University of Reading

EFFECT OF SULPHUR AND SILICON FERTILIZERS ON DISEASE CONTROL
AND YIELD AND QUALITY OF WHEAT

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Thesis submitted to School of Agriculture, Policy and Development Dept., The
University of Reading, in part fulfilment of the degree of Doctor of Philosophy

November 2016

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Declaration

I confirm that this is my own work and the use of all material from other sources has been properly and fully acknowledged.

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Abstract

Published research showed there was a link between the abundance of septoria diseases (*Zymoseptoria tritici* and *Parastagonospora nodorum*) and sulphurous acid in acid rain. Historical records showed that *P. nodorum* was at a peak in 1970s while *Z. tritici* became more abundant at 1980s. Another, small scale, study showed that both pathogens were affected by sulphur, but *Z. tritici* was reduced more by sulphur than *P. nodorum*. These studies suggested it could be useful to investigate the effect of sulphur fertilizer under larger scale, field, conditions. Thus, the aim of this project was to study if sulphur fertilizer affects the abundance of these pathogens, and to study if sulphur fertilization alters the yield and quality of wheat under disease pressure. A further question was whether silicon fertilizer affects both pathogens and the yield and quality of wheat. A final aspect of this thesis was to develop a way to quantify pathogens by using qPCR assay on large field samples.

A glasshouse experiment showed that all wheat cultivars were susceptible to available pure isolates of both pathogens, but the proportion of leaves infected by these isolates varied between cultivars. Cv. Paragon showed a high proportion of infected leaves in comparison with Gallant and Maris Huntsman (M. Huntsman).

Three successive field experiments were conducted. *Z. tritici* infected all plots, so it was not possible to estimate changes in competitive effects due to the fertiliser regimes. In the field experiments a variety of measurements were used at different growth stages to assess the diseases. The proportion of leaves infected with, and disease severity of, *Z. tritici* were not significantly affectedly by sulphur fertilization in 2013. In two further experiments, proportion of leaves infected with, and spore concentration of, *Z. tritici* were reduced by sulphur fertilization. In the 2014-2015 experiment disease severity of *Z. tritici* was significantly reduced by sulphur fertilization. Although autumn spray and seed inoculation with *P. nodorum* did not succeed, a spring spray inoculation was successful in the 2014-2015 experiment. In this experiment, proportion of leaves infected and disease severity of both fungi were reduced by sulphur fertilization. but *Z. tritici* was reduced more than *P. nodorum*. These results agree with the historical trends.

Yield and quality measurements results showed that sulphur fertilization increased yield and the wheat quality was improved. This result was obtained when the sulphur fertilization was split and applied at multiple times throughout the growing season of wheat (Table 6).

Acknowledgements

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1-1- Wheat crop: use, production and limitation:

Wheat, rice and maize dominate grain production worldwide. These grasses are primarily grown for their grain (family Gramineae, syn. Poaceae). Wheat is a term which describes a number of species and sub-species in the genus *Triticum*. Bread wheat (*Triticum aestivum* subsp. *aestivum*) is the most important member, which accounts for more than 90% of wheat production in the world. A further 5% of production is durum wheat (*Triticum turgidum* subsp. *durum*) (Gooding, 2009).

In temperate areas, wheat is the primary cereal. Due to wheat being the most widely adapted cereal, it is grown in widely different areas. It is grown from the Arctic Circle to the equator, in areas with a range of rainfall between 250-1800 mm, and from 0m to 3000m above the sea level. The cultivated land area of wheat is more than any other food crop. Half of the wheat area is in developing countries. Asia (China, India, Pakistan and Turkey), North America (the United State and Canada), Europe (France and Germany), the Russia Federation and Australia are the top 10 producer countries (FAOSTAT, 2016; Figure 1). The majority of wheat grown in South America is in Argentina. Most wheat produced in Africa is in the north (e.g. Egypt and Morocco), or in Ethiopia and Kenya in highland areas (Gooding, 2009).
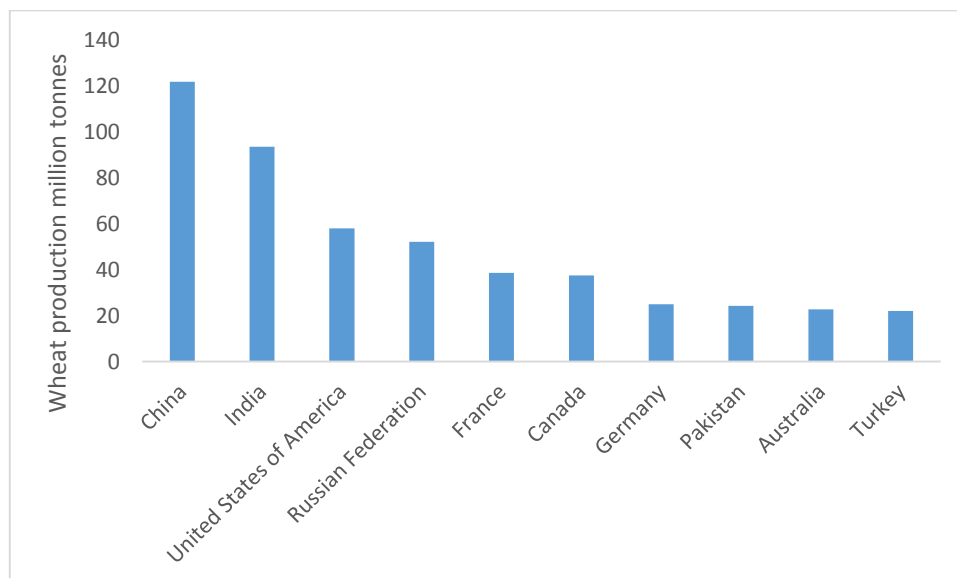
Figure 1. Worldwide wheat production, million tonnes in 2013. From FAO, 2016.

Wheat is cultivated over large areas where production is limited by water shortage, which is reflected in lower yield. Even in the areas of much more potential production, wheat yield can be

compromised due to the short duration of growth as a component of a rotation system. This system includes rice-wheat rotations in the south and east of Asia, and South America the rotation is wheat-soybean-maize. In commercial rain-fed fields the grain production can commonly exceed 10 t/ha in temperate, moist and long season areas which where intensive production derives from intensive use of fertilizers and agrochemicals. Light interception in these areas might be the more important limit factor, not water availability.

Wheat (*Triticum* spp) since 8000 years is a basic staple food, and was the first domesticated food crop in major cities of Europe, West Asia and North Africa (Monneveux *et al*., 2012). Wheat has many functional properties like other cereals, providing a concentrated source of carbohydrate and useful amounts of protein, fat, minerals, vitamins and fiber. The main source of calories for 1.5 billion people is wheat (Reynolds *et al*., 1999). Wheat can be used as human food or livestock feed; approximately 67% of production is used as human food and 17% for livestock feed. A large variety of processed foods are made from wheat, such as different types of bread, cakes, noodles, crackers, breakfast food, and biscuit (Monneveux *et al*., 2012).

In areas of the world where the local production of wheat exceeds the demand for food and livestock feed, wheat can be used in alcoholic beverages through starch fermentation. When the nitrogen content is low in wheat, that means it is less suitable for bread-making, but this type gives a high spirit yield for grain whisky production. It can be used to aid head retention in pasteurized beers in the barley-brewing industry. Furthermore, wheat grain is used to produce bioethanol as a source of renewable energy, by fermenting grain with a low nitrogen content. Commercial companies have achieved 40L of ethanol per 100 kg of grain, with experimentally up to 46.5 L (Rosenberger, 2005).

Wheat production increased by 3.3% per year between 1949 and1978. Increasing the genetic yield and expansion of the area that produces wheat were the reason for the initial rise of wheat production in this period. However, the main increase of yield from the start of the 1960s on was due to the use of fertilizer, improved cultivars, and pesticides. But the rate of increase of wheat production between 1982 and 1991 slowed to 1.5 % per year, one exception being China, which maintained a rate of increase and became the largest wheat producer in the world because its wheat production was still increasing at 2.6% per year. Also wheat production was increased by nearly 3% ber year in India and Pakistan at the same period (Monneveux *et al*., 2012). Since the green revolution in the mid-1960s, macro-nutrients have been adequately supplied in the west Asian countries. This was through the use of commercial fertilizers. But, with time, soils have started to

show deficiency in micro-nutrients (Bhatt *et al*., 2016). This is also reported in rice-wheat rotations (Biswas and Tewatias 1991). The advantage of applied sulphur for yield has been reported by Katyal (2003). However, the recommendation of sulphur quantity required in texturally divergent soils still needs more work (Bhatt *et al*, 2016). When a wheat crop grows after rice in coarse textured soil, wheat faces manganese deficiency, for example in Punjab. Chatterjee *et al*. (1987) found that wheat yield decreased due to deficiency of boron, in West Bengal soil and in a rice-wheat rotation. In the rice-wheat cropping system, selenium toxicity was apparent in the Hoshiarpur and Nawanshahr regions of Punjab.

Recently the wheat production in the world has been nearly 750 million tons per year (FAOSTAT 2016; Figure 2). Global wheat production is variable between countries. India and China have the highest wheat production in the world, then USA (Figure 1). There are many factors leading to increase in the wheat demand globally: increase in population, urbanization, development and growth in economies, and improved calorie intakes. Demand has been projected to increase to 900 million tonnes by 2050. The nations that expect to increase wheat consumption most highly are developing countries in Africa, and Asia, due to rapid increases in population, urbanisation and economic development. (Alexandratos and Bruinsma, 2012).
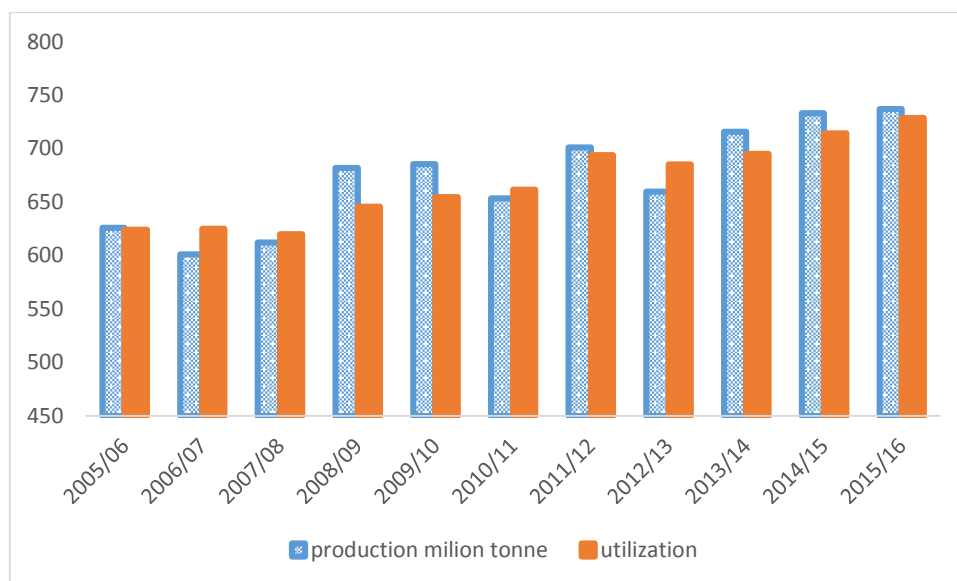
Figure 2. wheat production and utilization globally million tonnes/year. From FAO, 2016.

In the United Kingdom winter wheat is sown in September-November and then harvested between mid-August and mid-September. The production of wheat in the UK is around 15 million tonnes in a year (DEFRA, 2016) (Figure 3). The long growing season is because of the weather conditions which are suitable for this plant: for example, year-round rain fall. Another reason is that the day

length in the winter is short. This high yield is due to the moderate and wet conditions in summer that increase the period of grain filling. Currently, the UK is the third highest European wheat producer (USDA, 2015) (Figure 4). Yields in the UK could be increased still more through adopting appropriate rotation, cultivations and nutrient management. For example, the resistance of *Zymoseptorioa tritici* to triazoles makes researchers look for sustainable methods to reduce the proportion of this pathogen.
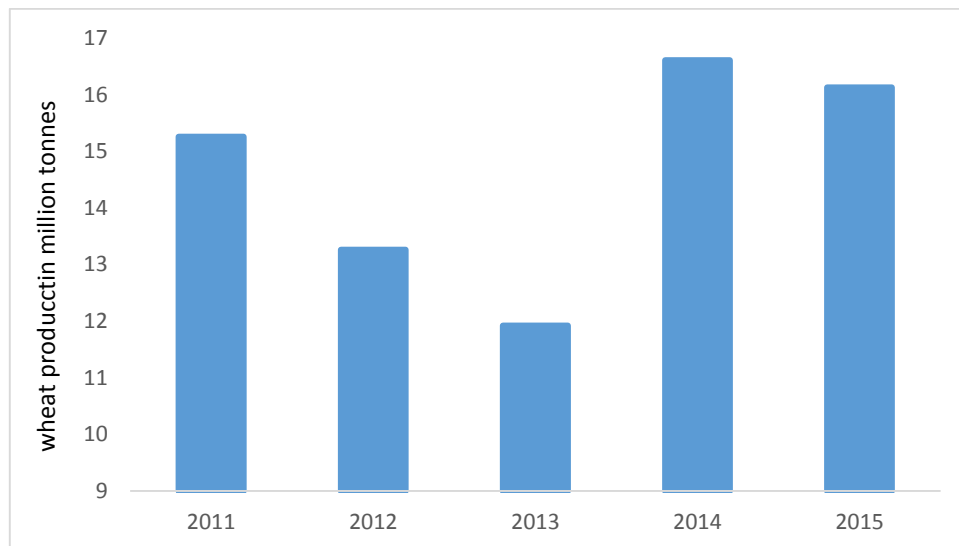
Figure 3. Total annual wheat production in the UK (from 2011-2015), Department for Environment Food and Rural Affairs (DEFRA, 2016).
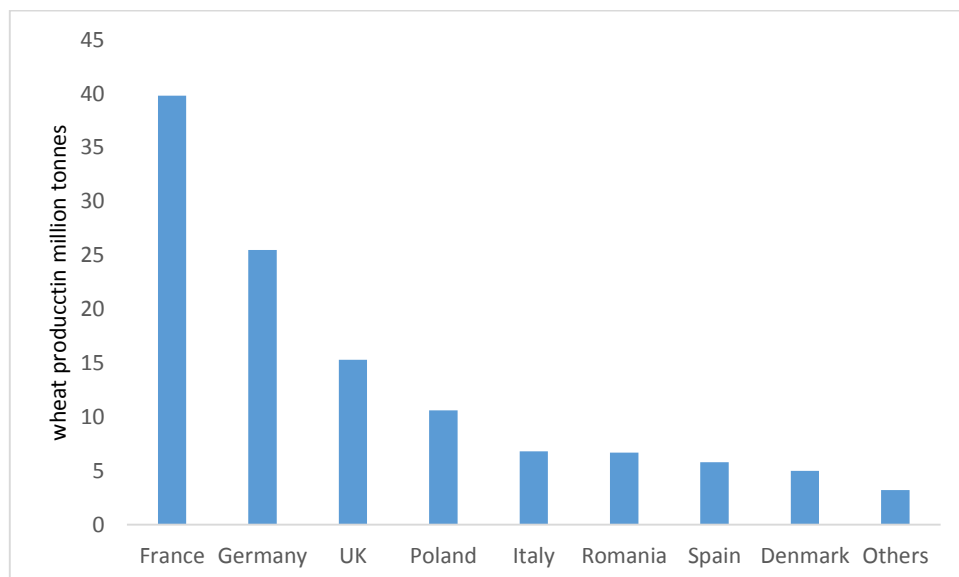
Figure 4. Estimated wheat production in Europe in million tonnes, 2015-2016. From US Department of Agriculture (USDA).

Disease can greatly reduce wheat production. Septoria diseases particularly (*Zymoseptoria tritici* and *Parastagonospora nodorum*) are two of the most common wheat diseases in the UK and Western Europe. Septoria diseases cause much damage in the tissue of leaves, reducing photosynthetic area, and this leads to yield losses (Gooding & Davies, 1997). In other words, the green area of the upper leaves is reduced by Septoria diseases and as a result of this the filling of grain before harvest will be reduced (Eyal, 1999). These diseases cause yield losses that can reach 50% in susceptible cultivars in bad years in conducive-disease climates (Eyal *et al*.,1987; Lilian *et al*.,2016; Andrew *et al*., 2016; Romain *et al*., 2016). Although full fungicide programs are used with most wheat cultivars, the economic losses can reach £200 million in the UK. (Fraaije, 2016).

1-2- Wheat breeding for disease resistance:

In the last decades, the technology of genomic selection as a breeding technique has been adopted in different species, after success with animal breeding. This is contributing to a reshaping of wheat breeding (Bassi *et al.*, 2016). Wheat breeding has improved recently, due to the accurate application of field experiment design and the development of in double haploids (Bassi *et al.*, 2016), among other things. The recommended list of wheat cultivars from AHDB recently has involved many cultivars with high yield and good resistance to disease. However, *Z. tritici* is still a serious disease that threatens wheat yield, in spite of breeding and the use of fungicides.

Nowadays, several types of pesticides are used to protect crops from pests and pathogens. As a result, some of these organisms have become able to resist these pesticides. For instance, *Zymoseptoria tritici* has the ability to resist triazoles (Mavroeidi and Shaw, 2006; Curvers *et al.*, 2015) and now succinate dehydrogenase inhibitors (SDHI) (Dooley *et al.*, 2016). To overcome this problem a variety of methods have been used. One of these methods is to enhance the ability of crops to resist diseases by themselves without using pesticides (Gupta and Pandey, 2014).

Recently an important target of wheat breeding throughout the temperate regions has been resistance to *Z. tritici*. The prospect of this disease damaging wheat crops has drawn international attention (Brown *et al*, 2015). Most wheat cultivars with high yield are susceptible to *Z. tritici*. These are two classes of resistance genes to *Z. tritici:* qualitative-genes that can control large fractions of genetic variation, and quantitative genes with small to moderate effect on *Z. tritici*. So far 21 qualitative genes (Stb genes) have been discovered and mapped. Most of these genes are genotype-specific, but the minority of *Z. tritici* isolates which are virulent have been affected by most of these genes. Some of the previously effective genes have been overcome due to the evolution of pathogen virulence, and most of qualitative resistances are unlikely to be durable. . In general, quantitative resistance with its weaker specificity has provided more durable resistance than qualitative genes. Recently eighty-nine genome regions have been identified which carry quantitative resistance traits. Some of the quantitative genes have been mapped. One of the interesting genes is *Stb*16q, which is effective against most *Z. tritici* isolates that have been tested so far (Brown *et al.*, 2015). Currently the individual *Stb* genes are not reducing *Z. tritici* populations in Europe (Arraiano *et al.*, 2009), which may be due to the rapid evolution of the pathogen. Breeding progress in *Z. tritici* resistance in the last few years may have happened by the accumulation of minor genes, and so the huge variation of resistance to *Z. tritici* in the field may be controlled by quantitative resistance (Torriani *et al.*, 2015). This quantitative resistance is durable in comparison to qualitative resistance, although the effectiveness of this type of resistance may be

eroded gradually (Mundt *et al.,* 2002; Krenz *et al.*, 2008). However, this happens slower than evolution in response to the use of quantitave resistance (Poland *et al.*, 2009; Brown *et al.*, 2015).

For the *P. nodorum* pathogen, some resistance to this pathogen was known to be gene for gene prior to 1980s, but no genes were mapped (Feng *et al.*, 2004). After 1981 many qualitative genes have been mapped but all were weakly effective and difficult to use in breeding programmes (Friesen and Faris, 2010).

Although breeding technology has had a beneficial effect against *Z. tritici*, the susceptibility of this pathogen to the most effective fungicides and resistance gene may be eroded. Thus, looking for another solution is required to against this pathogen, the use of fertilizers, for example.

1-3- The Pathogens:

Crops of wheat can be infected by many types of pathogens including bacteria, fungi and viruses. Fungal pathogens are one of the most common causes of crops' diseases. Wheat can be infected by many fungal species. *Zymoseptoria tritici* and *Parastagonospora nodorum* are common fungal species that can infect wheat crops (Eyal, 1999). These pathogens have been repeatedly renamed. *Zymoseptoria tritici* (Desm.) (Quaedvlieg *et al*., 2011) is the currently proposed name. The telemorph name *Mycosphaerella graminicola* (Fuckel) J. Schort in Cohn (Eyal, 1999) has also been widely adopted. The anamorph name *Septoria nodorum* (Berk.) in Berk and Broome was renamed *Stagonospora nodorum* (Berk.) by E. Castellani and E.G. Germanosy with the telemorph *Phaeosphaeria nodorum* (E. Muller) Hedjaroude, syn. *Leptosphaeria nodorum* E. Muller) (Solomon *et al*., 2006). The currently accepted name is now *Parastagonospora nodorum* (Berk.) Quaedvlieg, Verkley & Crous (Quaedvlieg *et al*., 2013). Both hexaploid bread wheat (*Triticum aestivum*) and tetraploid durum wheat can be infected by *Z. tritici* and *P. nodorum*.

