & Olivier var. *tritici* Walker (6.4%; Ggt) and eyespot due to *Ramulispora herpotrichoides* (Fron) Arx (4.5%; Ps).

Figure 1. Isolation frequency of wheat plants infected by root and crown diseases of different causal agents (F = *Fusarium* spp.; B = *Bipolaris sorokiniana*; Ggt = *Gaeumannomyces graminis* var. *tritici*; Rh = *Rhizoctonia* spp.; Ps = *Ramulispora herpotrichoides;* NI = not identified).

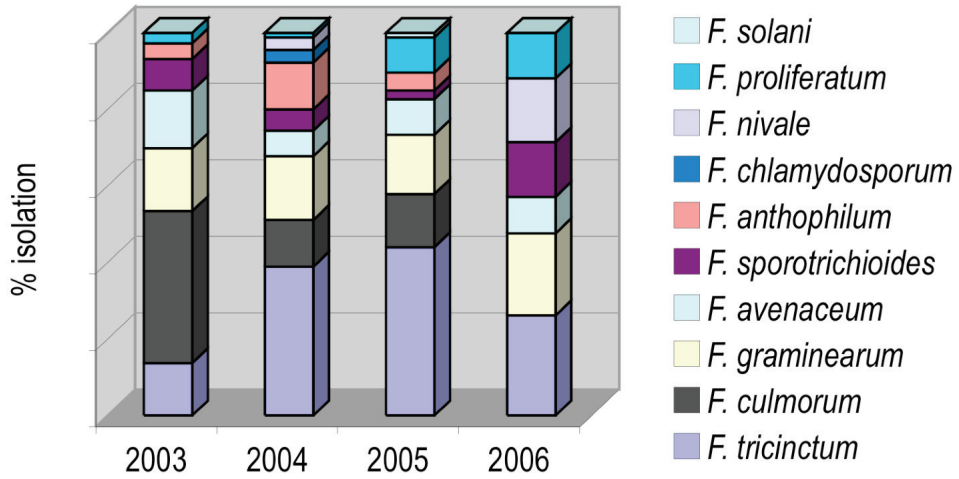


The isolated fusari mainly consisted of: *F. tricinctum* (Corda) Sacc. (35.2% of isolations, on average over the four years), followed by *F. culmorum* (W.G. Smith) (27.0%), *F. graminearum* Schwabe (15.3%) and *F. avenaceum* (Fr.) Sacc. (10.1%). The occurrence of other species (*F. moniliforme* Sheldon var. *anthophilum* (Braun) Wollenw, *F. chlamydiosporum* Wollenw & Reiking, *F.*

proliferatum (Mats) Niering, *F. sporotrichioides* Scherbankoff, *F. nivale* (Fr.) Ces., and *F. solani* (Mart.) Sacc.) never exceeded 5% of the total.

In the four years the pathogens colonized wheat with a various frequency (figure 1), probably due to different climatic courses. On average, the worst infections were recorded in 2004, the slightest ones in 2003 and 2006. The autumn-winter weather of 2002 was cold and rainy and should have favoured *G. graminis* (Ggt) and *R. herpotrichoides* (Ps), which are less thermophyl and prefer soaked soils. The subsequent hot and dry 2003 seasons should have stopped the secondary infections of these fungi and promoted fusarioses (F), especially those caused by *F. graminearum* and *F. culmorum*, and also by *Rhizoctionia* spp. disease, all of which are thermophyl pathogens. However, also the later infections of these blights on the upper parts of the plant should have been inhibited by the too dry conditions after wheat anthesis till harvest.

Figure 2. Isolation frequency of the different *Fusarium* spp. isolated in the four years on tissues at the base of wheat culm.

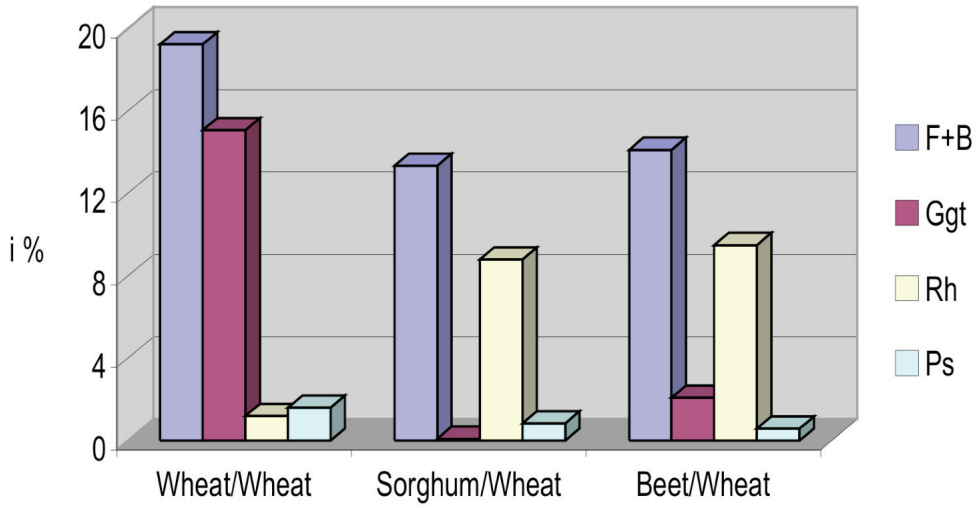


The following year (2003-04) the autumn-winter was milder, with more evenly distributed precipitations: the optimal conditions for the spreading of Ggt and Ps. Later, the cool and humid spring should have favoured also root rot; not caused by the same specie of the year before, but by less thermophyl ones,

e.g. *F. avenaceum* and *F. nivale*. Therefore 2004, on the whole, was particularly favourable to all fungi of the root and crown diseases on wheat. In 2005 the autumn-winter weather was similar to the previous year, favouring Ggt and Ps infections. In late spring, at wheat anthesis, the temperatures were already high, but the early drought did not allow a wide spreading of root rots, particularly of those caused by *F. tricinctum* and *F. culmorum* whose infections require high moisture to progress.

The rotation significantly influenced the pathogen spreading as well. With repeated shallow ploughings (25 cm deep, each year), which is the commoner tillage sequence in Northern Italy, the most troublesome pathogens in all successions were the *Fusarium* spp. and *Bipolaris sorokiniana* (F+B) (figure 3). They showed a higher damage degree in continuous wheat, but were also particularly noxious to wheat after sorghum and sugarbeet. Ggt was mainly found in continuous wheat, while Rh was prevalent in wheat after the other two crops. In all successions Ps caused very little infections to wheat.

Figure 3. Visual determination of the damage degree (i %) of the pathogens of the root and crown diseases as affected by crop precession in the soil continuously tilled to 25 cm depth (averages of four years). (F = *Fusarium* spp.; B = *Bipolaris sorokiniana*; Ggt = *Gaeumannomyces graminis* var. *tritici*; Rh = *Rhizoctonia* spp.; Ps = *Ramulispora herpotrichoides;* NI = not identified).



The importance of single pathogens in the determination of wheat yield was more thoroughly studied through a multiple regression analysis, based on all the four years' disease degrees, whose results is shown in table 6. The high corrected-R^2 of the regression (0,59, significant at P≤0.001) confirms that much of the observed variability in wheat production can be ascribed to the occurrence of the root and crown infections. Considering the b coefficient significance of each pathogen, the F+B and Rh influence on wheat yield (even positive, but statistically not significant) resulted far less than those of Ggt and Ps (both with negative b coefficients, significant at P≤0.001), which thus appeared the most dangerous pathogens in the experiment.

Table 6. Multiple regression analysis with y = wheat grain yield (t ha^{-1}) and x = the main wheat root and crown disease pathogens (damage degree %). The analysis was performed on the means of the four years (48 observations)

Multiple regression R = 0.79; R^2 = 0.62; Corrected R^2 = 0.59					
ANOVA	*d.f.*	*S.S.*	*M.S.*	*F*	*F signif.*
Regression	4	39.92	9.98	17.79	1.09E-08
Residue	43	24.12	0.56		
Total	47	64.04			
Coefficients	*Coeff.*	*St. error*	*Student t*	*t significance*	
Intercept	3.954	0.280	14.09	1.01E-17	
F+B	0.019	0.016	1.13	0.2664	
Ggt	-0.060	0.016	-3.66	0.0007	
Rh	0.037	0.020	1.79	0.0799	
Ps	-0.093	0.021	-4.33	8.74E-05	

The analysis of the inter-correlations between the damage degrees of the single pathogens (table 7) revealed a negative relationship between Ggt and Rh

that underlies some sort of antagonism between them. On the contrary, Ggt and Ps were strongly positively linked, which testify that they can both proliferate on a same plant, without any competition effects. Synergic phenomena can explain their high capacity of colonizing and damaging wheat yield.

