INTRODUCTION

Septoria tritici Rob. ex Desm. (teleomorph: *Mycosphaerella graminicola* (Fuckel) Schröter in Cohn.) causes leaf blotch, whereas ***Stagonospora nodorum*** (Berk.) E. Castellani and E.G. Germano (syn. *Septoria nodorum* (Berk.) in Berk., teleomorph: *Phaeosphaeria nodorum*) causes leaf blotch, glume blotch and seed diseases on wheat (Wiese, 1987). Both pathogens often occur simultaneously on wheat leaves, and build-up a Septoria/*Stagonosopra* **disease complex**. The *Septoria* diseases are also common on triticale, barley and rye (Nyvall, 1999; Eyal, 1999).

S. tritici and *S. nodorum* cause similar leaf **symptoms** and also have similar infection cycles. The initial symptoms of ***S. tritici*** are yellowish or chlorotic spots. The spots enlarge into irregular- lesions, brown-to-reddish brown in colour. Lesions age and become light brown to almost white and have small, dark pycnidia in the center. The presence of pycnidia is a good diagnostic characteristic. Leaf lesions of ***S. nodorum*** have light brown spots that are similar to those of *S. tritici* disease. As the lesions enlarge, they become dark brown and the centres turn grayish-white in color as tiny brown pycnidia develop within them. Pycnidia are present on the blotch leaf surface. On glumes the lesions of the fungus begin as either grayish or brownish spots. An exact **identifying** which disease is present on a given plant, measurement (morphology) of pycnidiospores and pycnidia is required (Agrios, 1997; Hoffmann and Schmutterer, 1999).

The primary **inoculum sources** of *S. tritici* and *S. nodorum* usually are the infected wheat debris and volunteer wheat plants. In some regions infected seeds also regarded as an important primary inoculums source for *S. nodorum* (Shah et al., 1999; Milus and Chalkley, 1997; Shaw, 1999). Pycnidia spores are the mainly inoculum of primary and secondary infection, ascospores served as inoculum were reported on both pathogens also (Nyvall, 1999).

Infection of *S. tritici* and *S. nodorum* may occur at any stage of plant development from seedlings to adult plants. Leaf infection of ***S. tritici*** usually occurs first on lower leaves in contact with the soil, and symptoms are usually detected in the fall and early spring. As wheat plant growing, fungus inoculums spread vertically to the upper

leaves by wind and rains. Disease development is favored by cool wet weather, and the fungus is more aggressive between 15–20 ^{o}C. The wheat plant is more susceptible to infection by *S. tritici* during the period of stem elongation to flag leaf emergence. *S. tritici* attacks the leaves of plants only. ***S. nodorum*** can infect the lower leaves of wheat, but the infection is more common on plants at the later growth stages, usually during and after heading; the glume and seed of wheat are also infected. *S. nodorum* prefers a more warm wet condition between 20–27^{o}C (Shaw and Royle, 1986; Shaw, 1987; Joerger et al., 1992). In condition of a prevailing wet weather, both *S. tritici* and *S. nodorum* diseases can result in a loss of yields on wheat.

Septoria*/Stagonospora* diseases are world-widely important diseases on wheat. During the past 15 years, changes on the patterns of occurrence of *S. tritici* and *S. nodorum* have been reported. In the early 1980s, *S. nodorum* was the most important disease in northern Europe, eastern USA and western Australia, while *S. tritici* was the most prevalent in Mediterranean climates and the Great Plains of North America. Recently, ***S. tritici*** has occurred with a greater importance in central Europe and UK (Bayles, 1991; Scharen, 1999). The yield losses caused by this disease were reported between 30 and 40% in Israel (Eyal, 1972), 12% in the Netherlands (Forrer and Zadocks, 1983), 25% in Northern Germany (Ceynova et al., 1993). The average annual yield loss in the UK was about £35 million during the period 1985–1989 (Cook et al., 1991) in relation to the achieved disease severity of *S. tritici*. In some states in USA, ***S. nodorum*** **blotch** is the most important component of foliar disease complex that attacks winter wheat (Shah and Bergstrom, 1999), and might cause yield losses of up to 50% (Nyvall, 1999). In Southern Germany, *S. nodorum* predominates for *Septoria* disease complex on wheat (Hoffmann and Schmutterer, 1999). In Northern Germany, for example, wheat leaf blotch pathogens were predominant with *S. nodorum* being prevalent in 1994 and *S. tritici* in 1995 (Hedke and Verreet, 1999). In wheat management systems in Germany, the two pathogens co-existed normally together (Eyal, 1999; Hede & Verreet, 1999; Müller & Luckhard, 2003).