1-3-1- Symptoms and life cycle:

The initial infection of a crop by *Z. tritici* occurs by air-borne ascospores produced in previous crop debris. Soon after seedlings emerge, infection occurs in autumn (for winter wheat) or in spring. The infection starts when ascospore germ tubes encounter stomata, which supply them with an entry to the sub-stomatal cavity either by direct growth or after appressorium production. With development of the fungus, and after switching from biotrophic to nectrophic nutrition, plant cells break down, and the lesions formed can be identified initially by small yellow flecks or blotches. Long, narrow, blotches often occur as the result of the expansion of a lesion in the direction of leaf veins. Within the necrotic area of the lesions, pycnidia start to develop around the stomata and release conidia in gelatinous, hygroscopic cirrhi. These spores spread to other leaves within same plant or on nearby plants by rain-splash. During the crop-free periods, the pathogens can survive as pseudothecia but also as pycnidia on crop debris (Ponomarenko *et al*., 2011). (Figure 5).
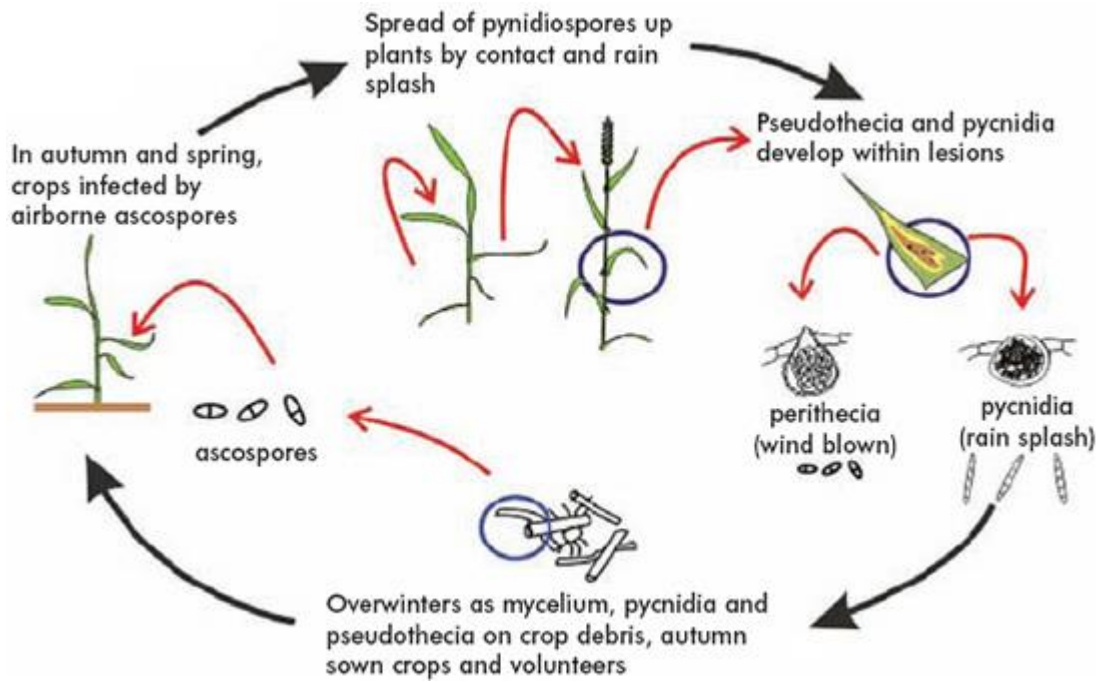
Figure 5. *Z. tritici* life cycle. (reproduced from AHDB, The Wheat Disease Management Guide 2010).

Symptoms of *Z. tritici* appear on the seedlings after emergence as small chlorotic spots (Figure 7, picture 1). When they enlarge, the lesion becomes a light tan colour and darker coloured fruiting bodies develop (Figure 7, picture 2). On mature leaves, the shapes of lesions are often long, narrow and delimited by leaf veins. However, they can also be shaped irregularly or can be elliptical, particularly on seedlings or leaves that were young when infected. Black or brown fruiting structures, sexual pseudothecia or asexual pycnidia, can be found in mature lesions. Within the substomatal cavities of the host, the pycnidia or pseudothecia develop, spaced regularly within the lesions.

P. nodorum can survive as pycnidia and pseudothecia on seed and crop debris. Infected seeds and ascospores are considered to be the major primary source of inoculation of *P. nodorum* (Shah *et al*., 2000). In autumn or spring, air-borne ascospores, which are released from pseudothecia and can travel a long distance on the wind, result in initial infections by *P. nodorum*. This usually happens in the case of absence of crop debris. Pycnidia start to produce pycnidiospores when the temperature and humidity increase, and spread by rain-splash transmission to other leaves within the same plant or on other plants. Spores produced from pseudothecia and pycnidia develop on the flag leaf and ears, and eventually the seed will be infected (Figure 6).
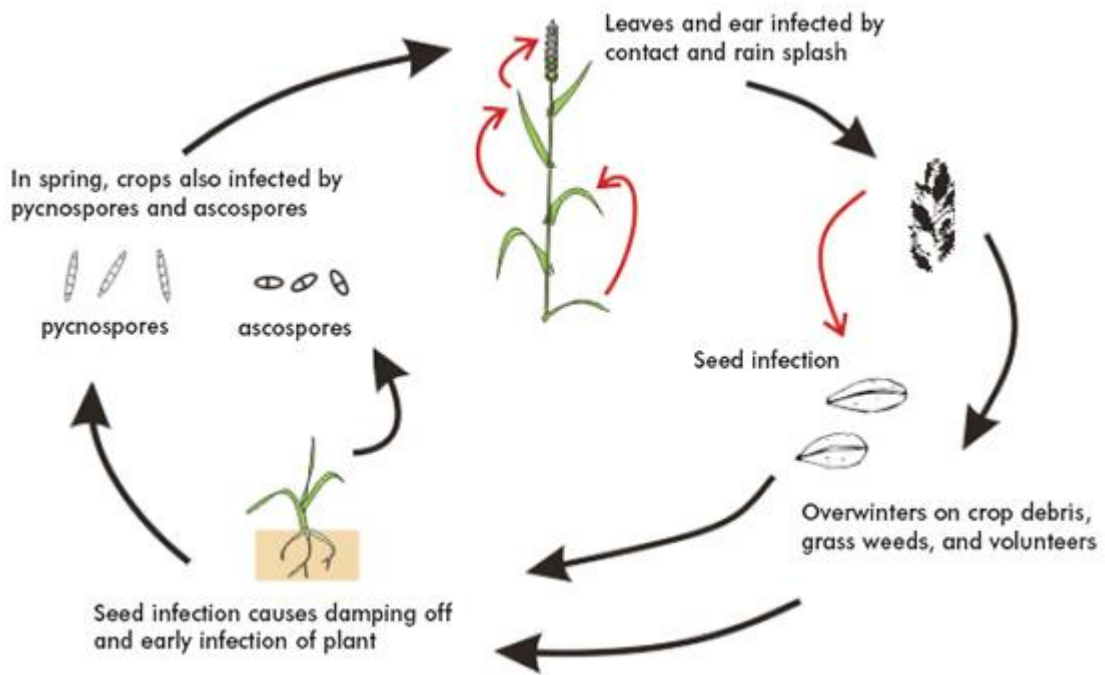
Figure 6. *P. nodorum* life cycle (reproduced from AHDB, The Wheat Disease Management Guide 2010).

P. nodorum can infect seedlings, leaves as they develop, and ears. The symptoms of *P. nodorum* on the seedling are dark green patches on the coleoptile, that then become necrotic. The first symptom on infected mature leaves is small necrotic lesions. These develop to become brown elliptical lesions with a yellow or chlorotic halo around them. Large areas of dead, dry and sometimes split tissue are produced from a frequent coalescence of these lesions. The pycnidia, which form in the infected tissue, are usually pale pink-brown in colour and difficult to see in the field even, by reflected light, with a hand lens. However, by viewing the lesions in transmitted light with a hand lens, pycnidia can be seen (Figure 7, picture 3). The symptoms in the ears are dark brown patches on the glume that then develop to become purple-brown. This symptom is easy to see on green spikes (AHDB; BASF) (Figure 7, picture 4).
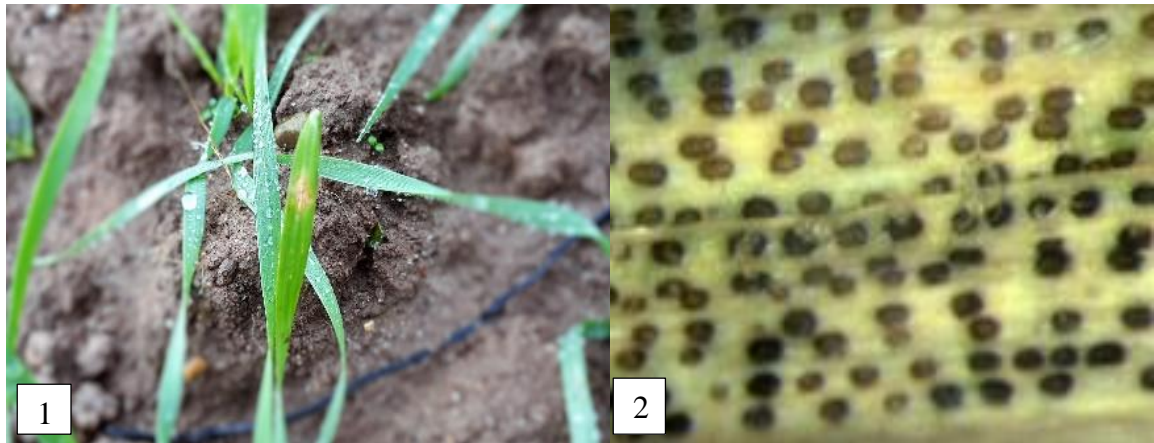
Figure 7, symptoms of (1,2) *Z. tritici* on seedlings in the field, (3,4) plant and spike infection with *P. nodorum*. All photographs are from winter wheat cv. M. Huntsman in a field experiment 2014-2015, Sonning Farm, University of Reading

1-3-2- Infection process:

1-3-2-1- *Zymoseptoria tritici*:

The dispersal of *Z. tritici* happenes on two scales: within a crop and between crops. Rain splash seems to be the factor that contributes most to spreading the conidia within crops. A single rain splash may disperse spores up to 1m away although the number dispersing reduces exponentially with distance. It is hard to estimate the number of spores moved by rain splash, but it is unlikely to be large. Between crops, it seems to be that the infection happens by ascospores which are carried by wind. This happens both from one growing season to another, and within the same season (Shaw, 1999).

The sexual stage of *Z. tritici* has been identified in many regions (Brown, 1975 in Australia; Scott *et al.*, 1988 in UK; Verreet *et al.*, 1990 in Germany; Garcia & Marshal, 1992 in USA; Kema *et al.*, 1996a in Netherland; Halama, 1996in France; Glazek & Sikora, 1998 in Poland; Cordo *et al*., 1999 in Argentina; Hoorne *et al*., 2002 in Canada; Eriksen & Munk, 2003 in Denmark; Pastircak, 2005 in Slovakia; Ben Hassine & Hamada, 2014 in Tunisia). Pseudothecia, the sexual fruiting bodies, are produced within the lesion. The fungus is known to have a bipolar, heterothallic, mating system. The mating types, designated *mat1-1* and *mat1-2*, mate when they grow on the same leaf. Underneath the host epidermis, pseudothecia are then formed. They are globose, dark brown in colour and 68-114μm in diameter. Asci, which contain ascospores, measure 11-14×30-40μm. The eight ascospores encapsulated by each ascus are hyaline, elliptical, and 2.5-4 x 9-16 µm in size, consisting of two cells of unequal length (Ponomarenko *et al*., 2011). Eriksen & Munk (2003) established that pseudothecia contain between 19-45 asci (average 32). Assuming that all asci reach maturity this then gave a number of ascospores of around 250/pseudothecia. These can be viable for 1-2 weeks after discharging if stored in darkness, but for two days when exposed to the light (Brown *et al*., 1978).

The production of pseudothecia can be regularly observed both in natural and artificial conditions, but a long time after the appearance of pycnidia on the infected leaf (Eriksen & Munk, 2003). The estimated incubation period of pseudothecia was estimated by these authors as 46-76 days, and the latent period 63-99 days, in the field. However, Kema *et al*. (1996a) estimated the period of pseudothecia incubation under artificial conditions to be only 35 days, while Hunter *et al*. (1999) estimated 84-132 days in the field. Morais *et al*. (2016) suggested that the initial airborne infection is not the limiting factor of the initial epidemic of *Z. tritici*, since there was no effect of local wheat debris in two years' experiments, in spite of high inoculum pressure in a 2012/2013 experiment. This was also observed by (Shaw & Royle, 1989).

Pycnidia are the structures producing asexual spores (conidia). Pycnidiospores are approximately 2.6×62.5µm, and typically have 3-7 septa. Spores can germinate from lateral or intermediary cells. Many cycles of reproduction via the asexual stage can occur through the growing season of a wheat crop (Ponomarenko *et al*., 2011).

During the epidemic period, pycnidiospores are the main source of *Z. tritici* infection (Eriksen *et al*., 2001). The viability of pycnidiospores remains in dry weather for an extended period, and could be a source of initial infection of a succeeding crop (Suffert *et al.*, 2011). The pool of pycnidiospores which are produced within pycnidia are released as a result of rain. Pycnidiospore release is at its peak when the wetting of leaf first happens. This release becomes fewer and fewer with subsequent wetting and drying cycles. After a release event, mature pycnidia are not able to regenerate new pycnidiospores (Eyal, 1971).

Shaw (1991) investigated the effects of light and humidity on winter wheat. Infection from *Z. tritici* needed a long time with 100% of humidity to succeed, but this could be interrupted by periods at lower humidity (50%). The infection was more likely to be reduced in long periods of reduced humidity and in light than in dark. Similarly, a 20-hour period of high humidity led to successful infection (Dewolf, 2008).

Macroscopic symptoms do not appear during the first nine days of infection, except some dead cells, but after 10 days the mesophyll cells die quickly in susceptible cultivars (Cunfer, 1999). Kema *et al.* (1996) used ELISA in their study. The biomass of fungus was similar in resistant and susceptible cultivars in first 6 days after inoculation. After that, biomass increased rapidly in the susceptible cultivar used in the test, but scarcely in the resistant cultivar.

1-3-2-2- Parastagonospora nodorum:

Wheat crops can be infected by *P. nodorum* in different ways: through infected seed, which is considered as the usual initial source of inoculum; through ascospores; and through pycnidiospores, which are considered the dominant inoculum during crop development. Luke *et al*. (1986), in Florida State, USA, found that 10% of infected seeds were able to supply sufficient inoculum to result in severe epidemics.

Transmission of pathogen from seed to seedling, then to the plant canopy, requires suitable conditions. Holmes & Colhoun (1971) reported that under glasshouse conditions, at 12°C with dry soil, transmission of pathogen was lower than in wet soil at 8°C or 17°C, but still enough to cause an epidemic. Shah & Bergstrom (2000) found that, at all temperatures, transmission occurred to the coleoptile. However, 100% transmission to the coleoptile at 9°C was reduced to 72% when the temperature rose to 25°C. There was less transmission to the first leaf. This was reduced from 37% to 2%, by increasing the temperature from 9 to 25°C. This means when the plant grows slowly at low temperatures the fungus population multiplies clonally rapidly. Babadoost & Hebert (1984) studied the incidence of *P. nodorum* in wheat seed and its effect on plant growth and grain yield in North Carolina, USA, and found that the proportion of seedlings with infected coleoptiles increased with the proportion of infected seed. As a result, seedling growth and grain yield were reduced. The development of pycnidia in diseased coleoptiles occurred shortly after planting the seed in the autumn, with lower leaves becoming infected in the spring. These authors also found that virulent fungus survived in stored seed for more than two years. Pycnidiospores can germinate in free water, usually present since exudation of pycnidia occurs in rain or dewy days (Eyal *et al*.,1987). Within a temperature range between 5-37°C germination can occur; the optimum range of temperature to germinate is between 20-25°C. In the laboratory, germination of pycnidiospores occurred two hours after their emergence from the pycnidia (Scharen & Krupinsky, 1970). Infection by *P. nodorum* occurs optimally at 22-24°C, and the symptoms appear after 7-14 days. High relative humidity (RH) is required for successful infection. In Wales, for example, infection occurred when the relative humidity was more than 63%. The latent period (period from inoculation to the production of mature pycnidia) can be achieved in 10 days at 22°C but can be shorter (6 days after inoculation) when the plants are kept in a continuously water saturated atmosphere. When plants were kept at 20°C with 12 hours of complete saturation alternating with 12 hours at 85-90% RH, the latent period was extended to ten days (Shearer & Zadoks, 1972).

Penetration may occur in a direct way, through cuticle, or an indirect way, through stomata. Penetration through stomata could be faster than direct penetration (Bird & Ride, 1981; O'Reilly &

Downs, 1986). Many studies have shown the enzymes secreted *in vitro* from *P. nodorum,* such as amylase, xylanase and pectin methyl esterase (Magro, 1984; Lehtinen, 1993). Baker & Smith (1978) found less penetration and hyphal development within leaves in resistant cultivars than susceptible cultivars. In same study, in all cultivars, the hyphae had disappeared from surface wax. Bird & Ride (1981) also observed that germ tubes grew slowly in resistant cultivars. Resistance of the host might relate to enzyme sensitivity (Magro, 1984). Faris (2016) suggested that specialist necrotrophic pathogens such as *P. nodorum* use necrotrophic effectors to exploit pathways often associated with resistance to biotrophs. Also the genetic variation in the Wheat-*P. nodorum* system appears mostly to involve host gene-necrotrophic effector interactions; nine have been identified. Oliver (2016) reported that the discovery of PtrToxA xenologue in *P. nodorum* ushered in a period of rapid improvement in understanding of the host-pathogen interaction. It has been established as a new behaviour that this pathogen exerted pathogenicity by producing a number of necrotrophic effectors that induced a defence-like a reaction in host germplasm carrying otherwise paradoxical sensitivity genes.

The dispersal of *P. nodorum* spores by rainfall has been researched. The dispersal of *P. nodorum* spores by rain was investigated by Griffiths & Hann (1976) in spring wheat by using funnel traps above the crops. They found that rainfall dispersal of spores was effective at heights less than 40cm and distance of 50cm between plants. Another experiment done by Wale & Colhoun (1979) found the highest dispersal was 2 m and the distance was more than 0.9m. These results show the important of droplet splash by rain in causing severe epidemics in infected crops, in favourable conditions. Wind contributed greatly to increasing the dispersal of smaller droplets and spores in the downwind direction (Eyal *et al*., 1987).

1-3-3- Interaction of *Z. tritici* and *P. nodorum* on the same host

Wheat crops can be infected by more than one pathogen. In epidemiology, the interaction of pathogen populations is rarely considered. Many pathogens can be present at the same time on a tiller of winter wheat (Weber *et al*., 1994). The attack of pathogens may occur one after another, depending on the environmental conditions and the aggressiveness of each pathogen. When a plant is infected by one pathogen it may become susceptible to another pathogen (Yarwood, 1959). Changes in pathogen prevalence and population dynamics could be strongly affected by presence of another pathogen in the host.

There are several pathogens causing foliar diseases in wheat, for instance *Zymoseptoria tritici* and *Phaeosphaeria nodorum.* The interaction of these two pathogens in past studies has shown complicated results. In one of these studies, done by Jones & Odebunmi (1971) in a glasshouse, two spring wheat cultivars at the heading stage were inoculated with suspensions containing different proportions of spores, but at the same concentration (10^6 spore/ml). After two weeks the total disease was assessed, but the species were not scored separately. Total disease was reduced at high *Z. tritici* proportions. Highest severity of disease occurred when *P. nodorum* was inoculated alone, and lowest when *Z. tritici* was inoculated alone. Harrower (1978) also studied, using a similar design, the interaction of *Z. tritici* and *P. nodorum* on three wheat cultivars. The disease was assessed at different leaf stages. It was found that *Z. tritici* produced more spores but less severe symptoms than *P. nodorum*. In addition, Harrower (1978) suggested that *P. nodorum* was more destructive than *Z. tritici*, causing faster necrosis.