Table 7. Correlations' r between causal agents of wheat root and crown disease. The analysis was performed on the damage degree (i %) means of the four years (48 observations)

Correlation r	F + B	Ggt	Rh	Ps
F + B	1			
Ggt	-0.021 ^{ns}	1		
Rh	0.158 ^{ns}	-0.364**	1	
Ps	0.106 ^{ns}	0.409**	-0.108 ^{ns}	1

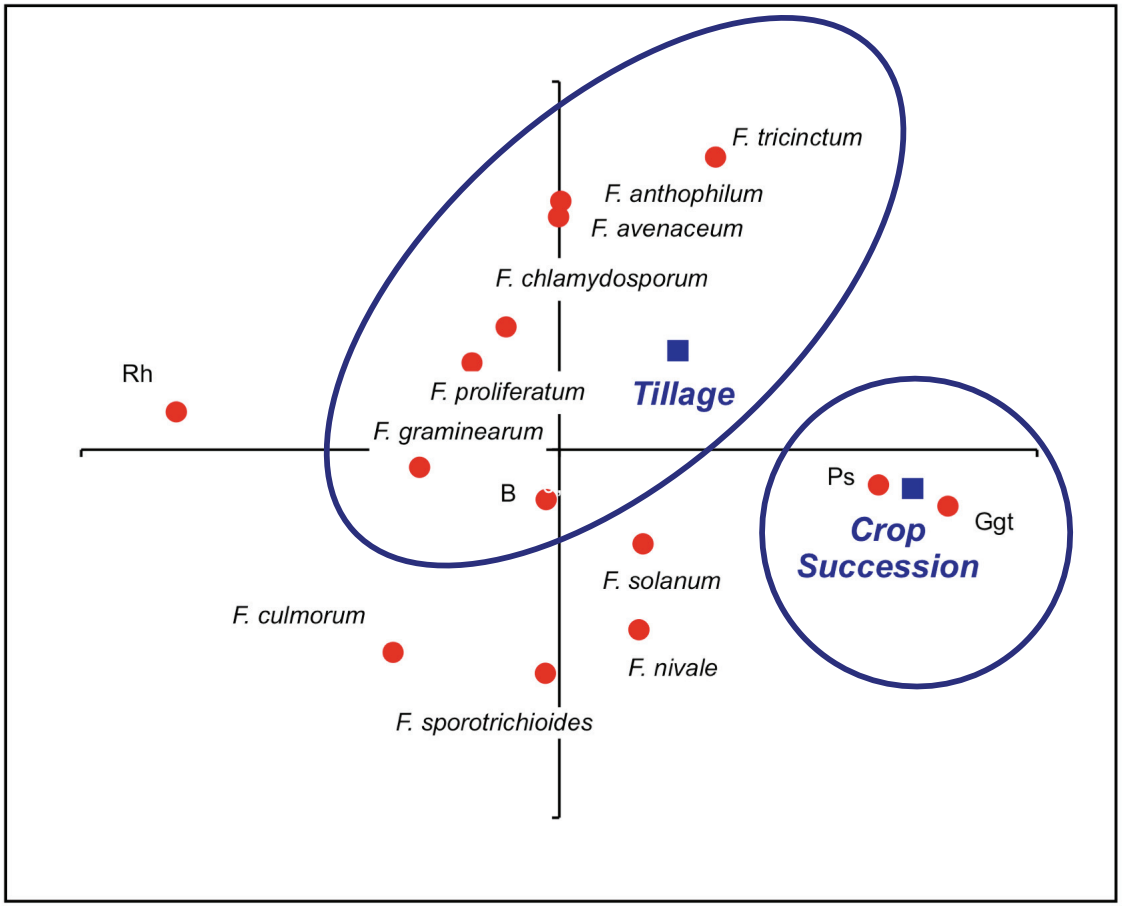
**, ^{ns} Correlation significant at P≤0,01 or not significant, respectively (with 46 d.f.)

The principal component analysis conducted on the basis of the damage degree means of all four years (figure 4) allows a better visualization of the relationships between the single pathogens of root and crown disease in wheat, the studied agronomic factors (soil tillage and crop succession), and between themselves altogether.

Three types of pathogens can be identified in that graph. A first, small group whose presence is mainly determined by crop rotation (*R. herpotrichoides* (Ps) and *Gaeumannomyces graminis* var. *tritici* (Ggt)), and a second group, mainly influenced by tillage, which is made up of many fusari (*F. tricinctum*, *F. avenaceum*, *F. chlamydosporum*, *F. proliferatum*, *F. graminearum, F. anthophilum),* together with *B. sorokiniana* (B). The presence of the other

casual agents resulted only slightly affected by both agronomic factors. In particular *Rhizoctonia* spp. (Rh) appeared the least influenced pathogen.

Figure 4. Principal component analysis of the two agronomic factors (soil tillage and crop succession) and the main agents of wheat root and crown disease. The analysis was based on the damage degree means of the four years. The two axes explain the 24% of total variability



Effects of crop succession and soil tillage on the causal agents of wheat root and crown disease

As shown above, the single agents of the root and crown syndrome of wheat showed different responses to the agronomic factors that we examined. Therefore it can be interesting to better illustrate how the main pathogens were influenced by the crop succession and soil tillage sequence.

Fusarium spp. (F) and *Bipolaris sorokiniana* (B)

The main agents of the "Common stem rot" resulted pooled in a unique group, which was mainly influenced by soil tillage. Therefore their responses are discussed together, particularly regarding the agronomic aspects (table 8).

Table 8. Damage degree (i %) on wheat of common stem rot disease caused by *Fusarium* spp. and *B. sorokiniana* in 4 years as affected by soil tillage sequences (25 or 50 cm ploughing for the preceding crop, followed by 25 cm ploughing or minimum tillage for wheat) (means of the crop successions).

Tillage	2003	2004	2005	2006	Means
25 / MT	10.28	22.52	15.13	9.21	14.29
25 / 25	12.68	19.81	13.20	13.48	14.79
50 / MT	nd§	17.38	13.43	10.90	13.90
50 / 25	7.40	nd	nd	nd	7.40
Means	11.48	19.90	13.92	11.20	

§ nd = Tillage sequence not present in that year, the means are calculated without this datum

The common stem rot disease was abundant in all four years. In 2004 wheat was severely damaged, probably because of spring conditions that were favourable to the pathogens. As a mean of the four years and of all the successions, a deep ploughing for the previous crop resulted in less stem rot than shallow tillage. On the contrary, the difference between minimum tillage and shallow ploughing for wheat were quite slight on the disease spreading on

the crop. Probably a deep burial of the propagules of the pathogens on wheat stubble for the seedbed preparation of summer crops can effectively reduce the inoculum presence for at least two years.

Gaeumannomyces graminis var. *tritici* (Ggt)

The take-all disease was particularly infectious in 2005 while was almost absent in 2003 (table 9). As expected, it always resulted strongly associated with wheat monosuccession. Surprisingly, sugarbeet was more favourable to the disease than sorghum, which was the crop that caused the least take-all infections to the following wheat. Probably sorghum residues create a microbial population on the soil surface that includes many antagonists of *G. graminis*.

Table 9. Damage degree (i %) on wheat of take-all caused by *Gaeumnannomyces graminis* var. *tritici* in four years as affected by crop succession (averages of the different tillage).

Succession	2003	2004	2005	2006	Means
Wheat/W.	0.69	20.87	22.60	9.87	13.51
Sorghum/W.	0.00	0.12	0.13	0.14	0.10
Maize/W.	0.00	1.45	nd	nd	0.73
Sugarbeet/W.	0.00	0.35	0.43	3.08	1.29
Means	0.23	7.48	11.37	5.00	

§ nd = Succession not present in that year, the means are calculated without this datum

Ramulispora herpotrichoides (Ps)

The eyespot was particularly damaging in 2004 (table 10), but the disease was common in all four years. As the previous fungus, the continuous wheat markedly favoured *R. herpotrichoides* and, again, sugarbeet resulted less effective against its colonizing ability than sorghum, but not so evidently as for take-all.

Table 10. Damage degree (i %) on wheat of eyespot caused by *Ramulispora herpotrichoides* in 4 years as affected by crop succession (means of the different tillage sequences).

Succession	2003	2004	2005	2006	Means
Wheat/W.	5.62	11.86	4.74	3.18	6.35
Sorghum/W.	0.15	2.90	1.01	0.07	1.03
Maize/W.	0.00	1.28	nd	nd	0.64
Sugarbeet/W.	0.14	2.01	1.15	0.00	1.05
Means	1.92	5.35	2.88	1.63	

§ nd = Succession not present in that year, the means are calculated without this datum

Rhizoctonia spp. (Rh)

Differently from all other pathogens, the most serious root rot by *Rhizoctonia* spp. was prevalently found on wheat following other crops, particularly maize. Instead the continuous wheat was less affected by *Rhizoctonia* spp. (table 11), probably because this pathogen is less competitive on wheat stubbles than other aggressive pathogens, like *G. graminis*.