S. *tritici* penetrates host by hyphae aggregates via the stoma. Inside the leaf, the hyphae grow intercellularly in the apoplast, and occasionally producing haustoria in mesophyll cell (Kema et al., 1996). After a latent period of between 2 – 6 weeks, it

switches from biotrophic to necrtrophic growth and visible symptoms (necrotic lesions) start appearing. The infection of *S. nodorum* is similar to that of *S. tritici,* but the fungus penetrates by penetration peg directly through the surface of the epidermal cells, does not produce haustoria and also has much shorter latent period, i.e. a period of 7–10 days under optimum conditions (Bird and Ride, 1981; Karjalainen and Lounatmaa, 1986).

During the infection process, pathogens intensively produce various cell wall degrading enzymes (**CWDE**) including xylanase, celllulase, 1,3-ß-glucanase, protease, polygalacturonase, pectinase and cutinase, in order to destruct the cell wall and/or cause tissue maceration (Keon et al., 1987; Cooper, 1984; Mishagi, 1982; Bateman and Basham, 1976; Wijesundera et al., 1984). CWDE play an important role in the infection process of pathogens and the host-pathogen interaction (Keon et al., 1987; Bateman and Basham, 1976; Agrios, 1997; Jørgensen et al., 1999). On the other hand, plant pathogens can also induce an increase in plant enzymes such as ß-1, 3-glucanases and chitinases as well as proteases (Ye et al., 1990; Krishnaveni et al., 1999; Segarra et al., 2002), which are involved in resistance of plants to fungal and bacterial pathogens and enlisted in the group of pathogenesis-related proteins (PR-proteins). In several pathogens, measurement of fungal enzymes allowed a fungal detection in host tissue and some enzyme activities have often highly correlated with disease incidence and further have been used for assessment of resistance of infected wheat varieties (Afshari-Azad and Wolf, 1992; Kopahnke et al., 1994; Volke et al., 1996, 1997).

To date, a number of cell wall degrading enzymes of ***S. nodorum*** have been isolated and characterized (Magro, 1984; Sieber, 1989; Lehtinen, 1993; Halama et al., 1999; Lalaoui et al., 2000; Carlile et al., 2000), which included xylanase, cellulase, polygalacturonase, protease, α-amylase, 1,3-ß-glucanase and chitinase so on. In contrast, less is know about CWDE production from ***S. tritici***. Activities of cellulolytic and pectic enzymes of the fungus *in vitro* and in infected plants were recorded by Cordo et al. (1990). Recently, Segarra et al. (2002) reported that activity levels of proteinase in *S. tritici* infected resistant wheat cultivar were higher than that in susceptible cultivar and has contributed this increased protease to PR-proteins.

The **objectives** of the enzymatic studies in the present work was to investigate and compare the production of six hydrolytic enzymes (xylanase, cellulase, protease, a-amylase, ß-1,3-glucanase and chtinase) from *S. nodorum* and *S. tritici in vitro* and *in planta*, and to determine a quantitative relationship between enzyme activity and disease severity for a possible application in assessing fungicide efficacies and resistance of wheat varieties to *Septoria* leaf diseases.