Another study showed the interaction between these pathogens is isolate-dependent (Nolan *et al.,* 1999). She also found the interaction might be antagonistic or beneficial, depending on the combination of the isolates studied. The results from field and growth chamber experiments showed that more spores of *P. nodorum* were produced when both pathogens were present together. This study suggested that *P. nodorum* inhibits *Z. tritici*, because the dual inoculation treatments reduced the spore production by *Z. tritici*.

Chandramohan (2010), studied the interaction between *Z. tritici* and *P. nodorun* in glasshouse conditions, using different concentrations of each pathogen. She found the symptoms of *Z. tritici* were reduced by *P. nodorum* at high concentrations. Low concentrations of *Z. tritici* increased the symptoms of *P. nodorum* and reduced *Z. tritici*, but with high concentration this did not occur.

1-3-4- The factors that affect pathogen progress:

1-3-4-1- Weather factors:

Rainfall and temperature are important for a number of reasons for both pathogens. Rainfall is important to disperse pycnidiospores; there is no spore germination without humidity; and moisture plays a role in asci growth to produce ascospores which then are spread by wind.

The size of droplets is important in determining which carry spores. Brennan *et al*. (1985) reported that most spores are carried by droplets over 1mm diameter. Another study by Fitt *et al*. (1989) showed droplets between 0.2 to 1mm carried the largest proportion of spores. The differences between results depend upon the weather conditions at the time when they did these studies.

Spores of both pathogens required similar optimal temperatures to germinate, between 20-25°C (King *et al*., 1983). There is a good relation between humidity or temperature and survival of spores, especially of *P. nodorum*, which was found to survive for 24 hours when humidity was at 80% and temperature 20°C (King *et al*., 1983). Another study by Beyer *et al*. (2012) studied spring air temperature to account for the bimodal temporal distribution of *Zymoseptoria tritici* epidemic in Luxemburg. They found the early epidemic was between 180- 210 day after sowing and at an average temperature between 12.4 – 14°C.

1- 3-4-2- Host resistance and escape:

Different factors may lead to resistant hosts; these factors can relate to plant physiology. One of these plant physiological factors is the height of plants. Taller cultivars have less disease on the upper leaves because spores spread by rain-splash reach the upper leaves (Lovell *et al*., 2001) inefficiently and the distances between leaves are longer. Semi-dwarf cultivars, with higher yield potential, have shorter distances between lower and upper leaves, especially the flag leaf. Thus, spread of pycnidiospore by rain-splash between leaves will be efficient. Consequently, pycnidiospores will appear earlier and more severely on the upper leaves of semi-dwarfs in comparison with taller cultivars (Eyal *et al*., 1987).

Mixtures of wheat cultivars can reduce disease. Cowger & Mundt (2002), over three year experiments, used four different wheat cultivars (moderately resistant and susceptible winter wheat cultivars) inoculated naturally with *Z. tritici,* and investigated the impact on disease progression under field conditions and the effect on pathogenicity. They found that severe epidemics and pathogen populations were reduced by 9.4% and 27% respectively, but only had one year's results.

Looking for specific resistance genes has increased, since *Z. tritici* populations developed resistance to fungicides (Fraajie *et al*., 2005; Mavroeidi & Shaw, 2006; Curvers *et al*., 2015). Many resistance genes to *Z. tritici* have been found and some of these are already mapped. Named genes run from *stb*1 to *stb*12. One of these genes, widespread in commercial cultivars, is *stb*6, demonstrated to have a gene-for-gene relationship with a pathogen locus (Brading *et al*., 2002). The resistance to *P. nodorum* is quantitative and manifested by reduced symptoms or delayed symptom expression, rather than by immunity. So in this state, the host may produce morphological changesor biochemical enzymes which defend but do not kill the pathogen (Goodwin, 2012). Transfer of genes to toxin insensitivity could give better resistance to *P. nodorum*. This pathogen produces host-selective toxins that act on wheat lines harbouring corresponding toxin sensitivity genes. The host-selective toxin ToxA is produced by this pathogen, and in wheat Tsn1 on chromosome arm 5BL confers sensitivity to ToxA. The Tsn1 gene has been cloned and appears to be a chimer generated by fusion of two other genes (Faris *et al*., 2010).

1- 3-4-3- Effect of nutrient:

It has been observed that nutrient supply can play an important part in increased or decreased susceptibility of wheat to pathogens, especially *Z. tritici*. Simón *et al.* (2003) studied the effect of nitrogen fertilization on *Z. tritici*. In conducive seasons they found that severity of disease was increased by nitrogen fertilization. They demonstrated also that high nitrogen fertilizer may produce short internodes in some cultivars, making the leaf layers closer together and so increasing the disease severity due to rain-splashed spore movement (Simón *et al.*, 2003). Shaw (1999) found the susceptibility of wheat to *Z. tritici* increased with increasing rates of nitrogen, but the susceptibility to *P. nodorum* was reduced.

Mann *et al.* (2004) studied the effect of foliar applications of potassium chloride on the upper wheat leaves against septoria disease. They found that the area infected by *Z. tritici* on leaf 2 was reduced by 50% in field experiments. In the same study, but by using scanning electron microscopy, they found that germination of conidia was inhibited by potassium chloride. Deliopoulos *et al.* (2010) compiled evidence that 34 types of inorganic salts were able to reduce 49 kinds of fungal species in 35 species of plant.

1-3-4-4- Other factors:

There are other factors, such as agricultural management, regional conditions, and infection time that could affect the pathogens' prevalence and severity. Eyal *et al*. (1987) reported that using rotations of 3-5 years decreased the incidence of *Z. tritici*, and 2 years' rotation with treated seed reduced *P. nodorum*. Bailey *et al.* (2001) found that rotation in general did not greatly reduce the severity of wheat septoria disease, but some diverse rotations such as cereal, pea, and flax - especially wheat after pea - reduced populations of *Z. tritici*. Schuh (1990) concluded that disease severity of *Z. tritici* was increased greatly by conventional tillage. Rodgers-Gray & Shaw (2000) studied use of straw and manure soil amendments. They found *Z. tritici*, *Erysiphe graminis* (powdery mildew), *Puccinia recondita* (brown rust), and *Fusarium* spp. (foot rot) were all reduced within the plots that received straw, but *Z. tritici* was not reduced by straw in an outdoor pot experiment.

The importance of different forms of inoculum can be modified by regional conditions. Pycnidiospore survival and viability on wheat debris depend upon the environmental conditions. The effect of wheat debris in Mediterranean area cannot be predicted easily (Suffert *et al*., 2011). In two Iranian regions pycnidiospores of *Z. tritici* were found to be the main source of primary inoculum, based on studies of the distribution of clonal haplotypes (Abrinbana, 2010).

1-4-Role of nutrients in regulating plant diseases.

Nutrient supply plays an important role in plant growth. Fertilizer is one of the most important sources of plant nutrition. Crops may be suffering from a shortage of some elements in soil, so providing nutrients such as fertilizer helps to provide adequate nutrition for crops. Furthermore, the reduction in the amount of elements of the soil that might happen with repeated cropping can be prevented by adding fertilizers. Sources of about twenty elements (macro and micro elements) are required for wheat growth. Macro elements are required in a kg/t amount and include nitrogen (N), potassium (K), sulphur (S), and phosphorus (P), whereas micro elements are required in very small amounts (g/t). They include, among others, copper, manganese, iron, boron and sodium (Gooding & Davies, 1997). Many studies have shown that inorganic fertilizer; such as silicon, chloride, potassium and phosphates have the ability to fight diseases (Mann *et al*., 2004; Kettlewell *et al*., 2000; Fauteux *et al*., 2005; Deliopoulos *et al*., 2010). In addition, the effect of sulphur as sulphur emission and sulphur dioxide on some diseases has been studied (Khan & Kulshrestha, 1991; Khan *et al*., 1998; Bearchell *et al*., 2005; Shaw *et al*., 2008). Walters & Bingham (2007) reviewed the evidence on how development of diseases caused by various pathogens was increased or decreased with applied fertilizers. With nitrogen treatment, disease was decreased in 168 cases and increased in 566. With phosphorus, 82 cases were decreases and 42 increases. With potassium treatment 144 cases were decreases and 52 were increases. With sulphur, 11 cases of diseases were reduced and 3 increased (Huber & Graham, 1999).

1-4-1- Sulphur:

Sulphur is an essential element that plays an important role in plants through its role in structure and function of proteins and co-enzymes (Kopriva *et al.*, 2009). There are different sources of soil sulphur including atmospheric deposition, fertilizer, parent rock, and biomass decomposition. Sulphur deposition comes from combustion of fossil fuel as sulphur dioxide, plants, and fertilizers, which are the greatest sources of sulphur applied to the land (Figure 8). In soil, sulphur can occur in both organic and inorganic forms. Organic sulphur is the largest part of the total sulphur in soil. Inorganic sulphur in the soil is relatively rare, but this is the major supply of sulphur to the roots. Plants can uptake sulphur as sulphate, SO_4^{2-}, and this is the most important source of S to plants. Atmospheric sources of S also can be used by aerial parts of plant. Uptake and regulation is dependent on demand; when sulphur is sufficient the absorption by roots is down-regulated, but deficiency results in rapid intake (Blak-klaff *et al.*, 2000).

Deposition of sulphur from the atmosphere into the soil can occur in two different ways: wet and dry deposition. Gaseous emissions include sulphur dioxide and hydrogen sulphide from activity of volcanoes. These combine with water in the atmosphere to form acid rain, which is wet deposition. Subsequently, sulphur dioxide oxidation occurs Other sources, such as applied fertilizer including combined sulphur and the residues of crops, contribute to supply of sulphur. Acid rain can transfer to a wide area from its sources; this is due to the sulphur dioxide mixing with water in the upper troposphere and then being carried great distances (Fowler *et al.*, 2007). After deposition sulphur is exposed to transformation in different ways that may make it available to uptake by plants or lead to leaching. Industrial sulphur emissions led to increased sulphur dioxide in the atmosphere and this in turn contributed to high rainfall acidity (Neftel *et al.*, 1985). Sulphur emission was at its peak due to huge consumption of fossil fuel without removal from exhaust gases from the mid-19th century until the late 20th century (Bearchell *et al.*, 2005). Zhao *et al.* (2003) studied the soil and crop sulphur content in long term experiments on Broadbalk at Rothamsted since 1845, showing sulphur deposition from the atmosphere was related to national emissions. Sulphur dioxide in the atmosphere - which was then formed to the sulphuric acid in rainfall - provided much of the sulphur available to crops.

Inputs: **atmosphere deposition (wet, dry)** **fertilizers / manures** **crop residues**

S

Adsorption / desorption / oxidation / reduction / mineralisation /

Transformation:

Losses: **leaching** **crop uptake**
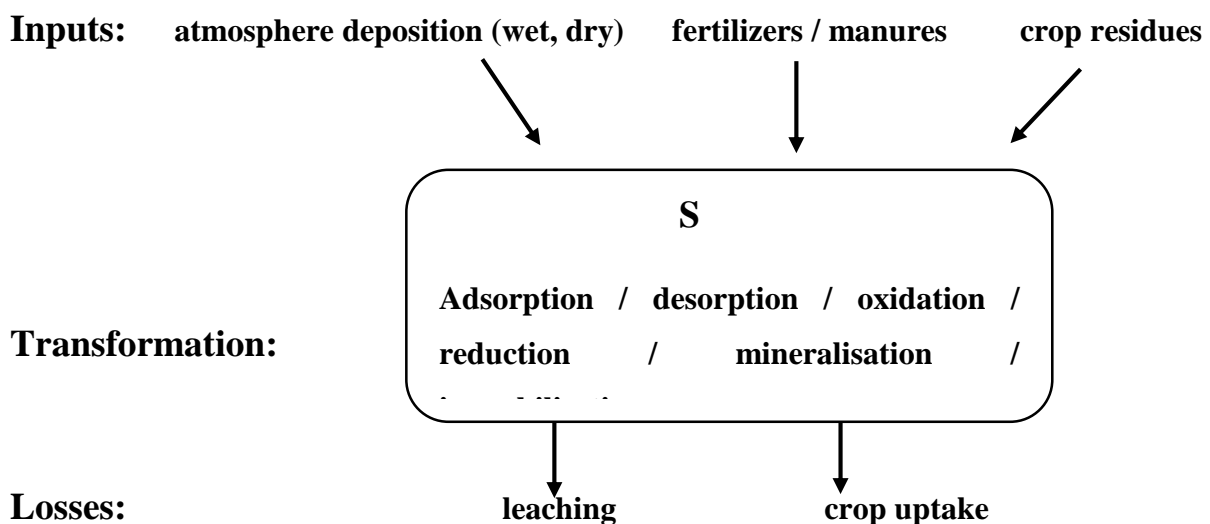
Figure 8. Sulphur cycle (reproduced from Chandramohan, 2010).

A lack of sulphur in soils in many regions of the world has become increasingly important as shown by surveys in those areas, such as the UK and Germany, which showed a decreased sulphur concentration in grains of wheat. These soil shortages are due to various causes including the use of fertilizer alternatives containing no sulphur, and a decline in deposition of sulphur dioxide

emission from atmosphere (Figure 9). Deficiency of sulphur in the UK was first detected in oilseed rape crops, then after a few years developed in other crops (Chalmers *et al.*, 1999). The decline in fertilizers containing S is due to replacement of ammonium sulphate with ammonia or ammonium nitrate to achevie high production of wheat (Gooding & Davies, 1997). Yield, quality and protein content cannot reach the potential amount in crops without adequate amounts of S, which also enhances nitrogen utilization (Sahota, 2006).
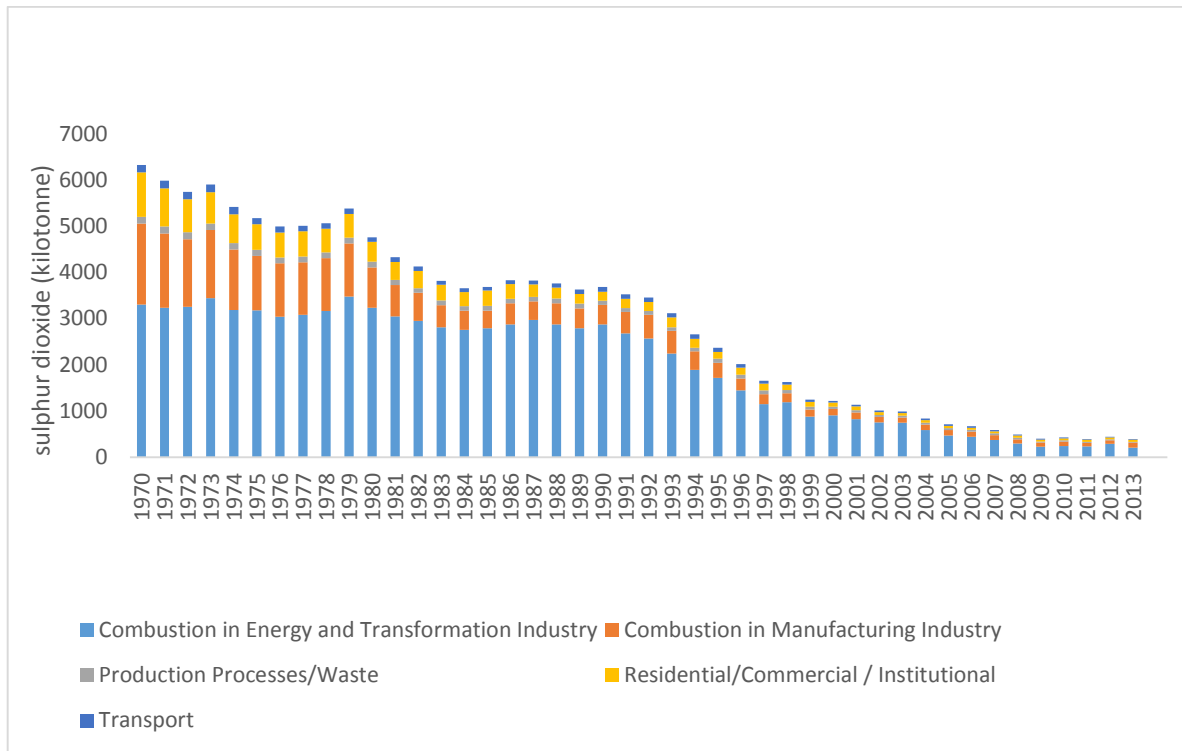
Figure 9. Sulphur dioxide emission from different resources in the UK (1970-2013). Reproduced from National atmospheric emission inventory.

1-4-1-1- Effects of sulphur on diseases

Sulphur has been known for many decades as one of the main elements that can inhibit or reduce infection by fungal diseases (Beckerman, 2008). The defence of plants against microbial pathogens is linked directly or indirectly with S containing compounds. There are many of these compounds, which include thionins, defensins, glucosinolates, phytoalexins, alliin and glutathione (Hell, 1997). Many different species produce phytoalexins; elemental S is the only inorganic phytoalexin. Many fungal pathogens are affected by the toxicity of S, including most ascomycetes and basidiomycetes, but not oomycetes or bacteria (Cooper & Williams, 2004).

Magan & McLead (1988) found the dominant mycoflora in sulphur dioxide fumigated plots was changed in different ways when they were compared with fungicide treatments. Khan & Kulshrestha (1991) reported that higher concentrations with longer exposures of sulphur dioxide inhibited spore germination of powdery mildew. Chandramohan & Shaw (2013) tested sulphurous acid at pH4 on two pathogens (*Z. tritici* and *P. nodorum*) and found that the percentage of conidia germination of *P. nodorum* was reduced more strongly than *Z. tritici*, while the mycelial growth of the two fungi in solution of sulphurous acid was similar.

1-4-1-2 Effects of sulphur on quality of wheat

Sulphur is an essential nutrient for plants because of its importance in the formation of protein: the amino acids cysteine and methionine contain sulphur (Gooding & Davies, 1997). Sulphur deficiency in wheat grain can be determined by the proportion of nitrogen to sulphur. A proportion of nitrogen to sulphur of 17 to 1 or higher indicates a lack of sulphur in the grain (Gooding & Davies 1997). Sulphur insufficiency in crop plants damages not only the growth of crops and grain yield, but also quality of the product, especially bread-making quality (Ryant & Hřivna, 2004). Protein plays a key role in the process of bread-making. Mature grain contains 8-20% protein: the gluten proteins which are gliadins and glutens constitute up to 80-85% of the total protein in the flour, and provide extensibility and elasticity, which are two of the properties necessary for bread flour (Kuktaité, 2004).

Sulphate is the main non-protein compound of sulphur in each of the stem, leaves and (in the stage of grain development) ears, while glutathione is the most important compound at the synthesis stage of sulphur-containing protein in the grain. The differences in sulphur concentration due to high or low sulphur fertilization became apparent after the milky stage of grain maturity. Steinfurth *et al*. (2012) found that late sulphur fertilization increased the concentration of S in flag leaf within a short period of about 2 weeks at ear emergence. It also prevented insufficiency of S in late stages

of wheat growth. Furthermore, it enabled more equal concentrations of S, glutathione and protein in all wheat organs, compared to application of S at sowing.

1-4-2- Silicon:

Silicon (Si) is the second most abundant element in the soil (Liang *et al.*, 2007; Richmond & Sussman, 2003). It is one of the minerals that has been shown to reduce incidence of disease caused by a number of pathogens. Silicon has been shown to lead to accumulation of phytoalexins and consequent signalling cascades in both monocot and dicot plants. In elicited cells silicic acid might act locally in inducing defence reaction. The production of stress hormones that induce systemic resistance is contributed to by silicon in the form of silicic acid. Thus, the performance of Si could be as an activator of strategic signalling proteins or as a potentiator of plant defence responses. Several key components of the plant stress signalling system might therefore interact with silicon to induce resistance against pathogenic fungi (Fauteux *et al.*, 2005).