Table 11. Damage degree (i %) on wheat of root rot caused by *Rhizoctonia* spp. in 4 years as affected by crop succession (means of the different tillage sequences).

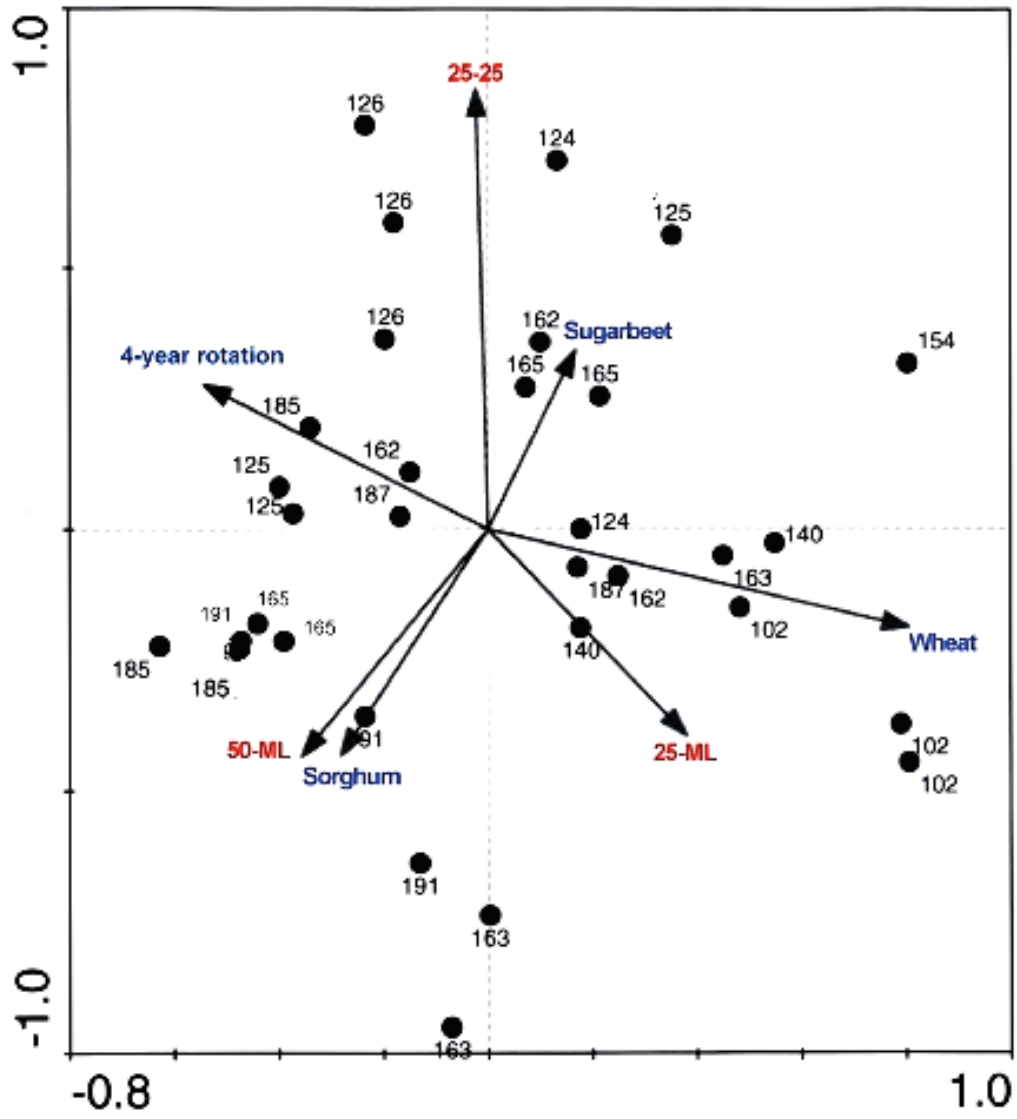
Crop succession	2003	2004	2005	2006	Means
Wheat/Wheat	1.21	2.09	0.56	0.17	1.01
Sorghum/Wheat	1.68	13.23	3.58	1.92	5.10
Maize/Wheat	6.73	12.33	nd	nd	9.53
Sugarbeet/Wheat	5.53	12.01	10.51	3.52	8.68
Means	3.21	9.22	2.07	1.04	

§ nd = Succession not present in that year, the means are calculated without this datum

Effects of crop succession and tillage on the soil fungi population

The DGGE analyses of microbial soil population that were performed in Wageningen (NL).

Figure 5. Ordination by discriminant analysis performed on the DGGE bands of soil samples collected in 2006. Means of 2 gels/sample. The graph shows the major factors of discrimination between fungi populations and the reciprocal distance between the different soil samples, each with its fungi population.



The analysis gave gels with different bands that can be used to determine if the agronomic factors modified the fungi population in the soil, thus influencing the root and crown disease of wheat.

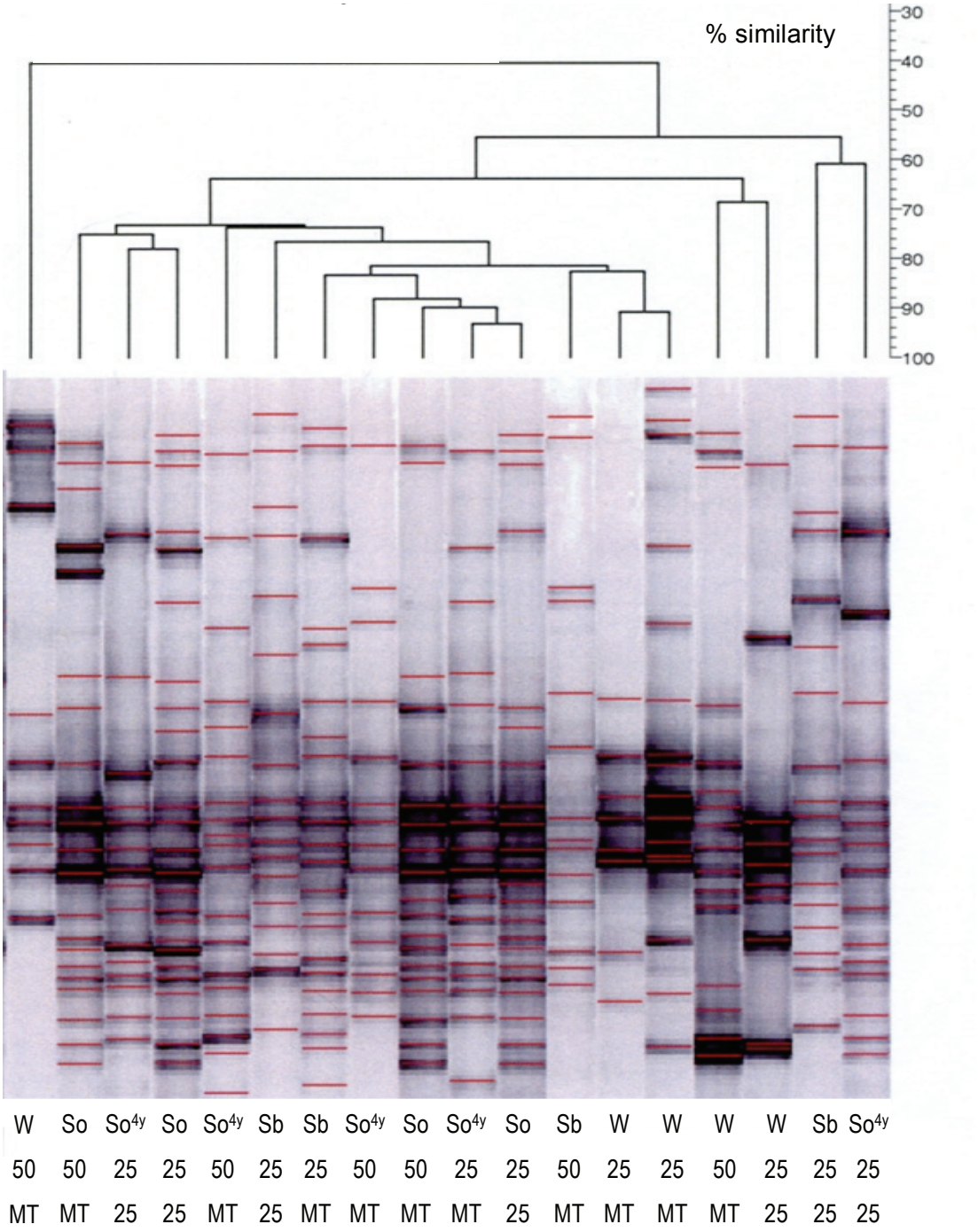
The results of the ordination of the soil samples that were collected in the experimental plots allowed to clarify which of the agronomic factors that we compared had a major influence in determining the composition of the fungi population in the soil.