Uses of fungicides and cultivation of resistant varieties play important roles in the controlling plant diseases. Although wheat cultivars carrying resistance to *Septoria* leaf diseases are available, at present, control of the *Septoria* diseases still mainly relies on the use of fungicides (Joerger et al., 1992; Cook, 1999). To obtain an **optimum control**, the most appropriate fungicide has to be applied at the correct timing and dose rate (Eyal, 1981). Even the most effective fungicide triazole (eradicating activity) is limited to the period of hyphal extension and colonization. As soon as pycnidia are initiated, none of the fungicides that are presently available are effective enough to control the generation of pathogens (i.e. the established lesions). Thus fora better fungicide timing, a **key requirement** is to identify the pathogens and to quantify the amount of inoculums present (Mittermeier et al., 1990). Furthermore, after the infection and during the early stages of hyphal colonization and initiation of pycnidia within leaf tissue, there are no visible signs but an infection occurred, i.e. latent periods. The varied latent periods, particularly for *S. tritici* that has a more long time of latent period, can also result in the differing of fungicides efficacy against the pathogen (Shaw, 1990). Therefore, **accurate** and **pre-symptomatic diagnosis** of pathogens is critical components of effective plant disease control programs of fungicides (Joerger et al., 1992).

However, an **early** and **accurate detection** of *S. tritici* and *S. nodorum* is often difficult by the naked eyes, because of the similar symptoms caused by those two pathogens as mentioned above and also by several other causative agents. Both *Bipolaris sorokiniana* (**spot blotch**) that occurs wherever wheat is grown and *Drechslera tritici-repentis* (**tan spot**) have the initial symptoms similar to lesions of the Septoria diseases (Nyvall, 1999; Obst, 1993). Recently *D. tritici-repentis* has changed to a great important disease on wheat in some regions in Germany, and causes mixed infection with the *Septoria*/*Stagonospora* diseases (Schmitz and

Grossmann, 1987; Müller and Habermeyer, 2001). **Pollen scorch** resulted from pollen remaining on leaves that provides a nutrient source for fungal saprophytes, has the symptoms similar to the Septoria leaf blotch (Hollomon et al., 1999). **Physical damage** caused by frost and hail as well as ozone can cause also necrotic symptoms similar to those caused by the leaf blotch pathogens (Tiedemann, 1992; Lamprecht, 2001). Moreover, the symptoms of *S. nodorum* are often **non-specific**, and the coalescing lesions and pale pycnidia both can result in difficulties to distinguish from natural senescence (Schnieder and Fehlmann, 1995; Hollomon et al., 1999). Furthermore, *Microdochium nivale* and *Fusarium spp*. cause also necrotic lesions on wheat leaves (Diehl and Fehrmann, 1989; Westphal et al., 1993). Consequently, when necrosis on wheat leaves occurs, it could be difficult to diagnose the exact cause. To achieve these aims, rapid, accurate, specific and precise **diagnostic technologies** are required (Hollomon et al., 1999).

Normally, investigations of *Septoria* and/or *Stagonospora* diseases have based on visual assessment of symptoms and count of the pathogen pycnidia. Counting **pycnidia** has been often used to investigate each of the pathogens in the disease complex (Loughman et al., 1996; Eyal, 1975, 1999b; Verreet and Hoffmann, 1993; Hedke and Verreet, 1999). However, the sporulation is heavily influenced by physiological stress, nutritional status, and other environmental factors (Beckman et al., 1994; Marjolein and Schots, 1995). Counting the pycnidia and/or pycnidiospores is time-consuming. Furthermore, in damp locations and wet seasons, *Didymella exitialis* can be found on damaged and senescing tissue where it causes brown oval or irregularly shaped lesions with brown or black pseudothecia and pycnidia, which are easily confused with the pycnidia of *S. tritici* (Hollomon et al., 1999; Hoffmann and Schmutterer, 1999).

Enzyme-linked immunosorbent assay (**ELISA**) has provided the possibilities of specific diagnose and quantitative detection of pathogens in plants. Immunological assay was first described by Engvall and Perlman (1971) and then widely used as a specific and rapid diagnostic tool in medicine and virology. Nowadays, this technology has been also much applied to crop fungi diseases (Miller et al., 1988; Sherald and Lei, 1991; Meyer, 1996; Jenny et al., 1999; Meyer et al., 2000; Kageyama et al., 2002), and several of the tests have been introduced to commercial