1-4-2-1- Role of Silicon in plant resistance:

Si fertilisation has been reported widely as an important way of improving yield and quality of rice, sugarcane and wheat. Silicon fertilizers have been used with rice for many years in order to improve yield (Savant *et al.*, 1997). Liang *et al.* (1994) reported wheat yields raised by 4.1-9.3 % in calcareous paddy soils. In addition, silicon fertilization has been shown to increase resistance to biotic stress (Ma, 2004). Silicon fertilisation in some plant species can alleviate the toxicity of Mn (Jŭnior *et al.*, 2010), and increase resistance to salinity and drought stress (Fauteux *et al.*, 2005). Rizwan *et al.* (2012) concluded that less cadmium could be found in wheat shoots when the concentration of soluble Si was increased in the soil. Rodgers-Gray & Shaw (2000) showed silicon fertilizers could increase the resistance of wheat grown in pots to a number of pathogens, especially powdery mildew. DeCamargo *et al.* (2013) studied the effect of silicon fertilizer in four different types of soil on rust disease of sugarcane in Brazil. They found that incidence of rust was influenced by both factors, but the silicon reduced the incidence of brown rust in all types of soil.

There are two mechanisms by which Si can enhance the resistance of plant to diseases. The first mechanism is that Si can act as a physical barrier. A double layer of Si-cuticle form is produced through deposition of Si under the cuticle. This can mechanically prevent fungi penetrating host cells and so disturb the process of infection (Fauteux *et al.*, 2005). However, a study by Kim *et al.* (2002) looked at the role of silicon in inducing cell wall fortification against blast disease in rice leaves in Korea. Plants treated with Si had only slight deposition in stomatal guard cells. The second mechanism by which Si may enhance resistance against pathogens is that soluble Si may

modulate host resistance. Many studies done in monocots (rice and wheat) and dicots (cucumber) have shown that some plants produce phenolic and phytoalexins when Si is supplied (Fawe *et al.*, 1998; Rodrigues *et al.*, 2004). Si can also activate other mechanisms of defence. For example, Chérif *et al.* (1994) found that when roots of cucumber supplied with extra Si were infected by *Pythium* spp, the activity of peroxidase and polyphenoloxydases increased rapidly and chitinase activity rose as well.

1-4-2-2- Silicon transport and accumulation in plants:

Silicon is the second most abundant element in soil after oxygen, but the form that plants can uptake is monosilicic acid $[Si(OH)_4]$ (Ma & Takahashi, 2002) which is often scarce. Si is taken up by roots, then translocated to the shoot by xylem. Chemically, silicic acid polymerizes to silica gel when the concentration exceeds more than 2mM, but the concentration of Si in xylem sap was higher than 2mM in rice and wheat. In spite of this, the Si form in xylem sap has been identified as monomeric silicic acid in both these plant species (Mitani *et al.*, 2005; Casey *et al.*, 2003). Concentrations of silicon accumulated in the shoot differ depending upon the plant species, with a range from 0.1% to 10% Si on a dry weight basis (Ma & Takahashi, 2002; Hodson *et al.*, 2005). Mechanisms of Si uptake differ between plant species. One study by Mitani & Ma (2005) used rice, cucumber and tomato. They found that the concentration of Si was 3 times higher in rice than in cucumber and 5 times higher than in tomato. In another part of this study they studied the transport of Si from cortical cells to xylem (xylem loading). The concentration of Si in xylem sap was 20 times higher in rice than in cucumber and 100 times higher than in tomato. The lower accumulation in cucumber and tomato may be explained either by the lower density of transporters to transport the Si from the external solution to the cortical cell or the absence of transporters to transport from the cortical cell to the xylem.

1-4-3- Potassium:

Potassium is a common element. It plays an important role in metabolism and water utilization by controlling the osmotic and salt balance (Gooding & Davies, 1997). Potassium can reduce the severity of disease caused by both biotrophic and nicrotrophic pathogens (Walters & Bingham, 2007).

1-4-3-1- Potassium in soil and plant:

Potassium plays an essential role in metabolism and in enabling phloem transport and stomatal movement (Kifkafi *et al.*, 2001; Scanlan *et al.*, 2015). The importance of K^+ is not only for optimal growth of the plant, but also for plant adaptive sensing of its environment (Anschütz *et al.*, 2014). Crops vary widely in K concentration, depending on crop species, location and soil. Askegaard *et al.* (2004) reported the K concentration in plants to range between 0.4-4.3%; Őborn *et al.* (2005) stated a narrower range (<2.5-3.5%). The critical range of K concentrations in dry matter is between 0.5-2.8%. The maximum concentration is in the range 3-10% in many crops (Leigh & Wyn Jones, 1984). The differing root structures in different plant species such as length, rooting depth, and root density may cause different absorption of K by different plant species. Jungk (2001) reported positive correlations between uptake of K in K-depleted soil and root density or root length in maize, tomato, and oilseed rape. Høgh & Pederson (2003) had similar results in red clover, barley, rye, and pea.

Most agricultural soils have a range of K as a total content of the 0.2m of upper surface between 10-20g/kg. A high percentage (90-98%) of soil K is combined with the crystal lattices of minerals, so this form is not available for plant to uptake. In addition, soil type plays an important role in K availability to plants due to the effect of physio-chemical properties of soil. Thus, applied potassium fertilizer in agricultural soil is often required to provide adequate soluble K to crops. Potassium fertiliser consumption is expected to rise from 30.1 million tonnes in 2013 to 34.4 million tonnes in 2018 (Figure 11) worldwide. Consumption depends upon the geographic region, with East Asia the largest consumer of K fertilizer (Figure 10) (FAO, 2015). Nowadays, potassium chloride is the form of fertilizer that is mostly used. There are other products such as potassium sulphate and potassium nitrate which are available commercially, but potassium chloride is cheapest. One application of potassium is usually adequate, applied at sowing or before.
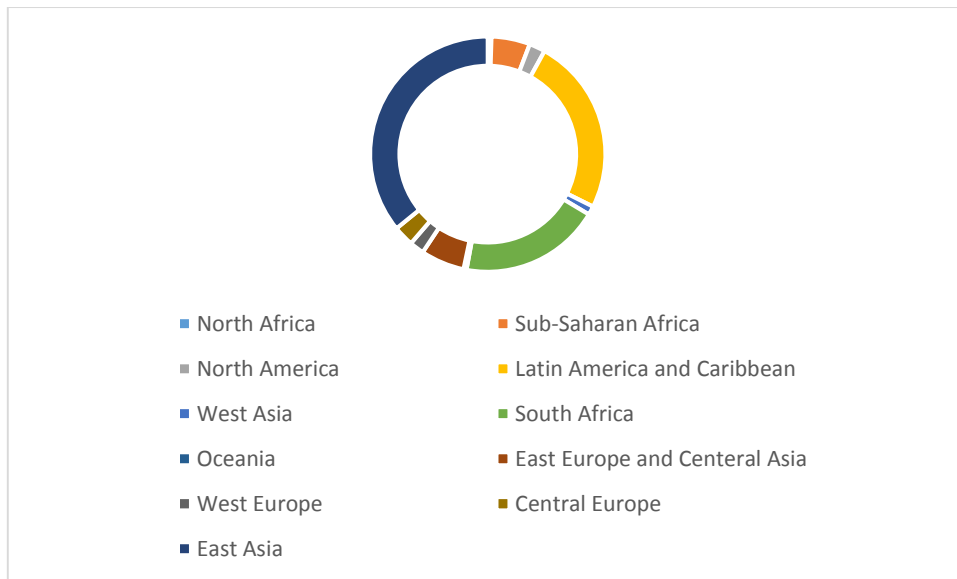
Figure 10. Percentage of K fertilizer used in each region of the world. (FAOSTAT, 2015).
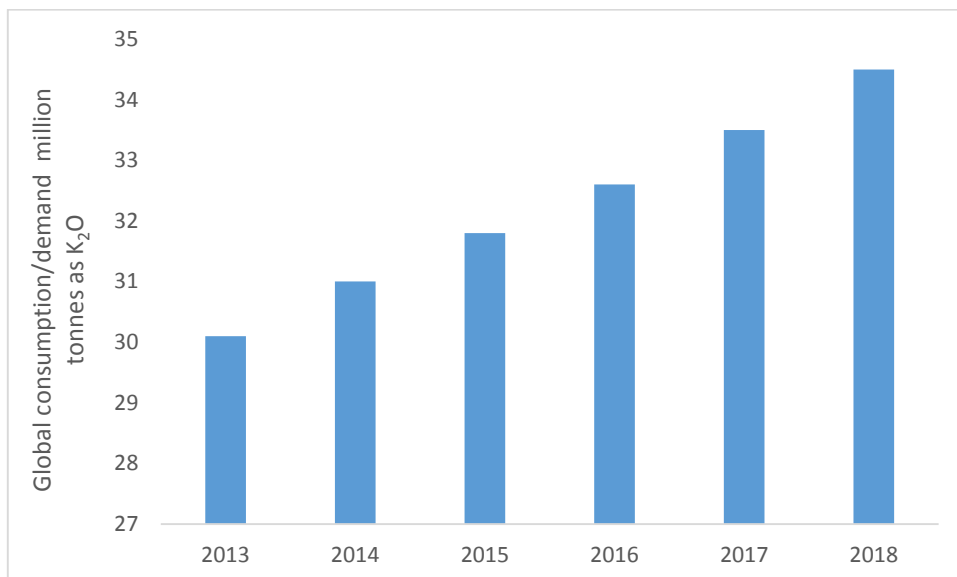
Figure 11. Global potash fertilizer consumption. FAO (2015).

The soil structural forms of K are mostly muscovite (potash mica or common mica), biotite and feldspars. K-feldspars may release K directly to soil solution (Zörb *et al.*, 2014). Singh & Goulding (1997) studied the changing of potassium concentrations with time in Broadbalk samples from 1856-1987. They suggested that plant–available K in soils may be produced from secondary soil minerals which are produced from the primary mineral parent by weathering. Sheng (2005) found the potassium content was increased by 30% in cotton and 26 % in rape which were growing in soils treated with both insoluble K and inoculum of bacteria *Bacillus edaphicus*. Basak & Biswas, (2009) found that the percentage of K recapture was increased in soil inoculated with bacteria (*Bacillus mucilaginosus*) and mica.

1-4-3-2- Impact of potassium on fungal diseases in plants:

Deficiency of potassium (K^+) in plants may lead to increased susceptibility to infection. For example, *Helminthosporium sigmodeum* is reduced in rice when potassium is applied (Ismunadji, 1976; (in Walters & Bingham, 2007)), and infection by rust, powdery mildew and take-all on wheat is likewise reduced ((Kovanci & Colakoglu, 1976; Boquet & Johnson, 1987) in Walters & Bingham, 2007)). Sweeney *et al*. (2000) reported that the severity of rust (*Puccinia triticina* Eriks.) decreased when potassium fertilizer was used with red winter wheat in central and southern Great Plains. They also found potassium fertilizer increased the grain weight and yield. Kettlewell *et al.* (2000) found that the spore germination and leaf area infected by powdery mildew were reduced by potassium chloride and polyethylene glycol, due to the osmotic effect on spore germination.

1-4-4- Calcium:

The production of wheat tends to reduce soil pH, and because use of some fertilizers, such as ammonium nitrate and urea, leads to increased pH applications of calcium nitrate are required to rectify the pH. The ideal pH for most wheat varieties is between 6.2-6.8. Within this range of pH, many plant nutrients are available and the toxic elements are maintained in insoluble forms. Soil type determines the necessary amount of lime to alter pH. A rise in pH by 0.5 can be obtained in sandy, medium loam, and heavy or organic soils when 1.6, 2.5, and 4 t/ha of lime respectively are applied (Gooding & Davies, 1997).

1-4-4-1- Calcium in soil and plants:

Calcium abundance in the soil is relatively high, and calcium is about 3.6% of the earth's crust. Gypsum, amphibole, and pyroxene are the forms of calcium present in soil minerals. Calcium is a positively charged ion (Ca^{2+}), and is held on soil clay and organic matter more tightly than magnesium (Mg^{2+}), potassium (K^+), and other exchangeable cations. Soil is usually formed from parent material which contains much more calcium than magnesium and potassium. Thus, large amounts of exchangeable calcium (soil calcium available for root uptake and leaching) (300-5000ppm) can usually be found in the soil. The concentration of exchangeable calcium in the forest soils of Europe and North America has decreased over the past decades ((Rodhe *et al.*, 1996) in Lawrence *et al.*, 1999)). This trend is mainly due to leaching by acidic deposition in regions with severe air pollution (Wesselink *et al.*, 1995).

Calcium is an essential plant nutrient, it has a structural role of cell wall and membranes and is an intracellular messenger in the cytosol ((Marschner, 1995) in White and Broadley 2003)). Plants can uptake calcium from the soil solution through the root and then transport it to the shoot via the xylem. Calcium may be crossing the root either through the symplast or the apoplast. To prevent

the accumulation of toxic cations in the shoot, control the rate of Ca delivery to the xylem and allow root cells to signal using cytosolic Ca^{2+} concentration, the movement of calcium through these pathways must be finally balanced (White & Broadley, 2003).

Calcium (Ca^{2+}) plays a vital role in physiology and biochemistry in all organisms (Nomura & Shiina, 2014). At high concentrations, calcium is toxic to the cell, including interference with nucleic acid metabolism. Eukaryotic cells therefore, translocate excess Ca^{2+} from their cytosol to organelles and extracellular compartments to maintain the very low free concentrations of Ca^{2+} in cytoplasm (~100 nM). The concentration of Ca^{2+} in the apoplast is considerably higher than cytoplasmic levels, more than 1 mM. Changes of cytoplasmic Ca^{2+} concentration are recognized by Ca^{2+} sensor proteins and activate downstream signal transduction pathways (Ca^{2+} signalling) (Nomura & Shiina, 2014). The level of free Ca^{2+} concentration in plant mitochondria is maintained less than 100nM, similar to the level in cytosol.

Currently it is unclear if the Ca^{2+} concentration and patterns in its oscillation between organelles transfers information capable of determining specific downstream responses. The alternative is that Ca^{2+} influx simply acts as a binary switch that, when the concentration of Ca^{2+} reaches a threshold, activates Ca^{2+} binding proteins (Chen *et al.*, 2015), while specificity is provided by parallel mechanisms (Seybold et al., 2014). In the plant immune system, Ca^{2+} dependent protein kinases (CDPKs) have emerged as a crucial sensor of Ca^{2+} (Romeis & Herde, 2014). For instance, CDPKs were rapidly activated when tobacco leaves were treated with the fungal elicitor Avr9 (Romeis *et al.*, 2000; Romeis *et al.*, 2001). When bacterial flagellin22 (flg22) was applied in *Arabidopsis,* several CDPKs (CDPK44, 5, 6 and 11) were stimulated, and use of Ca-channel inhibitors showed that this effect is Ca dependent (Boudsocq et al 2010). The overexpression of dominant active versions of CDPK3 and 11, and treatment with chitin octamers, indicate huge overlaps in transcriptional responses. These CDPKs might also be involved in innate immune responses to microbe-associated molecular patterns (MAMPs) (Boudsocq *et al.*, 2010).

1-5-Objectives:

Some studies have suggested there may be a causal link between sulphur and the relative abundance of *Z. tritici* and *P. nodorum*. Deposition of sulphur as sulphurous acid and sulphur dioxide is correlated with the abundance of these pathogens. In addition, it has an important role in improving yield quality. Furthermore, silicon has an effect on some diseases that infect crops; powdery mildew is one of these. Therefore, there is a need to test the effect of sulphur and silicon fertilisers on the septoria and powdery mildew disease complex under field conditions.

This project includes three main objectives.

1- Investigate the effect of sulphur fertiliser on *Z. tritici* and *P. nodorum*.
2- Investigate the effect of sulphur fertiliser on yield and quality of wheat under diseases pressure.
3- Investigate the effect of silicon fertiliser on pathogens, quality and yield of wheat crop.

1-6-Hypotheses:

1- Sulphur fertiliser reduces the combined abundance of these pathogens under field conditions.
2- Co-inoculation of *Z. tritici* and *P. nodorum* reduces their individual abundance compared to single infection.
3- Sulphur fertiliser will improve wheat yields and grain quality.
4- A- Sulphur fertiliser increases the proportion of *P. nodorum* in field plots with mixed infections of *P. nodorum* and *Z. tritici*.
   B- Silicon fertiliser increases the proportion of *P. nodorum* in field plots with mixed infections of *P. nodorum* and *Z. tritici*.
5- Silicon fertiliser reduces both these pathogens under field conditions; and improve wheat yield and quality.

Three field experiments were conducted to test these hypotheses, with associated lab work and technique development. Visual assessment, spore washing and qPCR as measures of the amount of pathogen were compared. The qPCR required development of extraction techniques suitable for field scale samples as well as optimisation of the assay.

CHAPTER TWO

Materials and Methodology

2-1- Glasshouse Experiment

This factorial experiment involved two pathogens (three local isolates of *Z. tritici* and one isolate of *P. nodorum*), two fertilizers (potassium sulphate and potassium chloride) and three wheat cultivars, with four replicates.

2-1-1- Wheat cultivars, compost, and fertilizers:

Three different wheat cultivars were used: cvs. Paragon as a spring wheat and two winter wheats (Gallant and M. Huntsman). Plastic pots 12 cm dimeter were used, filled with 1.2 L. of compost. The compost was three parts of John Innes type 2 compost (soil) with one part of perlite and 4g/litre of Osmocote (slow release nutrient as a source of NPK which includes N 12% as coated slow release, 12% of coated slow available phosphate P_2O_5, and 12% of soluble coated slow release potash K_2O). Five grains of wheat were sown at a depth of 2 cm in each pot. The plants were thinned to four seedlings per pot. The level of sulphaur fertilizer applied in this experiment was 60 kg/ha, 0.12g/pot (this amount fertilizer calculated according to the number of seedlings assumed in the field 200/m^2), of sulphur fertilizer, or 0.6 g/pot of potassium chloride. These amounts of fertilizers were dissolved in deionised water, and 10 ml of solution added to each pot. Pots were then irrigated approximately every 48 hours depending on the moisture content of the compost with an appropriate amount of water to avoid any through-draining.

2-1-2- Preparation of pathogen, suspension and spray:

Z. tritici isolates were provided by M. W. Shaw, isolated in autumn 2012 from the crops research unit fields at Sonning, Berkshire. *P. nodorum* was a gift from Syngenta, Jealott's Hill Research Station, UK. (Table 1).

Table 1. The source of each isolate that used in the experiments.

Isolate	Code	Date of origin	Source	Experiment
Z. tritici	St1	2012	Sonning farm, University of Reading.	Glass house, and field experiments
Z. tritici	St2	2012	Sonning farm, University of Reading.	Glass house
Z. tritici	St3	2012	Sonning farm, University of Reading.	Glass house
P. nodorum	K3428	1980	Syngenta, UK (1980).	Glass house, and field expeeriments

2-1-2-1- *Z. tritici* suspension:

Three isolates of *Z. tritici* (Table 1) were sub-cultured on potato dextrose agar (PDA) at 20 ^{o}C, with 12 h light and 12 h dark in an incubator for 2 weeks. Spores were then harvested into sterile deionized water by agitation with a glass rod.

Spore concentration was determined by using a haemocytometer. Table 2 shows the concentration of each isolate. The concentration applied to each pot was 1×10^6 spore/ml; 4ml spore suspension was used per pot. Spore suspension was sprayed on to the seedlings by a compressed air sprayer, then the seedlings were covered by clean polyethylene bags for 72 hours.

2-1-2-2- *P. nodorum* suspension:

The isolate of *P. nodorum* was sub-cultured on V8 juice agar and grown under 12 h UV light and 12 h in the dark. Then, after two weeks in an incubator with near UV illumination, spores were harvested into sterile deionized water by agitation with a glass rod. Spore concentration was determined using a haemocytometer (Table 2).

At GS 16 (6 leaves unfolded) plants were inoculated. After spraying, the seedlings were covered by clean polyethylene bags for 72 hours.

Table 2. Concentration of spores of each isolate that were sprayed on wheat seedlings.

Isolate	Spore concentration spore/ml
Z. tritici **1**	1×10^6
Z. tritici **2**	1×10^6
Z. tritici **3**	1×10^6
P. nodorum	1×10^6

2-1-3- Disease measurement:

At GS 25 (main shoot and 5 tillers) disease symptoms were assessed. Within this experiment, the proportion of infected leaves (through counting all healthy leaves divided by the leaves that showed visible symptoms) and proportion of dead leaves (number of leaves showing 100% senescence divided by the number of healthy leaves) were recorded.

2-1-4 Data analysis:

All data were analysed by GenStat 16th edition using general analysis of variance with a fully balanced randomized block design and three cultivars, two fertilizers and four isolates for the proportion of infected leaves as factors. Cultivar, fertiliser and four isolates and the unsprayed control was used for the analysis of percentage of dead leaves. The proportion of infected leaves was transformed to Log10 to get a normal distribution of the residuals.