The discriminant analysis results are reported in figure 5 as a two axes graph, showing arrows whose length is proportional to the factor influence. The factors that mainly determined the fungi population in the soil were tillage, particularly that for wheat: 25 cm deep ploughing vs. minimum tillage, and crop succession, mainly the difference between continuous wheat and wheat inserted in the 4-year rotation. Minor importance had the preceding crops (sugarbeet or sorghum) and the tillage performed for them (ploughing to 25 or 50 cm).

The observation of the analytic gels (figure 6) revealed that continuous wheat with respect to 4-year rotation and minimum tillage vs. ploughing for wheat both caused a drastic drop in the number of bands and an increase of their thickness.

This is a clear indication of a simplified soil microbial population that many authors consider unfavourable to cropped species (particularly to wheat) for the lack of pathogen antagonists. Indeed, on the basis of what we observed in the field, most of the more abundant fungi in continuous wheat presumably belong to two aggressive species: *G. graminis* and *Ramulispora herpothricoides*, which are generally overwhelmed by *Fusarium* spp. in the other successions.

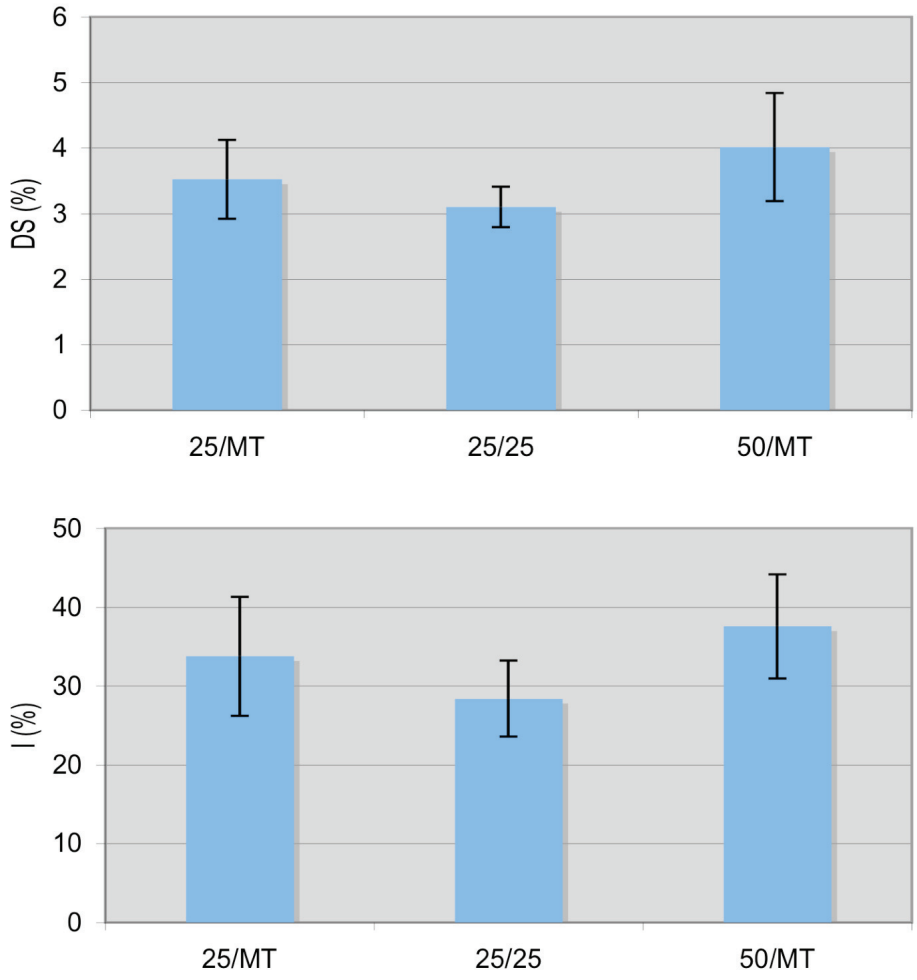
Figure 6. Classification of gel lines on DGGE bands corresponding to various soil samples



Interaction of Fusarium Head Blight (FHB) with wheat root and crown disease

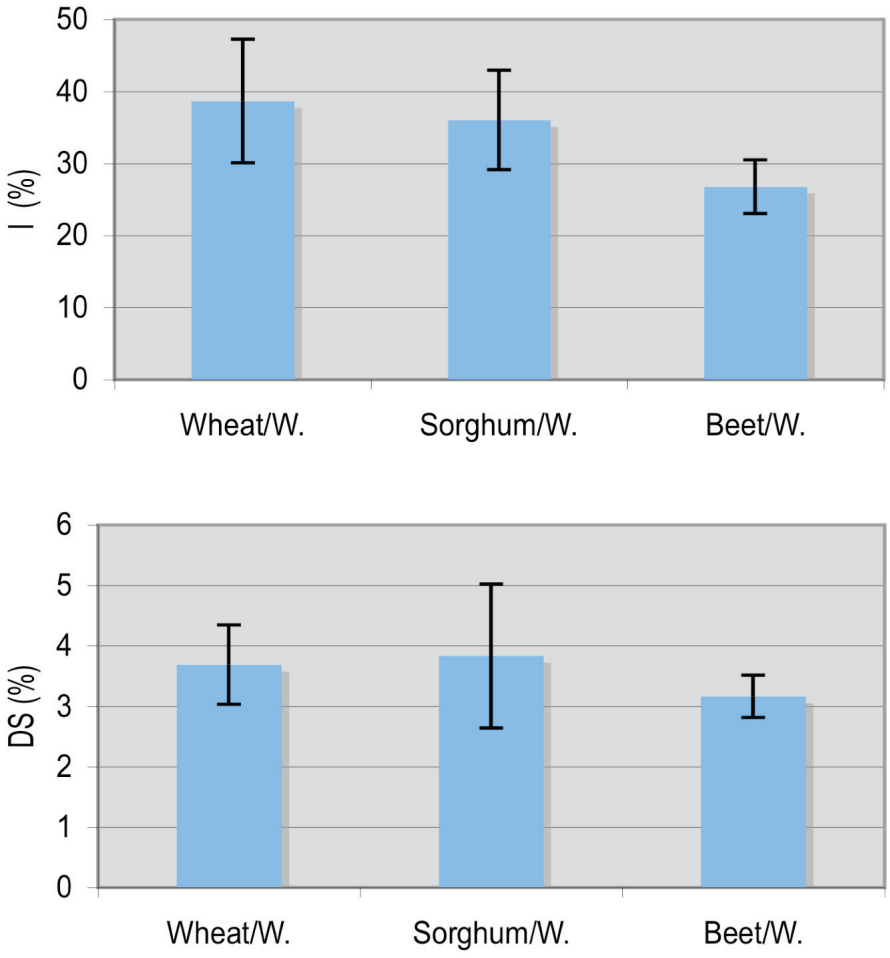
The results of the field determinations that we carried out in 2005 and 2006 after wheat flowering on the diffusion and severity of Fusarium Head Blight are shown in figures 7 and 8

Figure 7. Influence of crop succession on the incidence (I) and severity (DS) of Fusarium head blight. Averages of two years (2005 and 2006) and 6 tillage treatments ± standard errors



As an average of both years, with a similar occurrence of FHB, after sugarbeet head scab on wheat crop resulted sparser and less severe than after sorghum or wheat. The precession of the two cereals, instead, caused no significant difference on its diffusion and on severity of its damages (figure 7).

Figure 8. Influence of tillage sequence on the incidence (I) and severity (DS) of FHB (MT= minimum tillage; 25 or 50 cm ploughing depth). Averages of two years (2005 and 2006) and three crop successions ± standard errors



Both parameters appeared augmented when wheat was seeded in minimum tilled soil, maybe for a less vigorous growth of crop plants during stem

elongation that made them more prone to the *Fusarium* late infection. The tillage influences on FHB, however, were slighter than what we expected. The results of the correlation analysis between the incidence (I) and severity (DS) of scab and the detected root and crown pathogens (expressed as disease incidence [i%]) are shown in table 12.

Table 12. Correlations between Fusarium Head Blight (FHB) incidence (I) and severity (DS) on wheat and root and crown disease pathogens isolation frequency (%). Analysis based on 48 observations

	FHB (I%)	FHB (DS%)	*Fusarium* spp. (%)	*F. sporotrichoidies* (%)	*Rhizoctonia* spp. (%)	*B. sorokiniana* (%)	*G. graminis* (%)	*R. herpotrichoides* (%)
FHB (I%)	1							
FHB (DS%)	+0.53***	1						
Fusarium spp. (%)	-0.18ns	-0.15ns	1					
F. sporotrichioides (%)	+0.32*	-0.07ns	-0.09ns	1				
Rhizoctonia spp. (%)	-0.30*	-0.35*	-0.28ns	-0.17ns	1			
B. sorokiniana (%)	+0.12	+0.43**	0.00ns	-0.09ns	-0.41***	1		
G. graminis (%)	+0.45***	+0.71***	-0.30*	+0.04ns	-0.41***	+0.37**	1	
R. herpotrichoides (%)	+0.14ns	+0.54***	+0.24ns	-0.24ns	-0.13ns	-0.16ns	+0.29*	1

***,**,*,ns Correlation-r significant at P≤0.001, P≤0.01, P≤0.05 and not significant, respectively

We did not find any significant relationship between Fusarium head blight of small grains and most of the *Fusarium* spp. isolated at the wheat culm base and root, except in the instance of *F. sporotrichioides*. On the contrary, significant relations were found with *Rhizoctonia* spp. (Rh), *B. sorokiniana* (B), *G. graminis* (Ggt), and *R. herpotrichoides* (Ps*)*. While B, Ggt and Ps showed a positive correlation, meaning that when there is a heavy infection at the culm base it is probable to register a severe head scab disease. The relation of Rh with FHB was negative. Probably Rh infection does not weaken wheat plants, thus they can better resist to FHB. On the contrary, with heavy infestations of other root and crown disease pathogens (Ggt, Ps, B) FHB is more severe and Rhizoctonia spp. less present.

All these relationships can be probably due to the same antagonistic and synergic effects between the pathogen infections that we previously conjectured and can explain why most *Fusarium* and Rhizoctonia spp. at the culm base do not damage yield as the other pathogens. From this point of view *G. graminis*, *R. herpotrichoides*, and *B. sorokiniana* appear the most troublesome pathogens over the whole cycle of wheat crop, and they can effectively limit the diffusion of *Rhizoctonia spp*. On the other hand the *Fusarium* spp. and *Rhizoctonia* spp. that were so frequently found at the stem base do not seem significantly linked to the FHB damaging the wheat ear, which is so detrimental to grain yield.

Yield quality: effects of crop succession, tillage and diseases on wheat grain apparent specific weight

The compared agronomic practices had a marked influence on the quality of wheat caryopses. Their effect was clear on the apparent specific weight of the grain (tables 13,14,15,16) that varied similarly to wheat yield (table 17). This quality parameter was always lowest in continuous wheat with respect to other successions, and minimum tillage worsened it compared to ploughings. The best results were obtained in wheat following maize or sugarbeet with the alternation of ploughings to 25 and 50 cm depth. These tillage sequence and succession are also those that most limited the infections of wheat root and crown diseases.

Table 13. Effects of crop succession and soil tillage sequence on the apparent specific weight (kg hl^{-1} ± s.e. of the means) of wheat grain in 2003. (Tillage sequences: 25/Mt = 25 cm deep ploughing for the preceding crop followed by minimum tillage for wheat; 25/25 = repeated ploughing to 25 cm depth; 50/25 = 50 cm deep ploughing for the previous crop followed by ploughing to 25 cm for wheat). The interaction: Successions x Tillage was not significant

	wheat/w.	sorghum/w.	maize/w.	sugarbeet/w.	Tillage means
25/Mt	74.20 ± 1.35	77.10 ± 0.32	78.37 ± 0.59	76.27 ± 0.60	76.48 B
25/25	77.03 ± 0.96	78.07 ± 0.72	78.43 ± 0.27	78.30 ± 0.35	77.96 AB
50/25	78.27 ± 0.37	78.13 ± 0.34	79.03 ± 0.35	78.33 ± 0.35	78.44 A
Rotation means	76.50 A§	77.77 AB	78.61 A	77.63 AB	

§ Means followed by different letters are significantly different at P≤0.05 according to S.N.K. test

Table 14. Effects of crop succession and soil tillage sequence on the apparent specific weight (kg hl^{-1} ± s.e. of the means) of wheat grain in 2004. (Tillage sequences as in table 13). The interaction: Successions x Tillage was significant at P≤0.01

	wheat/w.	sorghum/w.	maize/w.	sugarbeet/w.	Tillage means
25/Mt	72.83 ± 1.30	78.73 ± 0.69	77.16 ± 0.22	78.90 ± 0.95	76.91 A
25/25	78.67 ± 0.58	78.63 ± 0.42	78.70 ± 0.24	78.87 ± 0.35	78.72 A
50/25	78.63 ± 0.29	78.27 ± 0.41	78.77 ± 0.35	79.40 ± 0.85	78.77 B
Rotation means	75.74 B§	78.54 A	78.21 A	79.06 A	

§ Means followed by different letters are significantly different at P≤0.05 according to S.N.K. test

Table 15. Effects of crop succession and soil tillage sequence on the apparent specific weight (kg hl^{-1} ± s.e. of the means) of wheat grain in 2005. (Tillage sequences as in table 13). The interaction: Successions x Tillage was not significant

	wheat/w.	sorghum/w.	beet*/w.	sugarbeet/w.	Tillage means
25/Mt	74.33 ± 1.96	78.77 ± 0.82	77.20 ± 0.21	77.53 ± 1.30	76.96
25/25	74.83 ± 0.42	78.60 ± 0.45	77.60 ± 0.37	77.87 ± 0.40	77.23
50/25	75.80 ± 0.18	78.23 ± 0.41	77.57 ± 0.17	77.60 ± 0.83	77.30
Rotation means	74.99 C§	78.53 A	77.46 B	77.67 B	

§ Means followed by different letters are significantly different at P≤0.05 according to S.N.K. test
* inserted in a 4-year course: sugarbeet/wheat/sorghum/wheat

Table 16. Effects of crop succession and soil tillage sequence on the apparent specific weight (kg hl^{-1} ± s.e. of the means) of wheat grain in 2006. (Tillage sequences = in table 13). The interaction: Successions x Tillage was not significant

	wheat/w.	sorghum/w.	sorghum*/w.	sugarbeet/w.	Tillage means
25/Mt	73.67 ± 1.96	78.43 ± 0.32	78.90 ± 0.04	78.33 ± 0.29	77.33
25/25	74.57 ± 0.44	78.57 ± 0.41	78.33 ± 0.31	76.87 ± 0.43	77.09
50/25	75.73 ± 0.15	78.80 ± 0.67	79.93 ± 0.20	77.93 ± 0.30	78.10
Rotation means	74.66 C§	78.60 AB	79.05 A	77.71 B	

§ Means followed by different letters are significantly different at P≤0.05 according to S.N.K. test
* inserted in a 4-year course: sugarbeet/wheat/sorghum/wheat

The grain apparent specific weight was better correlated to Fusarium head blight (FHB) severity than to most of the wheat root and crown disease pathogens (table 17), demonstrating the importance of the pathologies infecting the spike on the quality of produced grain. The significant negative correlation between grain specific weight and *R. herpotrichoides* incidence means that its disease also damages grain quality.

Table 17. Correlations between the grain apparent specific weight, the wheat yield, the Fusarium Head Blight (FHB) incidence (I) and severity (DS) and the isolated root and crown disease pathogens (%).

Correlation r	Apparent Specific Weight of wheat grain (kg hl^{-1})
Wheat grain yield (t ha^{-1}) §	+ 0.63***
Fusarium Head Blight incidence (I%) §	- 0.47***
Fusarium Head Blight severity (DS%) §	- 0.42***
Fusarium spp. on root and crown (%) #	- 0.02 ns
F. sporotrichioides (%) #	- 0.46***
Rhizoctonia spp. (%) #	+ 0.19 ns
B. sorokiniana (%) #	+ 0.14 ns
G. graminis (%) #	- 0.11 ns
R. herpotrichoides (%) #	- 0.33*

***,**,ns Correlation-r significant at P≤0.001, P≤0.01, and not significant, respectively,
§ based on 48 observations; # based on 24 observations