2-2- FIELD EXPERIMENTS LAYOUT AND METHODOLOGY:

Three field experiments were done, in 2013, 2013-2014 and 2014-2015, to test the hypothesis. The design of each experiment was adapted according to the results from each previous experiment. All experiments were conducted in the research unit field at Sonning, University of Reading, Reading, Berkshire, UK. (Latitude 51.472929 °N; Longitude -0.904234 °W; 476202 °E; 175454 °S; Grid reference SU762754).

2-2-1- Cultivars:

Three wheat cultivars were used. The first field experiment was sown in spring and used cv. Paragon. Two winter wheat cultivars were used in the second field experiment, Gallant and M. Huntsman. In the third field experiment M. Huntsman was used. cv. Gallant was used as a typical modern winter wheat cultivar; M. Huntsman was very widely grown in 1979 and for a decade before that.

M. Huntsman seed was provided by Germplasm Resources Unit, John Innes Centre, and Gallant was provided by Syngenta, UK. For both wheat cultivars the seed was untreated.

In all experiments the grain was drilled with a Hege 80 plot drill, mounted on a Hege 76 tool carrier, and the row spacing was 12cm. The seed rate in all experiments was 350 grains per square metre.

Soil analysis showed in Table (3).

Table 3. Soil pH, N aavailability, and the availability of each of P., K. and Mg in the field experiments.

Experiment	Soil pH	N kgN/ha (available N in depth given)	P (mg/L)	K (mg/L)	Mg (mg/L)
2013	6.5		60.6	110	68
2013-2014	6.6	18.4			
2014-2015	5.9	1.5	24.2	66	62

2-2-2- Spore suspension and spraying:

2-2-2-1- *Zymoseptoria tritici*:

A spore suspension was prepared from *Z. tritici* 1 isolate used in the glasshouse experiment. The fungus was cultured on PDA (39g/l, Sigma Aldrich, UK), and incubated at 20°C for 3 weeks. 10 ml of sterilised distilled water was added to the culture plate, which was then scraped with a glass rod. A haemocytometer was used to count the number of pycnidiospores that were present in this suspension. The spore suspension was stored at -20°C until use, two weeks after storage. Before spraying the spore suspension on to the seedling, the spore suspension was tested on PDA agar plated. Then the suspension was adjusted to 1×10^4 spore/ml in 2013 and 2013-2014. In 2014-2015 no inoculation was used because natural infection had consistently been abundant.

An air pressure sprayer (12L, Hozelock Knapsack garden sprayer/Homebase/ UK.) was used to spray the spore suspension on seedlings at growth stage 23 (main shoot and 3 tillers) for the 2013 experiment, and growth stage 13 (3 leaves unfolded) for the 2013/14 experiment.

2-2-2-2- *Parastagonospora nodorum*:

Two ways were used to inoculate the crops with *P. nodorum*. Seed inoculation was used in 2013. In 2013-2014 the spore suspension was sprayed on the seedlings at GS13 as for *Z. tritici*. In 2014-2015 both ways were used.

Spore suspensions were prepared from a culture of *P. nodorum* (isolate 3428 Syngenta Agrochemicals, Bracknell, UK; see ch 2). The fungus was cultured on CDV-8 agar (200ml V8 vegetable juice, 42.4g Czapek Dox agar (Sigma Aldrich), 3g calcium carbonate, 10g nutrient agar (Oxoid) in 1000ml de-ionized water) under 12 hour UV illumination alternating with 12-hour darkness for a fortnight (Cook & Jones, 1970). To collect spores, culture plates were agitated with a glass rod after adding 10ml sterile distilled water. A haemocytometer was used to count the number of pycnidiospores present in the wash solution. The spore suspension was adjusted to 1×10^6 spore/ml. Seed inoculation was done as follows, and an air pressure sprayer used as in *Z. tritici* (Figure 12). Grain weight was calculated according to the plot area. 25g of grain were soaked in 1ml of spore suspension then dried in an incubator cabinet, then the grain was sown (Imathiu *et al.*, 2010). immediately.

Figure 12. Spraying spore suspension of *P. nodorum* on the seedling, field experiment 2014-2015.

2-2-3-Experimental layout:

The experiments were to investigate the effect of sulphur and silicon fertilizers on quality and yield of wheat under ambient disease pressure, largely without use of fungicide. In the final experiment specific fungicides were used to manage rust across the whole experiment and against *Z. tritici* in specific plots. Silicon fertilizer was used in 2013 and 2013-2014 only. In the first two experiments *Z. tritici* and *P. nodorum* were sprayed on seedlings in subsets of the plots. In the final experiment only inoculation with *P. nodorum* was done, but by two methods. Fertilizers and the amount of each compound are given in Tables 4 and 5. All experiments were done with three replicates in randomised complete blocks. Each main plot was surrounded by 2m of barley to avoid dispersal of spores.

Table 4. Type and amount of each fertilizer used in the field experiments.

Fertilizer	Compound amount
Potassium sulphate (K_2SO_4)	333.3 kg/ha, (=60 kg S/ ha)
Potassium chloride (KCl)	288.5 kg/ha
Calcium silicate ($CaSiO_3$)	400 kg/ha, (=96 kg Si/ ha)
Calcium hydroxide($Ca(OH)_2$)	254.8 kg /ha

In 2013 experiment cv. Paragon, a spring wheat cultivar, was used. This factorial experiment involved two pathogens as a main plot factor (*Z. tritici*, *P. nodorum* and *Z. tritici + P. nodorum*), and four alternative fertilizers (Table 4 and 5) as subplots. Potassium chloride was used as a comparison for potassium sulphate and calcium hydroxide as a comparison for calcium silicate. The size of each main plot was 10×3.8 m, divided into four subplots (Figure 13), and the size of subplot was 5×1.9 m. Sowing started on the 16th of April. 150kg/ha of nitrogen as ammonium nitrate was applied in a split dose pattern according to normal practice (Table 6).
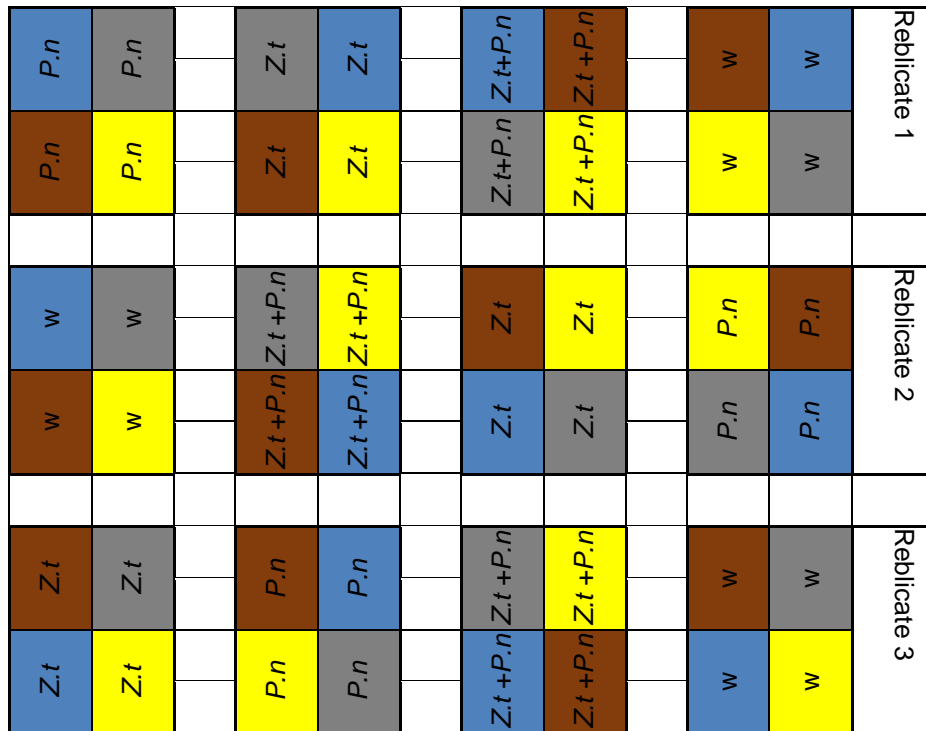
Figure 13. Field experiment layout in 2013. Spring wheat cv. Paragon was inoculated either with or without *Z. tritici* (*Z.t*) and *P. nodorum* (*P.n*), and co-inoculation and treated with one of four fertilizers. The colour of each sub-plot refers to a specific fertilizer. Blue refers to Calcium hydroxide; yellow to Calcium silicate; brown to Potassium sulphate; and grey to Potassium chloride.

In 2013-2014 M. Huntsman and Gallant, both winter wheats, were used (Figure 15). This factorial experiment involved factorial combination of two pathogens as the main plot factor (*Z. tritici*, *P. nodorum* and *Z. tritici + P. nodorum*), and each of four alternative fertilizers (Table 4) as subplots. All plots received potassium chloride except the plots treated with potassium sulphate, and all received calcium hydroxide except the plots treated with calcium silicate so as to balance potassium and calcium inputs across plots (Table 5). The size of each sub-plot was 10×1.9m. Seed was sown on the 1st October. 200 kg/ha of nitrogen and other fertilizers were added according to normal practice (Table 6). Due to the flooding (December till early February) in this season, which

most plots suffered from (Figure 14), the second application of fertilizer was applied again to reduce the effect of avoid leaching of sulphur fertilizer. Silicon fertilizer was applied once, on 5th of March (Table 5).

Figure 14. Field experiment picture 2013-2014, (1) field during flooding (2) field at late season after flooding.

Figure 15. Field experiment layout in 2013-2014. Winter wheat cv. Gallant and M. Huntsman were inoculated either with or without *Z. tritici* (*Z.t*) and *P. nodorum* (*P.n*), and co-inoculation, and treated with one of four alternative fertilizers (S was potassium sulphate; Si was calcium silicate; K was potassium chloride; Ca was calcium hydroxide). Each colour of main plot refers to cv.; green: cv. Gallant, yellow cv. M. Huntsman.

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In 2014-2015 the single cultivar M. Huntsman was used (Figure 16 and 17), because the earlier results showed that cv. M. Huntsman was less susceptible to yellow rust and sulphur fertiliser reduced *Z. tritici* within this cv. more than cv. Gallant. This factorial experiment involved one pathogen (*P. nodorum*) which was inoculated in two different ways, spray and seed inoculation. Plots were treated with and without sulphur fertiliser, but with more complex pathogen inoculations. Instead of *Z. tritici* spray inoculation, half the plots were treated with the fungicide tebuconazole (Table 4). The area of each main plot was 20×3.8m divided into two subplots Main plot factors were inoculation with *P. nodorum* and spraying with tebuconazole. The subplot factor was fertilizer. Sulphur fertiliser was applied four times (Table 5). 200kg/h nitrogen as ammonium nitrate was applied (Table 6). To avoid yellow rust and powdery mildew, the entire trial was sprayed with Comet and Flexity (Table 6).

						Reblicate 1
P.n 2+KCl	*P.n 2+KCl*	teb.+*P.n 2* + K₂SO₄	teb.+KCl	teb.+*P.n1* + KCl	*P.n 1 +* KCl	
P.n 2+ K₂SO₄	*P.n* 2+K₂SO₄	teb.+*P.n 2* +KCl	teb. + K₂SO₄	teb.+*P.n 1* + K₂SO₄	*P.n1 +* K₂SO₄	

						Reblicate 2
teb.+ K₂SO₄	*P.n2*+teb.+ K₂SO₄	*P.n 1*+teb. + K₂SO₄	w + K₂SO₄	*P.n 1 +* K₂SO₄	*P.n 2 +*KCl	
teb.+KCl	*P.n2*+teb .+KCl	*P.n1*+teb +KCl	w + KCl	*P.n 1 +* KCl	*P.n2 +* K₂SO₄	

						Reblicate 3
P.n1+KCl	w+KCl	*P.n 2 +* K2SO4	Teb+*P.n* 2 + KCl	teb.+ K2SO4	teb.+ *P.n* 1 + KCl	
P.n1+K₂SO₄	w + K₂SO₄	*P.n 2 +* KCl	Teb+*P.n2* + K₂SO₄	teb. +KCl	Teb+*P.n* 1 + K₂SO₄	

Figure 16. Field experiment layout in 2014-2015. Winter wheat cv. M. Huntsman inoculated either with two different ways of inoculation (*P.n* 1 was spray inoculation treatment; *P.n* 2 was seed inoculation treatment) of *P. nodorum* or without *P. nodorum* and sprayed or not with tebuconazole to control *Z. tritici*; subplots treated with or without sulphur fertilizer.

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Figure 17. Field layout aerial photograph of 2014-2015 experiment in July. Note the visible effect of sulphate fertilisation.

Table 5. Time and method of fertilizer application in each experiment:

experiment	Fertilizer	Date applied	Application method
2013	K_2SO_4	7/05/2013	Fertilizer was spread by machine
	KCl	7/05/2013	Fertilizer was spread by machine
	$CaSiO_3$	7/05/2013	Dispersed in 10 L water, spread on plot and the plant again washed with another 10 L water each plot.
	$Ca(OH)_2$	7/05/2013	Dispersed in 10 L water, spread on plot and the plant again washed with another 10 L water each plot.
2013-2014	K_2SO_4	28/11/2013 5/03/2013 12/03/2013 31/03/2013	Broadcast by hand
	KCl	28/11/2013 5/03/2013 12/03/2013 31/03/2013	Broadcast by hand
	$CaSiO_3$	3/03/2013	Dispersed in 10 L water, spread on plot and the plant again washed with another 10 L water each plot.
	$Ca(OH)_2$	12/03/2013	Dispersed in 10 L water, spread on plot and the plant again washed with another 10 L water each plot.
2014-2015	K_2SO_4	15/11/2014 6/01/2015 16/02/2015 6/04/2015	Broadcast by hand
	KCl	15/11/2014 6/01/2015 16/02/2015 6/04/2015	Broadcast by hand

Table 6. Date and details of agronomic treatments applied to the whole experimental area in each experiment

Experiment	Treatments	Treatment and date of application	Date
2012-2013	Drill	Discard areas between main plots were sown with Westminster spring barley	16/04/2013
	Nitrogen fertilizer	Ammonium nitrate 34.5% N at 75kg N per ha.	29/04/2013
	Nitrogen fertilizer	Ammonium nitrate 34.5% N at 75Kg N per ha.	31/05/2013
	Sprayed herbicide	Harmony, at 100g per ha in 220 L water.	17/05/2013
	Combine harvest		4/09/2013
2013-2014	Drill	Discard areas between main plots were sown with Flagon winter barley	8/10/2013
	Autumn herbicide	Crystal (flufenacet + pendimethalin) at 4 L per ha in 220 L of water.	26/11/2013
	Spring herbicide	Quantum SX at 30g per ha. in 220 L of water.	30/03/2014
	Nitrogen fertilizer	100kg N per ha as ammonium nitrate 34.5% N. 286kg ammonium nitrate/ha	31/03/2014
	Nitrogen fertilizer	100kg N per ha as ammonium nitrate 34.5% N., 286kg ammonium nitrate/ha	9/05/2014
	Combine harvest		1/08/2014

2014-2015	Drill	Discard areas between main plots were sown with KWS Meridian winter barley.	1/10/20104
	Autumn herbicide	Crystal 4L per ha. In 220 L of water.	25/10/2014
	Spring herbicide	Quantum SX 30g per ha. In 220 L of water.	17/03/2015
	Nitrogen fertilizer	100kg N as ammonium nitrate 34.5% N.	25/03/2015
	Fungicide	Comet (pyraclostrobin) ¼ rate 0.25 L per ha + Flexity (metrafenone)) ½ rate 0.25 L per ha, in 220 L water, against rust and mildew.	7/04/2015
	Fungicide	Fenpropimorph at 1 L per ha on all plots, Folicur (tebuconazole), against *Z. tritici* in designated plots.	20/04/2015
	Nitrogen fertilizer	100kg N per ha. As ammonium nitrate 34.5% N.	28/04/2015
	Combine harvest		7/08/2015

2-2-4- Disease measurement:

When plants were collected from a plot, a half metre was avoided from the edge of plot on all sides. Samples were then collected evenly along a zigzag path through the plot.

2-2-4-1- Disease measurements in 2013:

Disease measurements were taken at growth stage 31 (first node detectable), growth stage 59 (ears fully emerged), and growth stage 72 (milky ripe) on five plants collected from each plot. The number of leaves with obvious symptoms, after the completely dead leaves were removed, and the total number of leaves were recorded. At GS 31 and GS 59 the percentage of non-green leaf area was measured using win-DIAS 3.2 software, by selecting the chlorotic and or necrotic area manually. An attempt was made to measure total spore density, as follows. Spores were washed from plants by soaking each plant individually (stem and leaves) in a solution of 100ml of deionized water, 0.1 % of Tween 20 and 5ml ethanol. The jar was shaken intermittently for two three times. It turned out that spore concentrations were too low to measure, because 100 ml of deionized water per stem was too much.

2-2-4-2- Disease measurements in 2013-2014:

Within this year disease measurements were made on 10 plants taken at growth stage 31 and growth stage 59 from each plot. The number of leaves >50% green was recorded, and the number of those leaves that showed obvious symptoms.

A conical flask was used for spore washing by putting into it all plants from each plot that were used for visual measurement, and adding 100ml of deionized water containing 0.1 % Tween 20 and 5ml ethanol. Flasks were then shaken for 2-3 minutes and left overnight. The next day, the conical flask was shaken, the suspension poured into a 100ml Duran bottle, and the suspension then stored in a fridge at 4°C until the spores were counted using a haemocytometer.

2-2-4-3- Disease measurements in 2014-2015:

Ten plants were collected from each plot. Disease measurements were taken at growth stage 31, 59 and 77. The number of leaves >50% green was recorded, and the number of those leaves that showed obvious symptoms. Spore washing was performed as in 2013-2014. In addition, severity of disease, chlorosis and necrosis was measured using graph paper by taking the total number of squares occupied by the leaf and then recording the number of squares occupied by chlorotic or necrotic symptoms. The severity was measured by the ratio of the number of squares with symptoms and the total number of squares occupied by the leaf. For spore washing, the same procedure was used as in 2013-2014.

2-2-5- qPCR sampling and assay 2013-2014 season:

2-2-5-1- Collection of samples:

All leaves from five plants were used in this assay, collected at growth stage 59 from two out of three replicates only. The leaves were put it in paper bags, small hole was made, and then the bags stored at -20°C overnight. Leaves were freeze-dried for 48 hours, then milled (FRITSCH, Glen Creston Ltd. Stanmore, London, UK), and the powder stored at -20°C till use.

2-2-5-2- DNA extraction:

A sub sample of 0.02g from the freeze-dried sample was used. To make finer powder this subsample was put in a tissueLyser II (QIAGEN, UK). A DNeasy Plant Mini kit (QIAGEN) was used to extract the DNA following the manufacturers instructions. Bulk DNA concentration was measured using a Nano-Drop spectrophotometer (Thermo scientific, UK.).

2-2-5-3- qPCR assay:

Fungus isolated from 100mg of pure culture of *Z. tritici* was used to make a standard DNA concentration series. Samples were diluted to 10ng/ µl and stored at -20°C until use. Syber-green was used to quantify the amount of *Z. tritici*. Primers were as designed by Selim (2009), targetting a 60-bp fragment of the β-tubulin gene (Gen bank accession no. EF418622): 5'-CCTCGCCGAACTTACGATCT-3' and 5'-CGCGGACTTCCTTTCCTTG-3'.

2-2-5-4- Quantification of *Z. tritici*.

Fungal DNA was quantified by real-time PCR. A 20µl final reaction volume was used with 10 µl 2x Hi-Rox SYBR-green, and 0.4 µl forward primer 0.4 µl reverse primer, 4 µl DNA sample, and 5.2 µl molecular grade water. A holding stage of 95°C for 4 minuteswas followed by 40 cycles of 95°C for 15 sec. then 60°C for 20 sec. Finally, a melting curve was followed. A non-template sample control (NTC) and standard DNA for each fungus was included in each qPCR assay, all in triplicate. Data were initially processed by Step-one plus, Ver. 2.3 (Applied bioscience, UK.) software.

2-2-6- Yield and yield quality:

Total yield, Sodium dodecyl sulphate (S.D.S), hagberg falling number (H.F.N), sulphur concentration in dry weight of flour and in whole plants (S DW%), nitrogen concentration in dry weight of flour (N DW%), ratio of nitrogen concentration to sulphur concentration (N/S), and specific weight were measured in all three experiments. Harvest index (HI) was measured in the first two experiments.