Flour quality: Fusarium Head Blight and fungi content in wheat grain

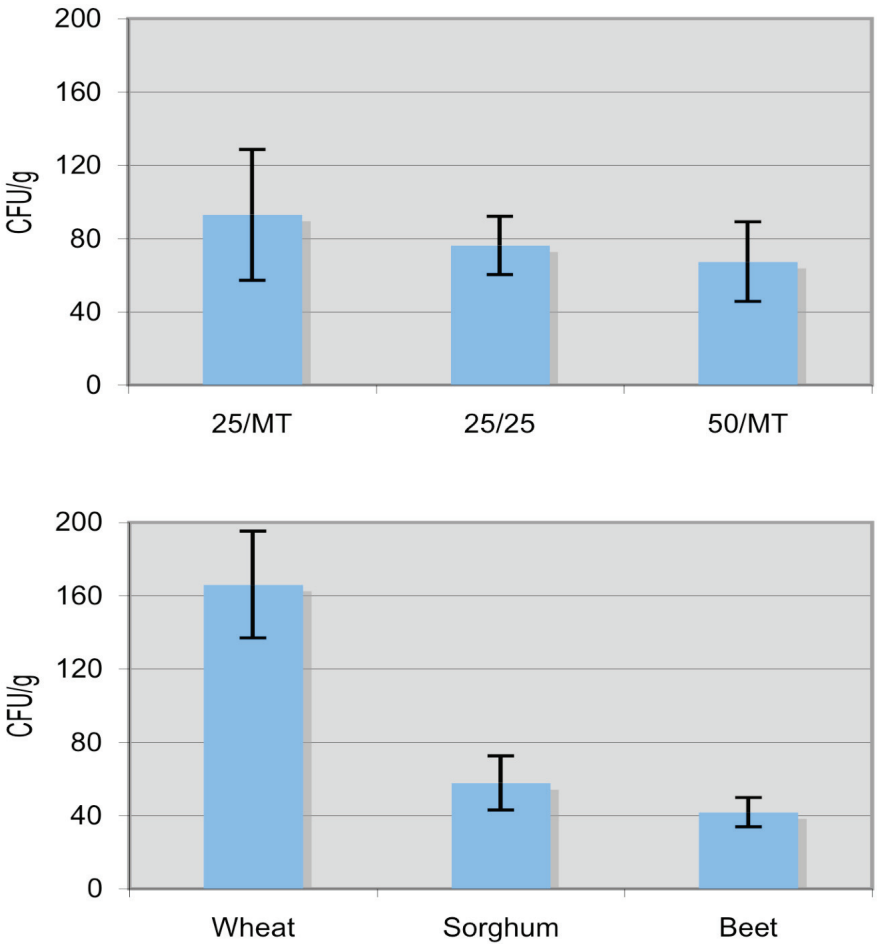
The determination of pathogens content in the grounded wheat grain revealed a vast quantity of fungi other than *Fusarium* spp., such as *Penicillium* spp., *Verticillium* spp. and *Aspergillus* spp., some of which resulted the most abundant contaminants of the flour (table 18). Most of them are ubiquitous saprophytes, whose spread shouldn't directly depend on the studied agronomic factors.

Table 18. Fungi in the flour of wheat (averages of the 3-years determinations, 48 samples per year)

Pathogen	% Isolations in flour samples
Penicillium spp.	38.51
Verticillium spp.	30.29
Fusarium proliferatum	15.57
Aspergillus spp.	10.89
Bipolaris spp.	1.67
Fusarium culmorum	1.64
Fusarium graminearum	1.31
Fusarium sporotrichioides	0.13

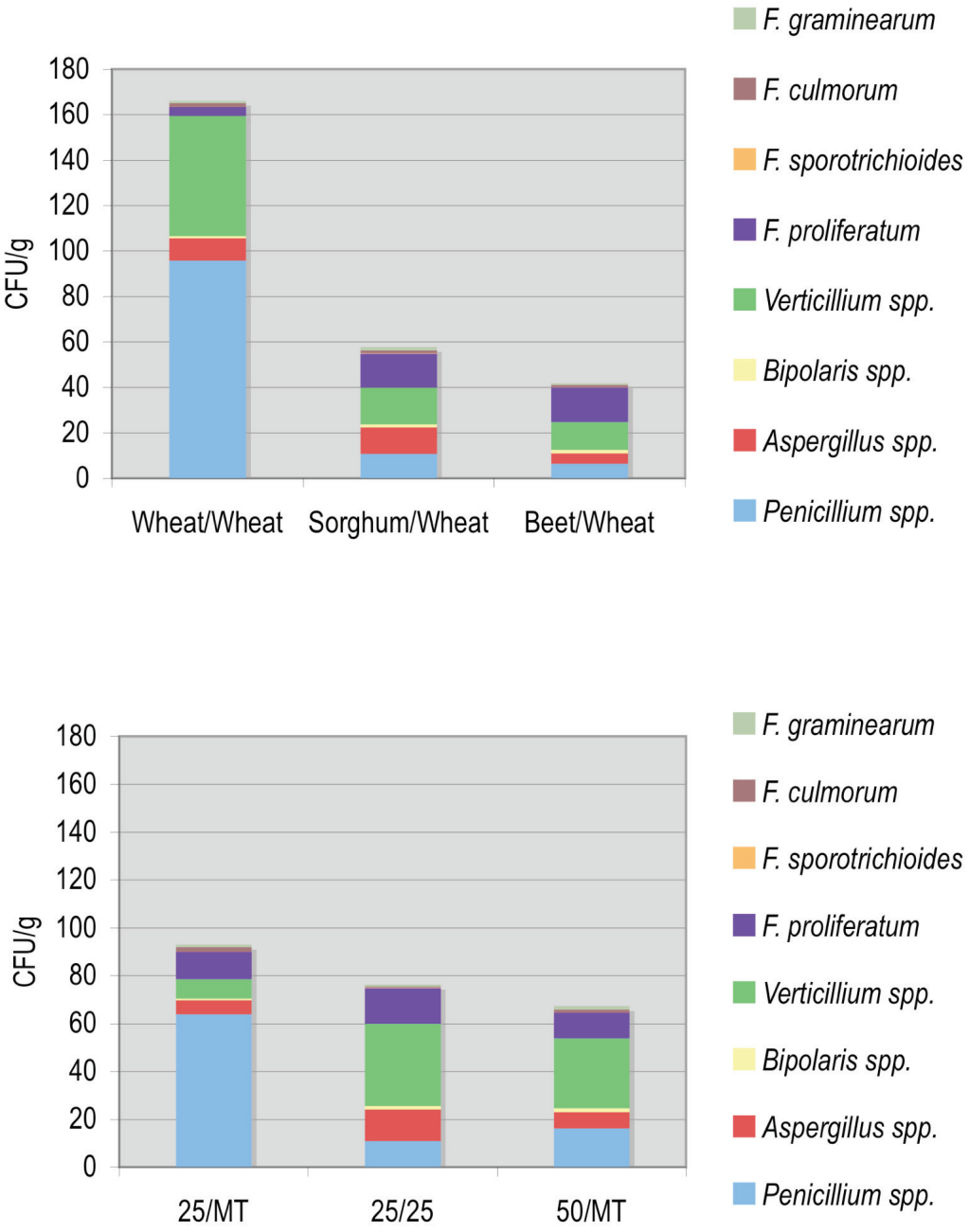
Tillage had no significant influence on the amount of fungi content in wheat flour (figure 9). On the contrary, crop rotation was determinant. In particular, continuous wheat caused a significant increase of UFC (Unit forming Colonies), while the precessions of both sorghum and sugarbeet caused much lower, similar, contents.

Figure 9. Influence of preceding crop and tillage sequence on the amount of fungi in wheat flour (means of 48 determinations ± standard errors of the means)



Regarding the frequency of single microrganisms (figure 10), it's clear that the difference of wheat monosuccession from the other rotations was due to *Penicillium* spp. and *Verticillium* spp. The fungi of both genera were favoured by continuous wheat, not showing any antagonistic effect. *Aspergillus* spp. were slightly less present in wheat after sugarbeet, also compared to sorghum precession, while *F. proliferatum* was found more in sorghum and sugarbeet precession than in continuous wheat.

Figure 10. Composition of the population of fungi that were detected in the wheat flour as affected by crop succession and soil tillage sequence (means of 48 determinations)



The tillage sequence, notwithstanding a scarce influence on the amount of fungi in the flour, markedly varied their composition. Surprisingly tillage did not influence the occurrence of *F. proliferatum*, which resulted indifferent even to the preponderance of *Penicillium* spp.

Table 19. Correlation-r between wheat grain yield and specific apparent weight, fungi in the flour (UFC/g) and Fusarium Head Blight (FHB) incidence (I%) and severity (DS%) on wheat spikes (means of 48 data).