2-2-6-1- Harvest Index (HI)

Two rows on each side of a 50 cm measuring stick were cut at ground level. This was replicated four times in each plot. Ears were separated from the straw, then dried in the oven at 80 ^{0}C for 48 hours. Weights of ears and straw was determined. Ears were threshed and the dry grain weighed again. HI was calculated as (dry weight of grain) / (total dry weight of crop).

2-2-6-2- Total yield

To prevent the contamination of each plot with another, approximately 0.5m around each plot was harvested and discarded. Then the length and width of each plot was measured. This remaining area was harvested. The grain for each plot was weighed, and then the yield was calculated according to the plot area.

2-2-6-3- Moisture content in grain and specific weight

A chondrometer was used to measure the specific weight. Sinar (serial number 200; model 6060, version 7) was used to measure the moisture content in the grain.

2-2-6-4- Thousand grain weight (TGW)

250 grains were counted and weighed. The total of four samples was taken to get the TGW.

2-2-6-5- Hagberg falling number (H.F.N.)

A sample of grain was taken then milled. A subsample of flour was weighed then dried in an oven at 80 ^{0}C for 48 hours and dry weight of flour recorded. The amount of fresh flour required for this test was calculated by this equation: (Moisture content $\times$ 0.1018g) + 5.4912g, or 7g when the moisture content was 15%. 25ml of deionised water was measured into a viscometer tube, and the calculated amount of flour added. The tube was shaken vigorously 20 times to obtain a homogeneous suspension, and then placed in water bath at 100 °C. The suspension was stirred for 60 seconds. The stirrer was then released at the top of the suspension; the falling number is the time taken, in seconds, for the stirrer to reach the bottom of the tube.

2-2-6-6- Sodium dodecyl sulphate (S.D.S.)

As the details mentioned in H.F.N., the required amount of fresh flour was obtained from this equation: (Moisture content $\times$ 0.0873g) + 4.7067g, or 6g when the moisture content was 15%. 50 ml deionised water was added to the test cylinder (volume 100 ml), then the flour. This was immediately shaken vigorously for 15 seconds. At 2min and 4 min, the cylinder was again shaken for 15 seconds. At 4min, after shaking, 50ml SDS reagent was added to the cylinder. The cylinder was inverted four times and again inverted four times at 8, 10 and 12 min. The cylinder was allowed to stand for 20 minutes. SDS value was recorded as the volume of visibly sedimented flour in ml.

2-2-6-7- Sulphur and nitrogen analysis (S% DM and N% DM)

Leco S (SC 144-DR) and Leco N (FP-328) machines were used to measure the sulphur and nitrogen content of fresh flour, by taking 0.2g of fresh flour milled from the grain, then following the manufacturer's instructions.

2-2-7- Sulphur analysis in leaves and stem:

Five plants were collected from each plot at growth stage 59 (ears fully emerged) in 2013-2014. In 2014-2015 plants were collected at two growth stages: the first at growth stage 31 and the second at growth stage 77. All roots were removed from the plant. The above-ground plants were then dried in an oven for 48 hours at 80°C. At growth stage 31 the samples were milled with a Culatti hammer mill (serial number 220309). At growth stage 77 a knife mill was used (Glen Creston P15 knife mill, Glen Creston Ltd., 16 Dalston Gardens, Stanmore, Middx, UK.). After this step the concentration of sulphur was determined by a Leco S machine as before (SC 144 DR).

2-2-8- Silicon analysis:

Plant and soil samples were used to determine silicon as monosilicic acid (H_4SiO_4), because this is the form that plants take up. Five plants were collected from across each plot, dried at 80°C for 48 hours, then ground to powder with a knife mill (P15 knife mill, Glen Creston Ltd., 16 Dalston Gardens, Stanmore, Middx, UK.). Soil samples were three cores (15 cm depth) from across a plot, kept in in a fridge in polyethylene bags with air expelled by hand until use. Before use soil samples were mixed well, dried at 80°C for 48 hours, and then passed through a 2mm sieve.

The digestion method followed was as below, using the oven-induced wet digestion method according to Kraska (2009).

100 mg of plant sample and 20 mg of soil sample were used to measure monosilicic acid, three replicates of each from each field sample.

Samples were weighed in a 50ml polyethylene screw-cap centrifuge tube. 5 drops of octyle-alcohol were added to reduce foaming. The sample was then wetted with 2ml of 30% H_2O_2, run down the side of the tube. All tubes were capped tightly and transferred to a convection oven at 95°C for 30 minutes. Then 4ml of 50% NaOH was added to the hot samples. The tubes were gently vortex mixed and put again in an oven at 95°C for four hours. All samples were removed from the oven and 1ml of 5-mM NH_4F added to form monosilicic acid. They were then quantitatively diluted to a final volume of 50-ml deionised water.

Si concentration was determined using molybdenum blue colorimetry described by Hallmark *et al.* (1982). A 15ml polyethylene screw-cap was used. A 1ml aliquot of diluted sample digest was taken. Six ml of 20% acetic acid were added, then two ml of 0.3 M ammonium molybdate. The tube was capped and shaken well. After five minutes one ml of 20% tartaric acid then 0.2ml of reducing solution were added then capped tightly. After 30 minutes the tubes were shaken well, then absorbance determined by spectrophotometer (Jascop, V-530, UV/VIS spectrophotometer, Oak Industrial Park, Chelmsford Rd, Dunmow CM6 1XN, UK.) calibrated at 650 nm.

A reducing solution was made according to Wei-min *et al.* (2005): 2g of Na_2SO_3 and 0.4 g of 1-amino-2-naphthol-4-sulphonic acid were dissolved in 25 ml of deionised water and mixed with 25 g of $NaHSO_3$ dissolved in 200 ml of deionised water. After mixing and the solution was adjusted to 250 ml with deionised water.

Standard solutions of Si were made by diluting the stock solution of Si1000ppm (Sigmaaldrich, UK.) to give solutions with 0, 2, 4, 6, 8 and 10 ppm of Si.

2-2-9- Titanium analysis:

A titanium assay was carried out to estimate plant contamination with adhering soil that happened due to taking the sample without effective washing. Titanium is present in a low concentration in soil but should be almost completely absent from plant tissues. Assays of plant and soil therefore show how much passive physical contamination of plant samples occurred. Analysis followed the steps below, according to Cook *et al.* (2009).

0.1 g of plant or 0.02 g of soil materials were weighed in 10 ml Erlenmeyer tubes. Seven ml of a mixed solution of 10:1 HNO_3: H_2SO_4 were added. Tubes were capped, transferred to a convection oven and heated at ~ 100°C for 5 hours. Samples were then diluted with 10 ml of 5% trace metal grade HNO_3, then filtered through #2 Whitman filter paper. Samples were diluted 1:5 in 2% nitric acid, 5 ppb rhodium solution, and Ti determined by ICP-MS (ICAP Q, serial number 02092R, Thermo Scientific, UK).

Standard solutions of Ti were diluted to 5, 10, 20, and 50ppb. Each standard was taken from a stock solution of Ti and diluted in 2% nitric acid including 5ppb rhodium to get 50ml standard solution.

CHAPTER THREE

INFECTION OF WHEAT IN GLASSHOUSE CONDITIONS BY *Z. tritici* AND *P. nodorum* ISOLATES, WITH AND WITHOUT SULPHUR FERTILIZER:

3-1-Introduction:

This experiment was done to investigate the different wheat cultivars intended for use in field experiments, to investigate whether available isolates of *Z. tritici* and *P. nodorum* were suitable for use in field experiments, and to test whether there was any effect of sulphur fertilization on disease produced by these isolates.

Currently, wheat cultivars grown are semi-dwarf wheat. These are more likely to be infected by *Z. tritici* than taller cultivars, due to the greater splashing of spores to upper leaves by rainfall (Shaw 1999, Simón *et al.*, 2003). The infection process has important differences between *Z. tritici* and *P. nodorum.* Spore germination and host penetration are much faster for *P. nodorum* than *Z. tritici*, and this has a substantial influence on disease epidemiology. (DeWolf, 2008). These pathogens interact when they infect together. For example, there was a reduction of *Z. tritici* spore production when they were both found in the same host (Nolan *et al.*, 1999).

Wheat cultivars can be differently susceptible to infection by different isolates; on the other hand, the ability of pathogen isolates to infect particular wheat cultivars may also be variable. Such interactions between pathogen isolates and host genotypes have been reported widely. The *Stb* genes are defined as major genes conferring resistance to *Z. tritici* (Chartrain *et al.*, 2009; Ghaffary *et al.*, 2012). Brading *et al.* (2002) describe the relationship of wheat to *Z. tritici* as gene-for-gene: specific resistance genes responding to specific virulence genes in the pathogen. Pathogen virulence and resistance in the host connected through gene-for-gene relationships has been suggested in both bread (hexaploid) and durum (tetraploid) wheat (Yechilevich-Auster *et al.*, 1983; Eyal *et al.*, 1985; Eyal & Levy, 1987; Danon & Eyal, 1990; Kema *et al.*, 1996 b). Specific resistances to some isolates of *Z. tritici* have been detected in many wheat cultivars. Kema *et al.* (1996 b and c) discuss the cultivar Obelisk, a Dutch wheat cultivar which was resistant to *Z. tritici* in 1985 but about ten years later was susceptible, due to a shift in allele frequencies in the pathogen population. Arina is a wheat cultivar that is resistant to many *Z. tritici* isolates (Abrinbana *et al.*, 2012; Chartrain *et al.*, 2004).

Sulphur is an essential element in all organisms through its role in protein and co-enzymes (Kopriva *et al.*, 2009). Its availability can affect disease in wheat (Beckerman, 2008). As an element, it can be an effective fungicide against various pathogens such as powdery mildew, rose black spot, and rust (Beckerman, 2008).

3-2- Methodology:

Please see chapter 2.3-3-Results:

3-3-1- Proportion of infected leaves:

All cultivars were affected by all isolates which were used in this experiment. (Table 7). Please refer to Tables 2G-1 and A-1 (Appendix) for more detail.

Table 7. The back-transformed mean values and Log10 of the proportion of infected leaves from three different cultivars inoculated with one of three isolates of *Z. tritici* or one isolate of *P. nodorum* under glasshouse conditionst. P<0.001; df 95.

	Variety		
	Gallant	M. Huntsman	Paragon
Proportion of infected leaves	0.05	0.034	0.081
Log_{10} (Proportion leaves)	-1.313	-1.468	-1.089
S.E.D.of log_{10}(Proportion leaves)	0.06		

3-3-1-1 Effect of fertilizers on proportion of infected leaves:

Sulphur fertilization reduced the proportion of leaves by 40% averaged over plants inoculated with all isolates (and both species of the pathogen) (Table 8). Please refer to Table 2G-1 (Appendix) for more detail.

TTable 8. Back-transformed mean values and Log_{10} of the proportion of infected leaves from three different cultivars inoculated with one of three isolates of *Z. tritici* or one isolate of *P. nodorum* under glasshouse conditionst.   P<0.001; df 95.

Fertilizers	K_2SO_4	KCl
Proportion of infected leaves	0.042	0.062
Log10	-1.374	-1.206
S.E.D.of log_{10}(Proportion leaves)	0.05	

3-3-1-2- Isolates:

Averaged over hosts, there were significant differences between the isolates of *Z. tritici* and the isolate of *P. nodorum*. Within the *Z. tritici* isolates, there were no differences between isolates (Table 9). Please refer to Table 2G-1 (Appendix) for more detail.

Table 8. The back-transformed mean values and Log10 of the proportion of infected leaves inoculated with one of three isolates of *Z. tritici* or one isolate of *P. nodorum* averaged over cvs. Gallant, Huntsman and Paragon under glasshouse conditionst. $P<0.001$; df 95.

Isolates	*P. nodorum*	*Z. tritici 1*	*Z. tritici 2*	*Z. tritici 3*
% leaves	0.36	0.032	0.023	0.026
Log10	-0.446	-1.499	-1.637	-1.579
S.E.D.of $\log_{10}$(% leaves)	0.07			

3-3-1-3- Interaction between wheat cultivars and isolates:

The disease produced by isolates of the same species on each cultivar was similar, except that Paragon had more disease with *Z. tritici* 1 by 80% and 53% than with *Z. tritici* 2 and 3 respectively (Figure 18). Please refer to Table 2G-1 (Appendix) for more detail.
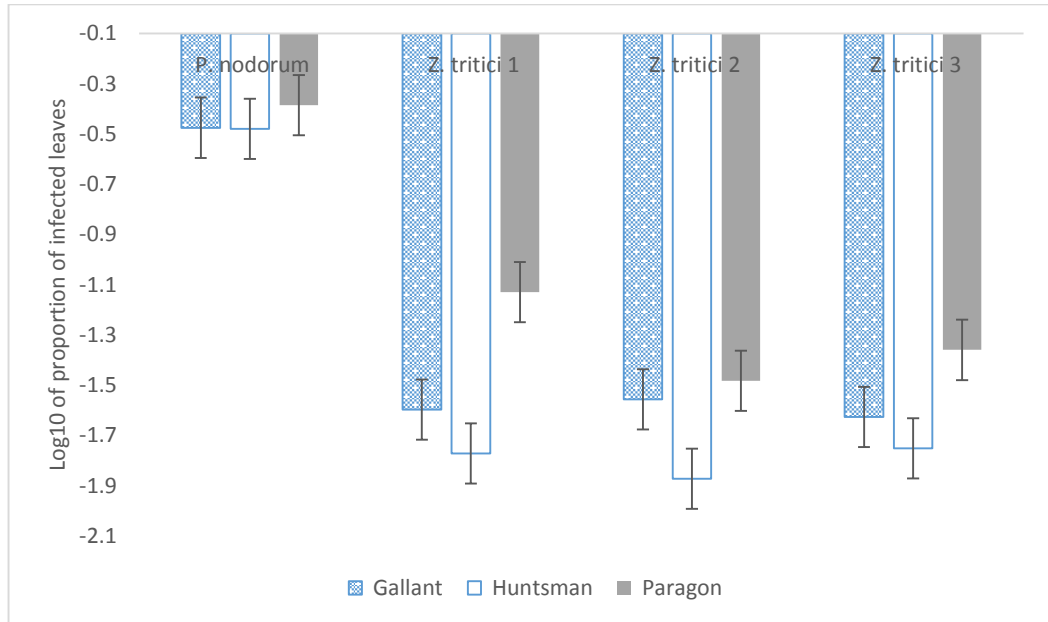
Figure 18. The interaction between isolates of *P. nodorum* and *Z. tritici* and wheat cultivars Huntsman, Gallant and Paragon under glasshouse conditions at GS 25. (Error bar = ± S.E.D, *P*. = 0.04, d.f. = 95).

3-3-2- Percentage of dead leaves:

Cv. Paragon had a greater percentage of dead leaves than Gallant and M. Huntsman. (Figure 19). Paragon was more susceptible to *Z. tritici* than Gallant and M. Huntsman, but similar to them in susceptibility to *P. nodorum*, so there were significant differences of interaction between isolates and wheat cultivars (Figure 20). Please refer to Table 2G-2 (Appendix) for more detail.
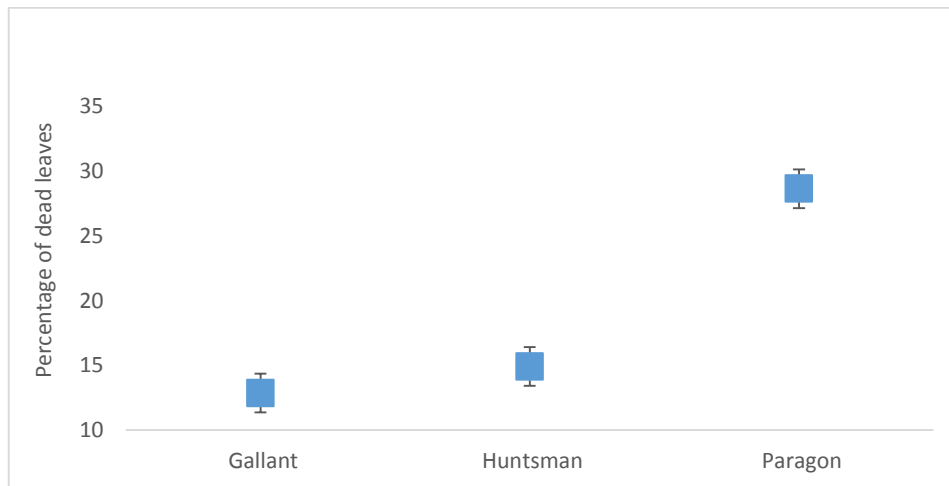
Figure 19. The percentage of dead leaves at GS 25 on wheat cultivars Gallant, Huntsman and Paragon averaged over inoculation and fertilisaer tratments. (Error bar = ± S.E.D, *P.* = <0.001, d.f. 119).
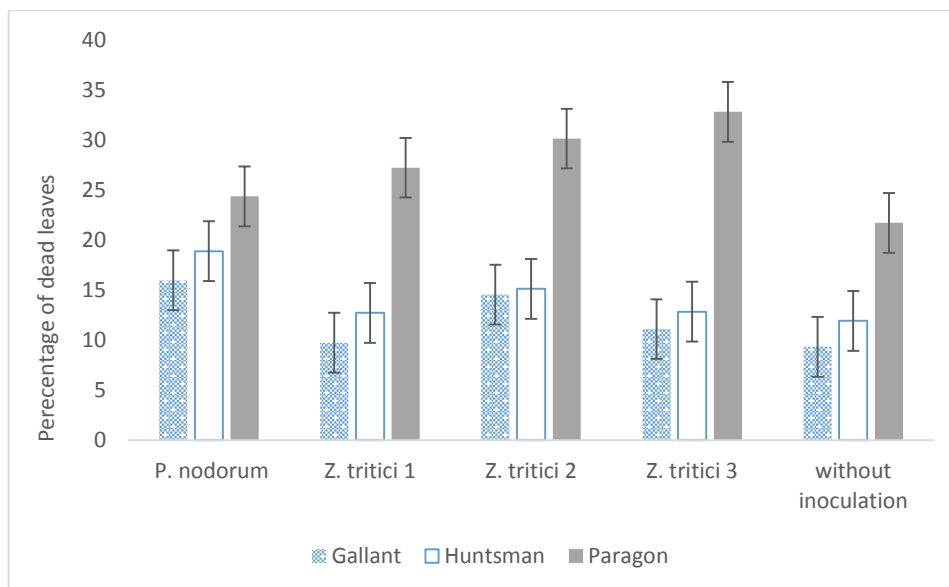
Figure 20. Percentage of dead leaves at GS 25 on wheat cultivars Gallant, Huntsman and Paragon and inoculated with one of three isolates of *Z. tritici* or an isolate of *P. nodorum*. (Error bar = ± S.E.D, cv. pathogen interaction *P* = 0.04, d.f. = 119).

3-4-Discussion:

The *P. nodorum* isolate was able to infect all wheat cultivars to an equal extent. All three *Z. tritici* isolates were able to infect all three cultivars, but there was some host-isolate specificity. Sulphur fertilizer reduced the proportion of infected leaves, which confirms the data of Chandramohan & Shaw (2013).

3-4-1- Wheat cultivars *and Z. tritici* isolates:

Wheat cultivars showed significant differences in response to the *Z. tritici* isolates. Although Paragon is stated to be slightly resistant to this pathogen (grade 5.6 according to AHDB (2012)) whereas Gallant is listed as a susceptible winter wheat (4.6 grade in AHDB (2013)). So Gallant under field conditions might be more susceptible than when growing in a glasshouse, and the reverse could be true for Paragon. Šíp *et al.* (2015) concluded that the resistance character was influenced by environment factors and not only the reaction of cultivars to isolates in controlled conditions. Similarly, M. Huntsman showed more resistance than the other two cultivars, but this was in glasshouse conditions, and might be different under field conditions. The interaction of the isolates and fertilizers on the proportion of infected leaves was not significant. This might be because the two different species are affected differently by fertilizers. Chandramohan (2010) showed that both pathogens reduced by sulphur fertilizer, but *Z. tritici* was reduced more than *P. nodorum*.

3-4-2- Percentage of dead leaves:

Paragon had a higher percentage of dead leaves than the other two cultivars, and the differences between it and the other two were much greater when inoculated with *Z. tritici*. Rapid death of leaves can restrict spore production and act as an effective field resistance mechanism. Paragon's greater leaf death in response to *Z. tritici*, but not *P. nodorum* could account for its resistant (AHDB, 2012).

CHAPTER FOUR

EEFECT OF SULPHUR AND SILICON FERTILIZERS ON DISEASE CONTROL.