	Wheat Yield t ha^{-1}	Grain spefic wt. kg hl^{-1}	*Penicillium* spp. *UFC/g*	*Aspergillus* spp. *UFC/g*	*Bipolaris* spp. *UFC/g*	*Verticillium* spp. *UFC/g*	*F. proliferatum UFC/g*	*F. sporotrichioides UFC/g*	*F. culmorum UFC/g*	*F. graminearum UFC/g*	FHB (I%)	FHB (DS%)
Wheat Yield t ha^{-1}	1											
Grain specific wt. kg hl^{-1}	0.63a	1										
Penicillium spp. UFC/g	-0.80a	-0.72a	1									
Aspergillus spp. UFC/g	-0.20	0.11	-0.07	1								
Bipolaris spp. UFC/g	0.25	-0.04	-0.20	-0.20	1							
Verticillium spp. UFC/g	-0.18	-0.20	-0.18	0.30b	-0.09	1						
F. proliferatum UFC/g	0.34b	0.21	-0.30	0.09	0.22	0.04	1					
F. sporotrichioides UFC/g	-0.15	0.08	-0.05	0.86a	-0.02	-0.04	0.21	1				
F. culmorum UFC/g	-0.02	-0.20	0.23	-0.21	0.17	-0.09	0.08	-0.18	1			
F. graminearum UFC/g	0.01	0.27c	-0.05	0.28c	-0.35b	0.11	-0.03	0.17	-0.29c	1		
FHB (I%)	-0.57a	-0.47b	0.56a	-0.11	0.07	-0.04	-0.16	0.00	0.08	-0.14	1	
FHB (DS%)	-0.33b	-0.42b	0.20	-0.21	0.24	0.07	-0.08	-0.10	0.38b	-0.43a	0.53a	1

§ a, b. c, Correlation-r significant at P≤0.001; P≤0.01 and P≤0.05, respectively

Instead we found much more *Penicillium* spp. in the sequence 25 cm deep ploughing followed by minimum tillage for wheat. In the other sequences the *Penicillium* place was taken by *Verticillium* spp., which, on the contrary,

appeared scarce with less intensive tillage. These responses of fungi population to tillage remain obscure. They could be due to competition effects between the two saprophytic genera, which have similar environmental requirements and behaviour, when they develop on a substratum more or less contaminated by other fungi.

The correlations between FHB, the pathogens found in the flour, grain yield and specific weight are shown in table 19. A significant negative relation was found between the wheat yield quantity and quality and the *Penicillium* spp. flour content. Because *Penicillium* spp. is not a pathogen we didn't expect this result. However *Penicillium* in the grain was strongly positively correlated with FHB, which was the more detrimental disease to wheat yield and its quality. Thus *Penicillium* damage to yield could be only due to indirect effects.

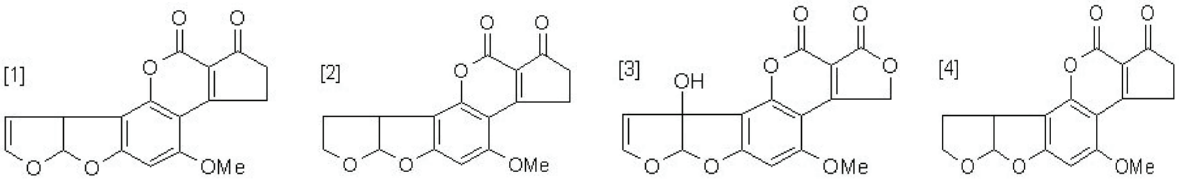
This conjecture is also confirmed by the fact that the great diffusion of fusari in continuous wheat and in shallow tilled plots brought about a high flour content of *Penicillium* spp. On the other hand, the preponderance of saprophytic *Penicillium* spp. and *Verticillium* spp. fungi in the flour would have masked any straight relationships between the manifest FHB in the field and the grain content of *Fusarium* spp.

Product quality: pathogen content in wheat flour and mycotoxins contamination

We decided to search for mycotoxins on the basis of pathogens that were mostly found in wheat flour. Thus, we analyzed aflatoxins G1, G2, B1 and B2 because of *Aspergillus* spp. presence; fumonisin B1 and B2 because of *F. proliferatum*, DON and ZEA because we had found *F. sporotrichioides*, *F. culmorum* and *F. graminearum* (Figure 11). In the three years' determinations all mycotoxins concentrations were below the detection limits of our instruments, with the exception of fumonisin B2 (table 20). The presence of this latter can be explained by the high content of *F. proliferatum* that we found in the grain, little influenced by the agronomic practices and by the presence of

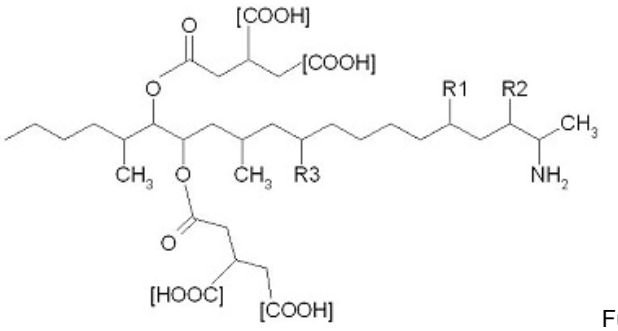
other fungi on the flour. However also fumosinin B2 was detected within a concentration range (0.05-0.10 ppm), which is well below the recent European regulations about winter cereal grains.

Figure 11. Determined mycotoxins



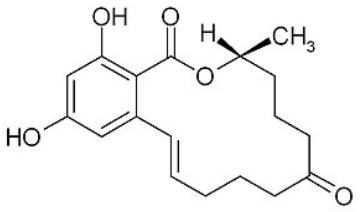
1.-Aflatoxin B1 2.-Aflatoxin B2

3.-Aflatoxin G1 4.-Aflatoxin G2

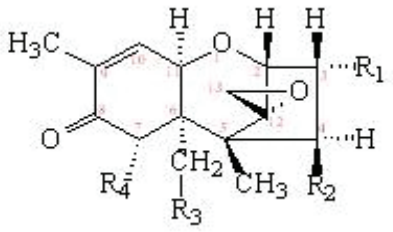


Fumonisin B_1: R1= OH; R2= OH; R3= OH;

Fumonisin B_2: R1= H; R2= OH; R3= OH;



zearalenone



B. trichothecenes

Don. (R1.=.OH, R2. = H, R3.=.OH, R4 = OH)

Table 20. Results of the analysis of fungi (CFU countings) and mycotoxins (HPLC) in wheat flour (averages of 12 detections per year).

		Years			Detection
		2004	2005	2006	Limits
Fungi in wheat flour					
Aspergillus spp.	CFU/g	3.42	1.46	15.71	-
Fusarium proliferatum	CFU/g	0.25	14.79	9.75	-
Fusarium sporotrichioides	CFU/g	0.08	0.04	0.17	-
Fusarium culmorum	CFU/g	0.96	2.08	0.50	-
Fusarium graminearum	CFU/g	1.58	0.48	1.58	-
Mycotoxins in wheat flour					
Aflatoxin G1	ppb	nd	nd	nd	<0.12
Aflatoxin G2	ppb	nd	nd	nd	<0.03
Aflatoxin B1	ppb	nd	nd	nd	<0.09
Aflatoxin B2	ppb	nd	nd	nd	<0.03
Deoxinivalenole (DON)	ppm	nd	nd	nd	<0.15
Fumonisin B1	ppm	0.05-0.1	0.05-0.1	0.05-0.1	<0.05
Fumonisin B2	ppm	nd	nd	nd	<0.05
Zearalenone (ZEA)	ppb	nd	nd	nd	<6.00

nd = Less than the detection limit of the instrument (HPLC)

Conclusions

The results that we obtained in this preliminary research show that durum wheat yield quantity and quality can significantly vary according to the crop rotation and the intensity of soil tillage sequence carried out for many years. The crop succession was the major factor in this sense, causing significant differences in wheat yield in all the four years. Continuous wheat substantially differed from the other rotations, because it always gave lower yields (1-3 t ha^{-1} less) and scanty kernel quality. The productivity of the other successions (wheat preceded by maize, sorghum or sugarbeet) didn't differ much among them. Tillage influence was less marked and unsteady. It resulted significant only two years out of four. The interaction between tillage and rotation treatments was seldom found significant. Therefore the lower productivity of monosuccession couldn't be significantly improved by any soil tillage intensification.

Many factors can substantially vary the responses of wheat yield to tillage; for example, its effects heavily depend on weather, which can affect not only the efficacy of this practice, but also crop growth and all crop adversities, including soil mycopathogens. However, wheat grown on minimum tilled soil yielded always less than after shallow ploughing. Instead the tillage performed for the previous crop had only a slight influence on grain production.

Much of the observed yield variability, and principally that linked to the continuous wheat vs. other crop successions, can be explained by the occurrence of pathogenic fungi infecting wheat, many of which survive in crop residues.