4-1- Introduction:

As explained in the general introduction (chapter 1) sulphur deposition on agricultural land in the UK rose during the 19th and early-mid 20th centuries, before falling in the last 40 years with the introduction of stricter pollution controls. The abundance of *Zymoseptoria tritici* altered during the period 1850-2003. It was at a peak from the mid-1980s till now, while the abundance of *Parastagonospora nodorum* in the UK was at a peak in 1970 (Bearchell *et al.*, 2005). It has been suggested that atmospheric SO_2 decreased the abundance of *Zymoseptoria tritici* and increased *Parastagonospora nodorum*. Chandramohan & Shaw (2013) found both pathogens were reduced by sulphur under glasshouse conditions, but *Z. tritici* was more reduced than *P. nodorum*, which is consistent with this hypothesis. However, glasshouse experiments ignore plant-plant spread and multi-generational effects which occur in the field. The objective of this chapter was to investigate the effect of sulphur fertilization on disease, the abundance of these pathogens under field conditions and their co-inoculation. Silicon was initially included in the experiments because of its role in defence against biotic and abiotic stress, the repeated regular application of chemical fertilizers, such as nitrogen, phosphorus, and potassium to crops may deplete the amount of Si that is available to plants in the soil. So effect of silicon fertilizer on both pathogens was investigated.

This chapter presents disease results from these field experiments; yield and quality results are discussed in Chapter 5. The glasshouse experiment (Chapter 3) showed that wheat cvs. Paragon, Gallant and M. Huntsman were susceptible to all isolates of both pathogens *Z. tritici* and *P. nodorum*. In addition, sulphur affected disease susceptibility to both pathogens. Hence the effect of sulphur and silicon fertilizers on disease susceptibility of all cultivars needed to be tested for both pathogens under field conditions.

An initial experiment (2013) was carried out with spring wheat cultivar Paragon to investigate the effect of fertilizers on disease control, yield and quality. The second field experiment (2013-2014) was conducted with two winter wheat cultivars Gallant and M. Huntsman. M. Huntsman responded to sulphur fertilizer more than Gallant. The proportion of leaves infected with *Z. tritici* was less than in Gallant. A third experiment (2014-2015) to confirm and extend the results of 2013-2014

was carried out with winter wheat cultivar M. Huntsman only, because M. Huntsman gave higher green leaf area and less disease in plots treated with sulphur fertilizer, which led to improved yield and quality, and Gallant was very susceptible to current strains of yellow rust.

4-2- Methodology

See chapter two.

4-3- Results

4-3-1 2013 season (cv. Paragon):

4-3-1-1 Growth stage 31:

At this growth stage the seedlings had not been sprayed with *Z. tritici*, and no symptoms were observed. The symptoms of *P. nodorum* and *Blumeria graminis* were measured at this stage. Fertilizers did not have any detectable effect on the proportion of leaves having visible symptoms of either disease, or on disease severity (Table 10; refer to Tables 2F-1, 2F-2 and 2F-3 (Appendix) for more detail).

Table 9. Effect of fertilizers on proportion of leaves of spring wheat cv. Paragon that showed symptoms at growth stage 31 (first node detectable). *P. nodorum* and *B. graminis*.

Disease measurements at GS 31

Fertilizers	Proportion of infected leaves	Disease severity	Proportion of infected leaves
	P. nodorum	*P. nodorum*	*B. graminis*
$Ca(OH)_2$	0.13	3.4	0.61
$CaSiO_3$	0.08	2.4	0.58
KCl	0.09	3.3	0.62
K_2SO_4	0.07	3.4	0.43
S.E.D	0.05	1.6	0.15
P value	0.5	0.9	0.5

4-3-1-2- Growth stage 59, 2013:

The proportion of both plants and leaves infected with *P. nodorum* was reduced by sulphur fertilizer by 85% and 93% respectively, in comparison with potassium fertilizer. In addition, calcium silicate fertilizer reduced the proportion of both plants and leaves infected with *P. nodorum* by 25% and 42% respectively, in comparison with calcium hydroxide. Disease severity was reduced by sulphur fertilizer by 76% in comparison with potassium chloride fertilizer (Table 11; refer to Appendix Table 2F-4, 2F-5 and 2F-6 for more detail).

Potassium sulphate and potassium chloride reduced the proportion of plants infected with *Z. tritici* equally, by 58% and 62% in comparison with calcium silicate and calcium hydroxide respectively. The proportion of leaves infected with *Z. tritici* was reduced by potassium sulphate and potassium chloride fertilizers 69% and 65%; 76% and 73% in comparison with calcium silicate and calcium hydroxide respectively. There was no effect of sulphur fertilizer on disease severity (Table 11; refer to Appendix Table 2F-7, 2F-8 and 2F-9 for more detail).

The proportion of leaves infected with powdery mildew was increased by potassium sulphate fertilizer by 71% in comparison with potassium chloride (Table 11; appendix Table 2F- 10).

Table 10. Effect of fertilizers on proportion of infected leaves showing visible symptoms, and disease severity at growth stage 59 (Ear completely emerged). of *Parastagonospora nodorum, Zymoseptoria tritici* and Powdery mildew (*Blumeria graminis*).

Disease measurements at GS 59

Fertilizers	proportion of infected plant		Proportion of infected leaves			Disease severity	
	P. nodorum	*Z. tritici*	*P. nodorum*	*Z. tritici*	*B. graminis*	*P. nodorum*	*Z. tritici*
Ca(OH)$_2$	0.24	0.26	0.33	0.26	0.07	3.4	5.0
CaSiO$_3$	0.18	0.24	0.19	0.29	0.06	9.1	7.0
KCl	0.20	0.10	0.28	0.07	0.05	9.7	9.2
K$_2$SO$_4$	0.03	0.10	0.02	0.09	0.12	2.3	5.7
S.E.D	0.06	0.06	0.07	0.08	0.02	2.8	5.2
P. value	0.02	0.05	0.01	0.03	0.08	0.04	0.8

4-3-1-3- Effect of *Z. tritici* inoculation on proportion of infected leaves of *P. nodorum* at GS 59:

Inoculation with *Z. tritici* at the seedling stage reduced the proportion of leaves infected with *P. nodorum* at GS59 from 25% to 17% (Figure 21; refer to Appendix Table 2F-4 for more detail).
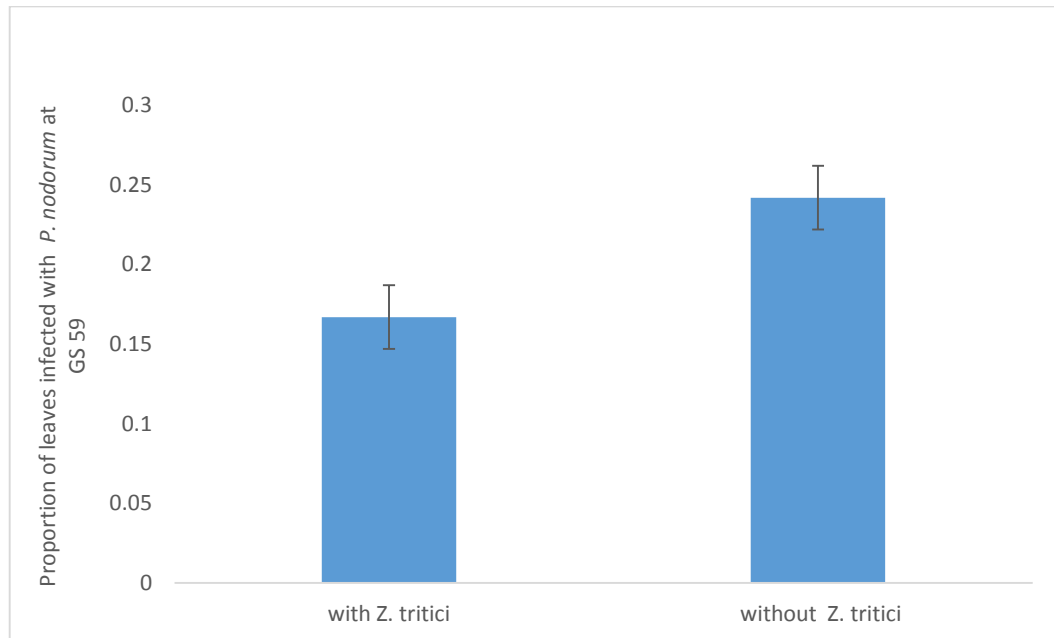
Figure 21. Proportion of leaves infected with *P. nodorum* at growth stage 59 (2013) in plots inoculated with or without *Z. tritici*. (*P* value = 0.05; Error bar = ± S.E.D, d.f. = 47).

4-3-1-4- Co-inoculation effect on proportion of infected leaves of *Z. tritici* at GS 59:

The proportion of leaves infected with *Z. tritici* was increased by Z. *tritici* inoculation, but it was less when inoculated with *P. nodorum*, whilst the proportion of *Z. tritici* was affected by *P. nodorum* inoculation in the treatments without inoculation of *Z. tritici*. (Figure 22; P = 0.024; refer to Appendix Table 2F-8 for more detail).
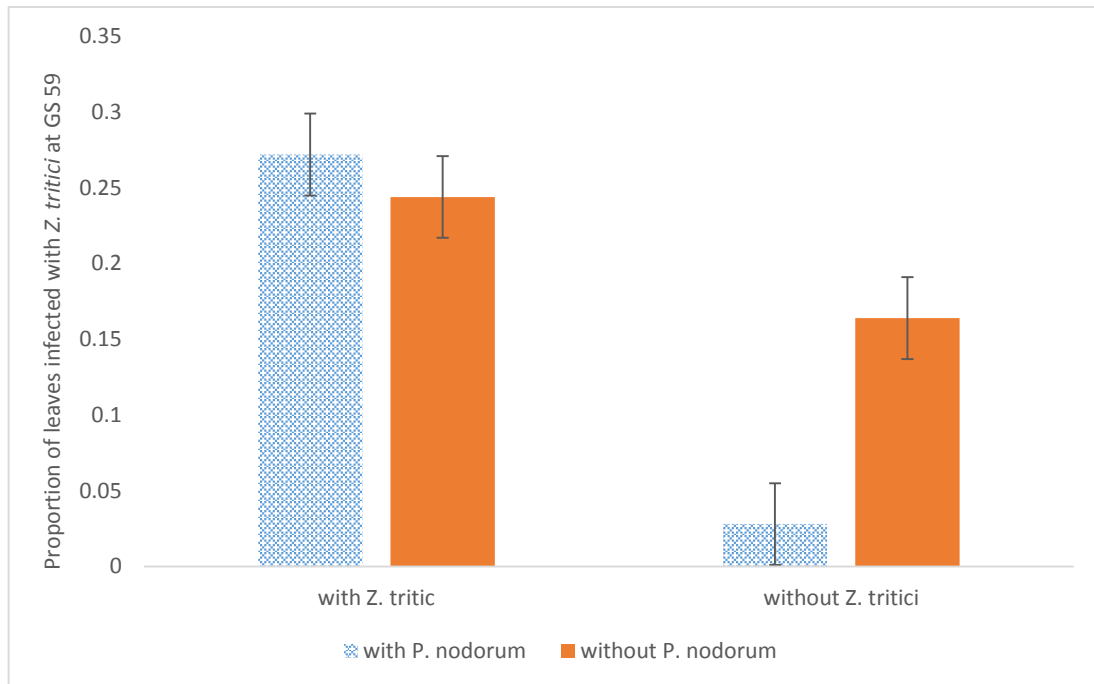
Figure 22. Co-inoculation effect of *Z. tritici* and *P. nodorum* on proportion of leaves infected with *Z. tritici* at growth stage 59 (2013 experiment on cv. Paragon). (*P*. value = 0.024, Error bar = ± S.E.D, d.f. = 47).

4-3-1-5- Growth stage 72, 2013:

The proportion of leaves infected with *Z. tritici* was, on average, similar in plots treated with calcium or potassium based fertilisers. However, Calcium silicate rather than calcium hydroxide reduced the proportion of leaves with symptoms of *Z. tritici* from 26% to 4%. Potassium sulphate increased the proportion of infected leaves from 3% with potassium chloride to 14% (Table 12; refer to Appendix Tables 2F-11 and 2F-12).

Table 11. Effect of fertilizers on proportion of leaves showing visible symptoms at growth stage 72 (early milk).of *Parastagonospora nodorum* (*P. nodorum*) and *Zymoseptoria tritici* (*Z. tritici*).

Fertilizers	Proportion of infected leaves	
	P. nodorum	*Z. tritici*
$Ca(OH)_2$	0.19	0.26
$CaSiO_3$	0.13	0.04
KCl	0.16	0.03
K_2SO_4	0.12	0.14
S.E.D	0.06	0.07
P. value	0.6	0.04

4-3-2- 2013-2014 season (cv. Gallant and M. Huntsman):

4-3-2-1- Growth stage 31, 2013-2014:

Gallant was more susceptible to Z. tritici than M. Huntsman measured by either spore concentration recovered (Figure 23, B; P = <0.001) or proportion of leaves with symptoms (Figure 23, A; P = 0.02). Please refer to Tables 2F-13 and 2F-14 (Appendix) for more details.
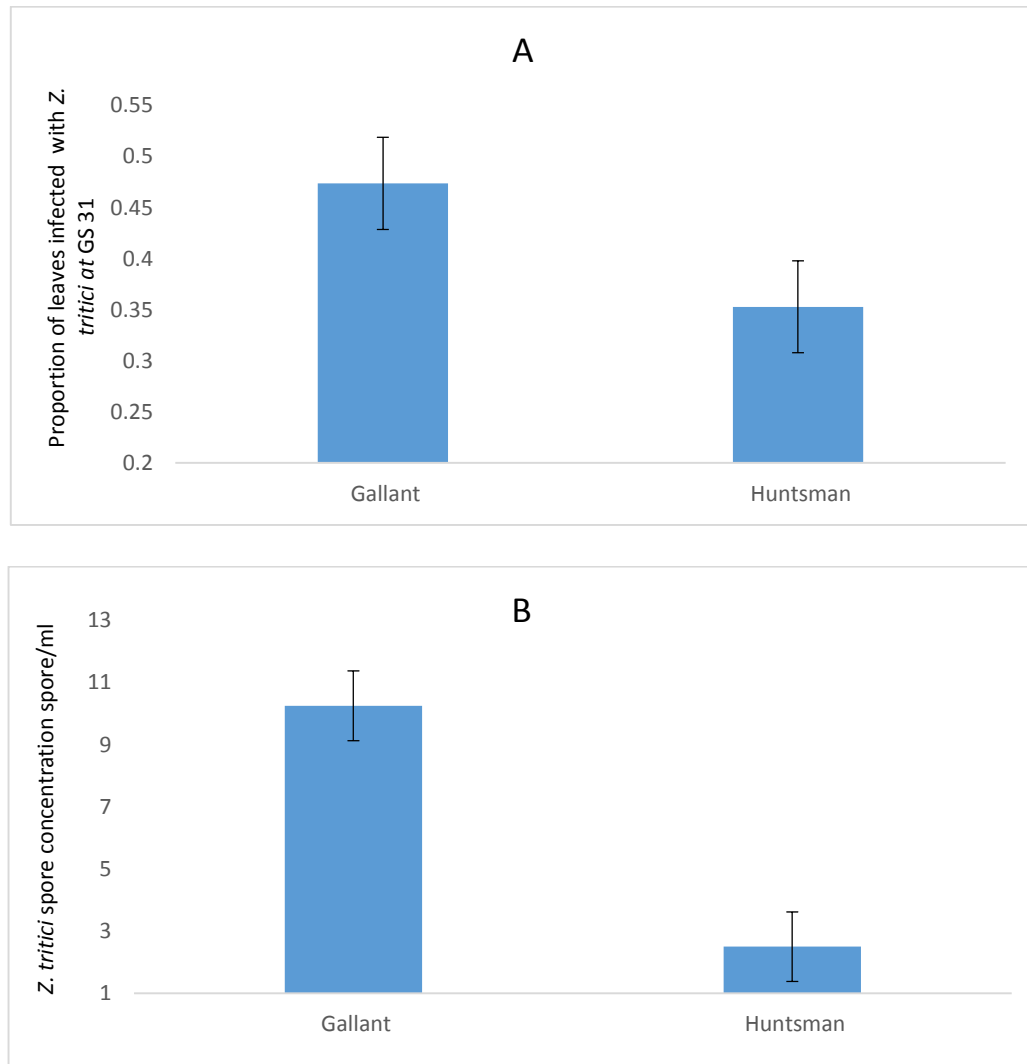
Figure 23. (A) Proportion of leaves infected with *Z. tritici* at growth stage 31 (2013-2014), in wheat cultivars Gallant and M. Huntsman (*p*=0.02, Error bar is ± S.E.D). (B) Spore concentration /10^3 of *Z. tritici* at growth stage 31 in wheat cultivars Gallant and M. Huntsman (*p.* <.001, Error bar= ± S.E.D, d.f. = 71).

Overall, the proportion of leaves with symptoms of Z. tritici at GS31 was reduced by approximately 8% with sulphur fertilizer. However, the effect was only present in plots inoculated with *P. nodorum* (Figure 24; interaction P = 0.006) in which sulphur fertiliser reduced the proportion of leaves infected with *Z. tritici* by about one-third.
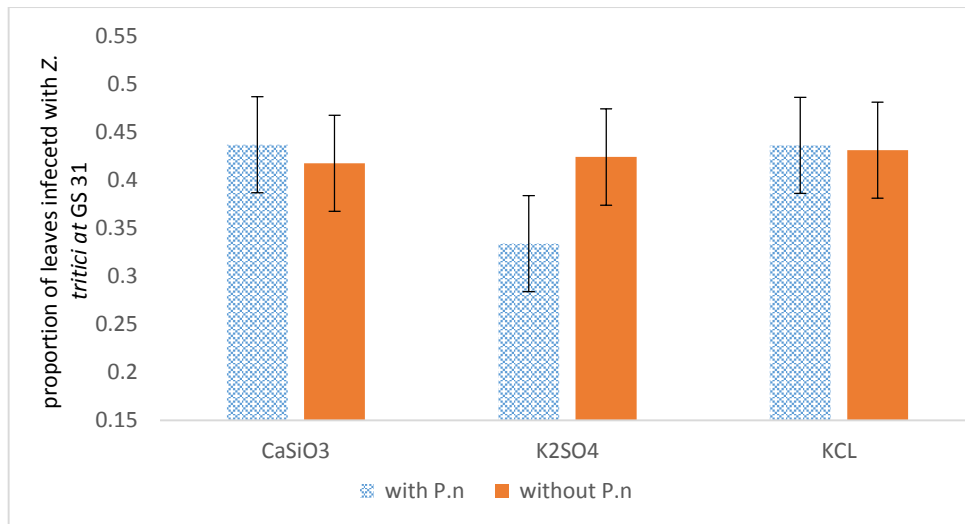
Figure 24. Proportion of wheat leaves infected with *Z. tritici* at growth stage 31 (2013-2014) in plots treated with three alternative fertilizers and inoculated with or without *P. nodorum* as a factorial combination. (*P.* value = 0.006, Error bar= ± S. E. D, d.f. = 71).

The effect of fertilizer on the proportion of leaves infected also differed in the two varieties. In M. Huntsman plots, fertilization with sulphur reduced the proportion by 25% in comparison with potassium chloride (Figure 25; P = 0.002). There was no detectable effect in Gallant plots. Please refer to Tables 2F-13 (Appendix) for more detail.
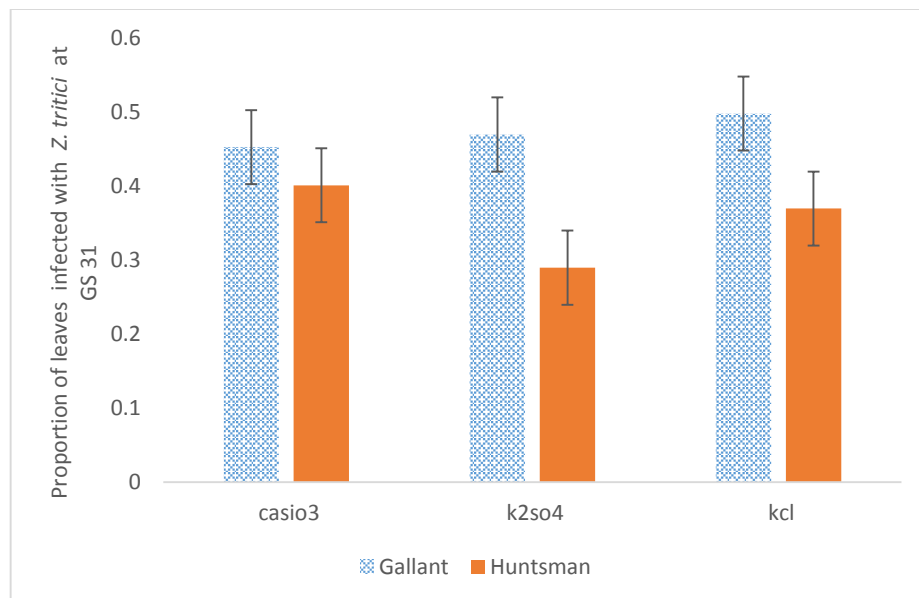
Figure 25. Proportion of leaves infected with *Z. tritici* at growth stage 31 (2013), for wheat cvs. Gallant and M. Huntsman treated with one of three fertilizers in factorial combination (*P.* value = 0.002, Error bar= ± S. E. D, d.f. = 71).