In our field experiment, which was conducted in rather wet conditions, in northern Italy, on a plain at the foot of the hills, most infections on the root and culm base were caused by the *Fusarium* genus, and, to a lesser extent, by *B. sorokiniana*. This result confirms what was already found in many other investigations on the wheat root and crown disease in Italy (Corazza *et al*., 1987

and 1998; Rossi *et al*., 1995). Also Innocenti *et al*. (2000) reported a similar pattern in another research, conducted in the same Ozzano farm. Thus it seems that fusari are always abundantly present in our wheat fields. Probably this genus can easily adapt to a wide range of environmental conditions, thanks to the different requirements of its several species. However, luckily, their infections didn't markedly reduce wheat yield. Their occurrence was more intense in wheat monosuccession than in any other rotations. *G. graminis* as well was more present in continuous wheat, but it was more noxious to the crop. Its infections did not show any trend throughout the experiment: in a year it caused very high damage degrees, the next one almost no symptom at all. This irregularity doesn't agree with the TAD theory that forecasts a progressive decrease of take-all intensity in cereal monosuccessions with time. The contrasting results can be simply due to different weather conditions. The crop preceding wheat had a significant effect on *G. graminis* diffusion. In particular, this pathogen developed better after sorghum than after sugarbeet and maize.

Our investigation revealed the great importance of *R. herpotrichoides* in wheat root and crown disease in Italy. Up to now this pathogen has been scarcely reported in our country; probably it received little consideration because *Fusarium* spp., which are often simultaneously present on the same wheat culm, can easily mask its symptoms. Moreover, it is difficult to isolate because on agar medium many fusari grow more rapidly and can even stop its mycelium development when they are grown on the same rich substratum, such as PDA. Therefore, *R. herpotrichoides* can actually be in Italy as serious a cereal pathogen as it is in France and in other Central European countries, but it is often undervalued. In our research its occurrence was positively correlated with the other most troublesome wheat pathogen (Ggt) and, together, they caused the worst yield reductions. The most serious infections of *R. herpotrichoides* were observed in wheat monosuccession; sorghum precession was slightly more favourable to the fungus than sugarbeet. Maize gave the least disease on the subsequent wheat, but this crop precession was tested only for two years.

We also found *Rhizoctonia* spp., whose spreading is increasingly reported in other Europe countries (Colbach *et al*., 1995). However, its dangerousness didn't appear high: the correlation between its damage degrees and wheat yield was never significant.

The diseases caused by the detected fungi were influenced by soil tillage and even more by crop precession. The rotation influenced mainly *G. graminis* and *R. herpotrichoides*, while tillage treatments showed major effects on most *Fusarium* spp. and *B. sorokiniana*. In particular minimum tillage for wheat favoured fusari. Ggt and Ps mainly occurred in monosuccession because they are wheat host specific and have a reduced capacity of saprophytic life. On the contrary the propagation of many *Fusarium* spp. is less dependent on the presence of crop residues because they can easily survive free in the soil. Both tillage and rotation treatments had no significant influence on *Rhizoctonia* spp., which are typical polyphagous pathogens, widespread on all the crops and can also survive for some years in the soil without plant debris.

Anyway, in our research the importance of crop residues as the main survival mean and the primary source of inoculum for most soil-borne pathogens was confirmed. Tillage mainly modifies the crop residues distribution in the soil profile, while crop precession determines their characteristics. But there can be an interaction between the two effects. If a residue from a host crop is left on the soil surface, where the crop seed will be planted (as it happens in a succession of host/host or bridge/host crops with minimum tillage) it is highly probable that the subsequent crop plants will be soon infected. On the contrary, if what remains on the soil surface is made of not-host residues (like when minimum tillage is performed for a not-host/host succession), primary infection will be limited both for a smaller inoculum amount and for a higher competition from the microflora that is linked to the not-host crop. This can explain why in the rotation: renewal crop-cereal, minimum tillage is unfavourable to many pathogens, while in the successions host/host (i.e. continuous wheat) or bridge/host, ploughing remains the most effective control practice against the two most frequent root and crown diseases of wheat:

Fusarium spp. and *R. herpotrichoides*. The sod turning brought about by ploughing, besides diluting the crop residues in the soil profile, carries them far from the seed and deep into the soil, where the pathogen propagules find sub-optimal growth conditions. Moreover, ploughing creates a macroporosity that speeds up the residue degradation, thus depriving pathogens of their main propagation means (Giardini, 1982). A long period from ploughing to sowing can drastically reduce the inoculum soil content, and this is what usually happens on clay soils, like those typical of the Po Valley. This phenomenon is more decisive for the scarcely saprophytic pathogens (Wiles, 1987). Our results on *R. herpotrichoides* confirm this theory. Its disease on wheat was more serious after minimum tillage than after ploughing, and its infections were particularly low when ploughing for wheat followed deep ploughing for summer crops. On the contrary, residue burial by ploughing didn't significantly affect Ggt, even in 2004 and 2005, when this pathogen resulted particularly damaging.

The Fusarium Head Blight of small grains (FHB) on wheat spikes was particularly noxious to the quantity and quality of grain yield. Its incidence and severity appeared little influenced by fungi that we isolated at the culm base, even if the literature reports the possibility of an upward movement of *Fusarium* spp. along the wheat culm. The major FHB infection source should be the amount of inoculum in the soil, more than the severity of *Fusarium* spp. diseases at the culm base. Relatively to other pathogens of wheat and crown disease, FHB was found positively related with Ggt, *B. sorokiniana* and *R. herpotrichoides* and negatively with *Rhizoctonia* spp. These relationships can be ascribed to the fact that a plant that is weakened by aggressive pathogens, like Ggt and Ps, is more susceptible to other later diseases, like FHB. On the contrary, less aggressive pathogens, like *Rhizoctonia* spp. don't favour further infections.

To reduce FHB sugarbeet appeared a good precession, better than the summer cereals. Sorghum caused FHB infections on subsequent wheat not different from wheat precession. Tillage type had a slight influence on the disease. However, 25-cm ploughing for wheat seemed to discourage the

infections more than minimum tillage. This confirms the hypothesis that much of the FHB infection starts from the *Fusarium* spp. in the soil, that are reduced by ploughing before wheat seeding. However these phenomena can be also determined by indirect influences between populations of fungi in the soil-crop continuum, which are difficult to clarify. There can be great effects due to climatic factors; indeed, the diffusion of Fusarium head blight seemed also determined by the weather in mid spring.

The wheat grain quality was significantly reduced by a wrong crop succession (continuous wheat) and by too a shallow soil tillage (minimum tillage for wheat in certain years with heavy autumn rain). As expected, the grain specific weight, which is well correlated with the semolina yield, was lowest in wheat monosuccession and with minimum tillage, similarly to what we observed for the grain yield. Moreover, it was strongly affected by the incidence and severity of Fusarium head blight of small cereals. The fungi contaminating the flour were also different from the causal agents of FHB symptoms. In our research many of them belonged to *Verticillium*, *Aspergillus* and *Penicillium* genera, which include typical saprophytic species. They are ubiquitous not-pathogenic organisms, also present on the aerial parts of the plants, where they can survive without revealing any symptom of their presence. In wheat flour they find an optimum substratum and, in the absence of other competitors, they can rapidly develop large populations. In the instance of wheat plants that are stressed because of nutrient or water deficiencies, or due to early infections of *G. graminis*, those saprophytic fungi can contaminate the kernels more efficiently than FHB *Fusarium* spp.

The rotation had a great influence on the fungi flour content. Monosuccession gave the highest amount of saprophytic organisms, probably due to a more severe crop stress. We found a strong negative correlation between *Penicillium* spp. occurrence and the crop qualitative and quantitative production that can be due to an indirect influence on Penicillium and FHB symptom intensity.

Tillage had a slight influence on the amount of fungi on the flour, but greatly affected the composition of their population by favouring either *Verticillium* or *Penicillium* as a consequence of turning or not the soil sod for wheat. Also at the flour level, thus, our research revealed complex relations between various microrganisms, which should be always considered when better control means of certain diseases are to be chosen.

The low content of *Fusarium* spp. and *Aspergillus* spp. that we always found in wheat spikes and flour could explain why mycotoxins were almost completely absent in wheat final product. Some traces were found only of fumonisin B, probably originated by the *F. proliferatum* infecting the flour. The mycotoxin problem, however, remains serious. In our research the presence of *Penicillium* spp. on the kernels that were produced by weakened plants is worrying. Indeed durum wheat in Italy can be easily stressed by water shortage or nutrient deficiencies or even by infections of fusari or other pathogens during its whole cycle. Many *Penicillium* spp. can produce ocratoxins, which are toxic as well. The search for these toxins in the flour coming from fusari diseased ears should be an interesting future step for a better understanding of the risks of mycotoxins contaminations of durum wheat grain with the aim to prevent any intoxication to humans.

From our experiment it can be evinced the difficult choice that a farmer should take regarding the control of wheat pathogens in Italy. Would it be more profitable to control wheat root and culm disease than Fusarium head blight? On the basis of our results perhaps it would be better to prevent any stress to cropped plants due to soil-borne pathogens, to reduce the possibility of later infections on the spikes by fungi that can contaminate grain production with mycotoxins.

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Summary