The proportion of infected leaves with *Z. tritici* at growth stage 31 was positively correlated with the spore concentration at the same growth stage (Figure 26; r = 0.5; P = <0.001).

Figure 26. Relationship between proportion of infected leaves and spore concentration of *Z. tritici* at growth stage 31. (r = 0.5; P = <0.001).

4-3-2-2- Growth stage 59, 2013-2014:

The proportion of leaves infected with *Z. tritici* was reduced by 6 % with sulphur fertilizer in comparison with calcium silicate and by 9 % in comparison with potassium chloride (Figure 27 A). No other effects were significant. Effects on spore concentration of *Z. tritici* were greater but followed the same pattern: spore concntration was reduced by 42 % in comparison with calcium silicate and 63 % in comparison with potassium chloride. (Figure 27 B; P = <0.001). Please refer to Tables 2F-15, 2F-16 and A3, A6 (Appendix) for more details.
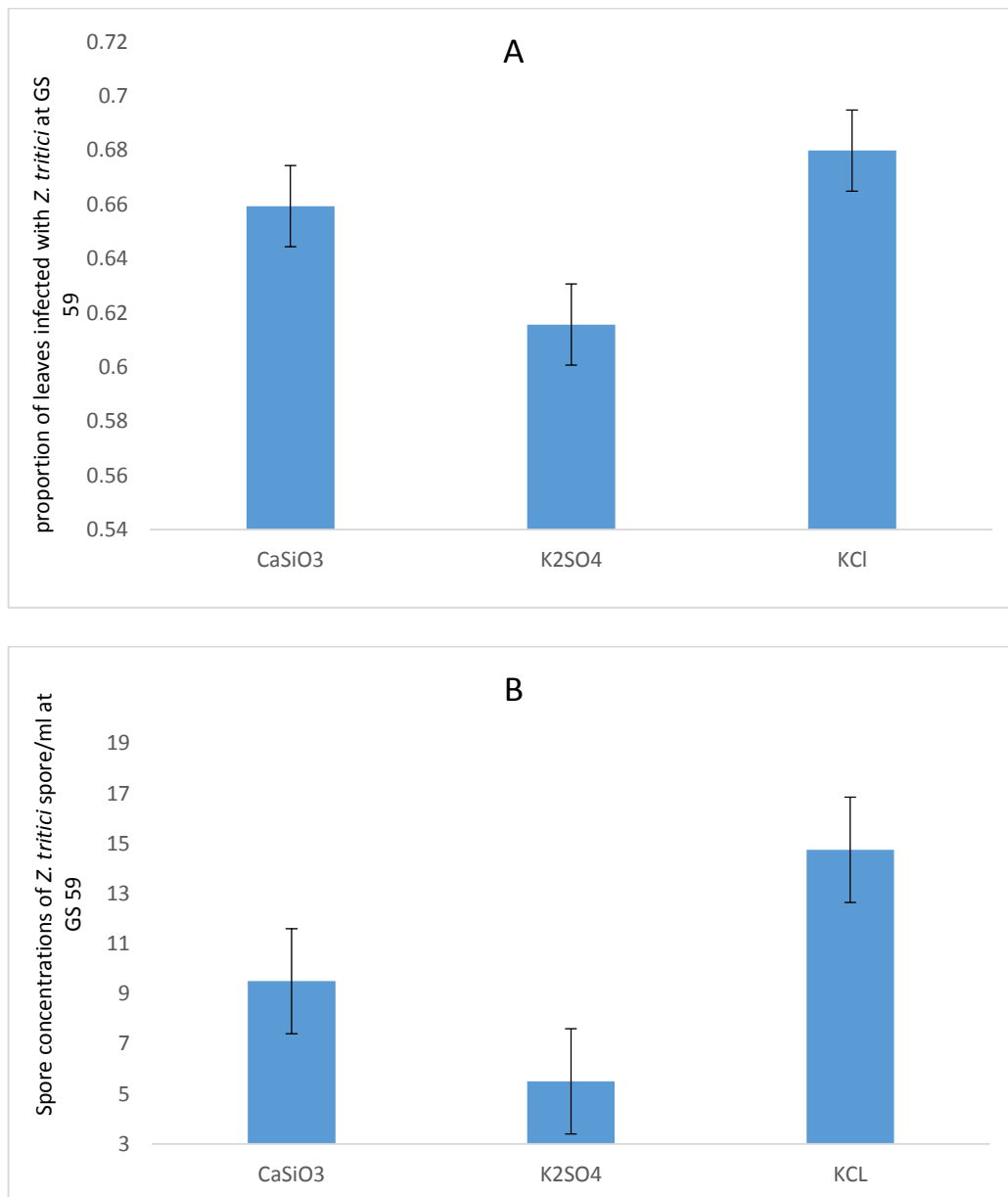
Figure 27. (A) Proportion of leaves infected with *Z. tritici* at growth stage 59 (2013-2014) treated with three alternative fertilizers as a factorial combination (*p.* <0.001, Error bar= ± S.E.D). (B) Spore concentration 1×10^3 of *Z. tritici* at growth stage 59 treated with three alternative fertilizers as a factorial combination (*p.* **<0.001**, Error bar= ± S.E.D, d.f. = 71).

The relationship between proportion of infected leaves with *Z. tritici* and spore concentration at growth stage 59 was positively correlated (Figure 28; r = 0.3; P = 0.04).
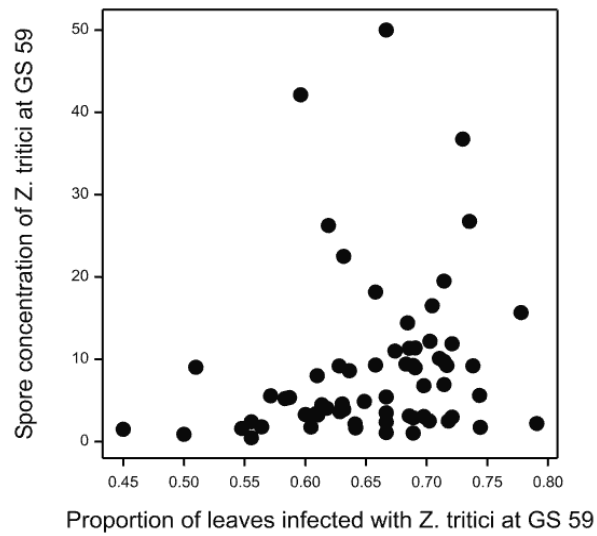
Figure 28. Relationship between proportion of leaves infected with *Z. tritici* at growth stage 59 with spore concentration of *Z. tritici* at growth stage 59 (r = 0.3; P = 0.04).

4-3-2-3- DNA amount of *Z. tritici* at growth stage 59, 2013-2014:

The 4-way interaction (i.e. all factors in the experiment) was significant (P = 0.009; Table 27). The amount of *Z. tritici* DNA was 29% greater in Gallant than in M. Huntsman (Figure 29; P = 0.02). (Compare Figures 30 and 31). Please refer to Table 2F-17 (Appendix) for more detail.
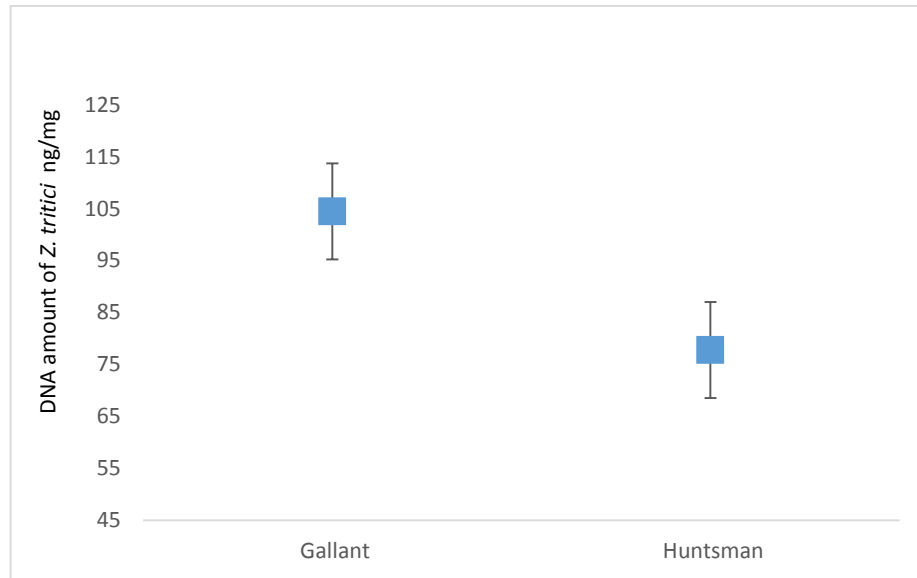
Figure 29. Amount of *Z. tritici* DNA at GS 59 (2013-2014) in two wheat cultivars. (*P.*= 0.02; Error bar = ± S.E.D, d.f. = 47).

In Gallant, despite the greater overall amount of *Z. tritici* DNA, there were few consistent effects. In plots inoculated with either pathogen potassium sulphate had significantly less *Z. tritici* than either alternative treatment, while the plots treated with calcium silicate had substantially more *Z. tritici* DNA than any other plots in the experiment in case of no either pathogen. In plots inoculated with both pathogens, the potassium chloride treatment had just distinctly more DNA than the other two treatments.

71
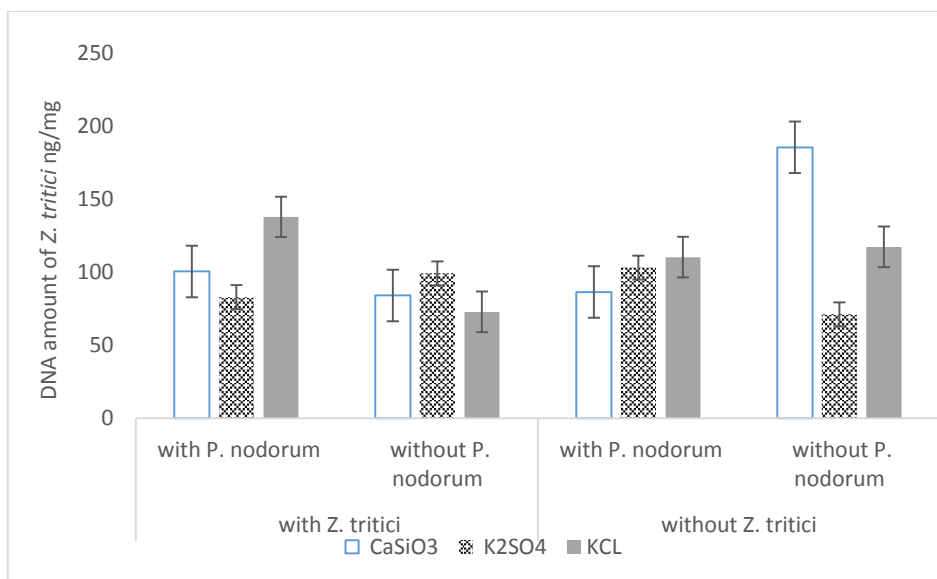
Figure 30. DNA amount of *Z. ritici* at GS 59 (2013-2014) in cv. Gallant treated with three alternative fertilizers and inoculated either with or without each pathogen (*Z. tritici* and *P. nodorum*) and with co-inoculation of pathogens as a factorial combination. (P. value= 0.009; Error bar = ± S.E.D, d.f. = 47).

In M. Huntsman, potassium sulphate fertilizer reduced the amount of *Z. tritici* in all treatments, except in plots co-inoculated with both pathogens.
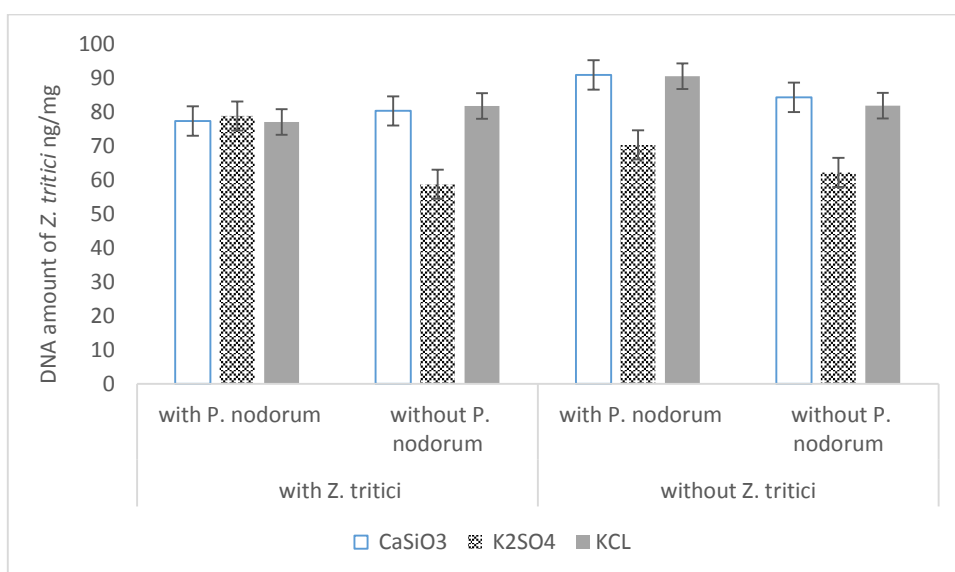
Figure 31. DNA amount of *Z. ritici* at GS 59 (2013-2014) in cv. M. Huntsman treated with three alternative fertilizers and inoculated either with or without each pathogen (*Z. tritici* and *P. nodorum*) and with co-inoculation of pathogens as a factorial combination. (P. value= 0.009; Error bar = ± S.E.D, d.f. = 47).

4-3-2-4- 2013-14 Yellow Rust *Puccinia striiformis* at growth stage 59

The proportion of leaves infected with yellow rust at growth stage 59 was 12% higher in Gallant than in M. Huntsman (P = <0.001). Sulphur fertilisation led to marginally more infection than the other two fertilisers; the effect was slightly larger in Gallan than M. Huntsman (Figure 32; P=0.03). Please refer to Tables 2F-18 and A4 (Appendix) for more detail.
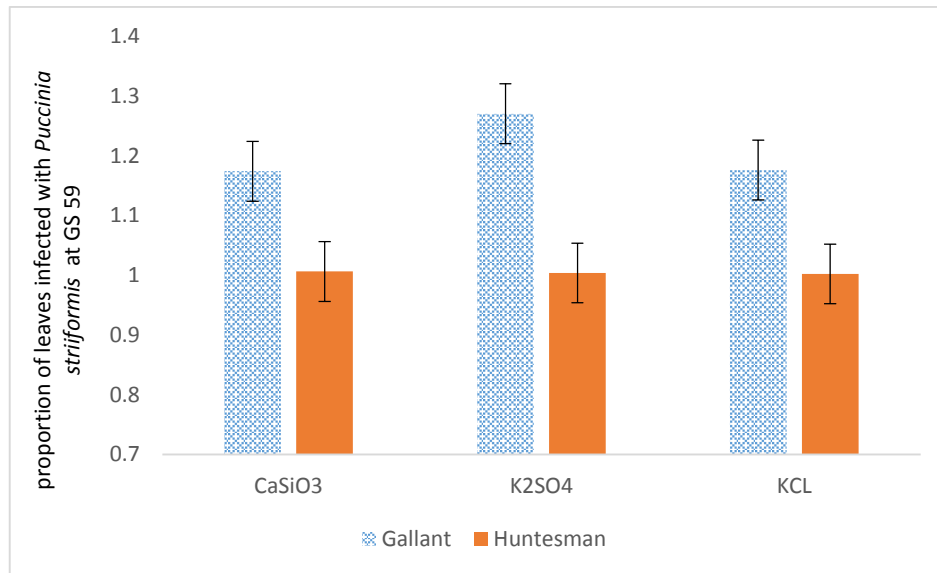
Figure 32. Proportion of leaves infected with yellow rust at growth stage 59 (2013-2014) in two different wheat cultivars Gallant and M. Huntsman altered with three alternative fertilizers as factorial combination (*p.* 0.03, Error bar= ± S. E. D, d.f. = 71).

4-3-2-5- Correlations between disease measurements, with S DW%, and with green leaf area in 2013-2014:

There were negative relationships between concentration of dry weight of sulphur in whole plant at growth stage 59, and both proportion of leaves infected with *Z. tritici* at growth stages 31and 59 and spore concentration of *Z. tritici* at GS 31 (r = -0.3; P = 0.03, -0.4; P = 0.002, and -0.4; P = 0.0014) (Figure 33 A, B, and C) respectively.

In addition, there was negative correlation between green leaf area and the proportion of infected leaves of *Z. tritici* at growth stage 31, 59 (r = -0.4; P = 0.002 and -0.3; P = 0.05) (Figure 34 A and B) respectively, and the correlation with spore concentration at growth stage 31 related negatively with green leaf area, but it was higher than the propoertion of infected leaves (r = -0.6; P = <0.001) (Figure 34 C). This is an expected result.

Correlation between disease measurements at different stages were imperfect but highly significant. The proportion of infected leaves of *Z. tritici* at GS 31was positively and highly related with the proportion of infected leaves at growth stage 59 and with the spore concentration at GS 59 was not statistically significant (r = 0.4; P = <0.001, 0.2; P = 0.09), (Figure 35; A, B) respectively.
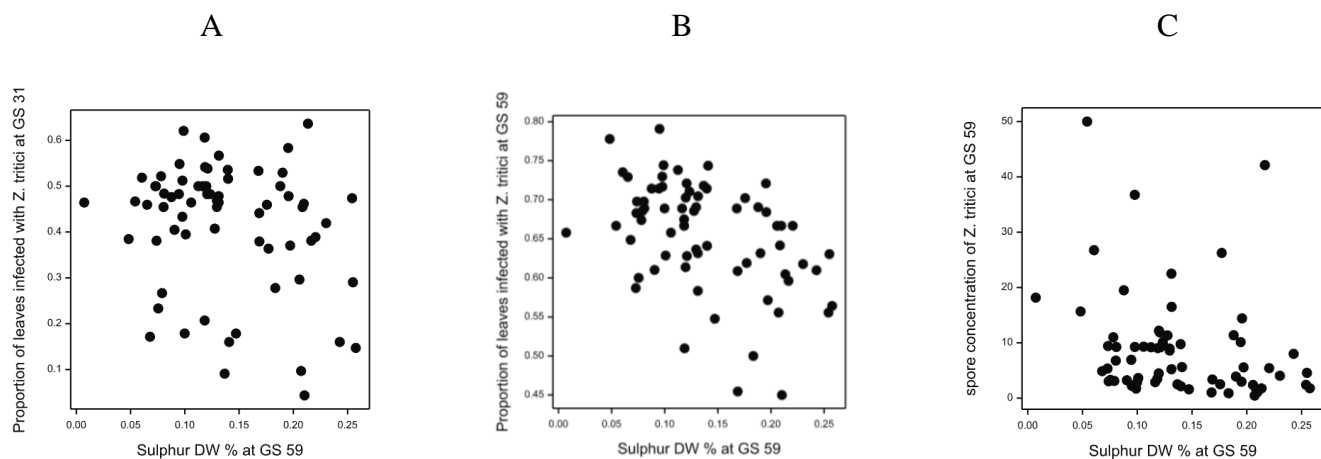
Figure 33. Relationship between whole-plant sulphur concentration as percentage of dry weight at groth stage 59 and each of (A) proportion of leaves infected with *Z. tritici* at growth stage 31 (r = − 0.3; P = 0.03) and (B) at GS 59 (r = − 0.4; P = 0.002) and (C) spore concentration of *Z. tritici* at growth stage 59 (r = − 0.4; P = 0.0014).
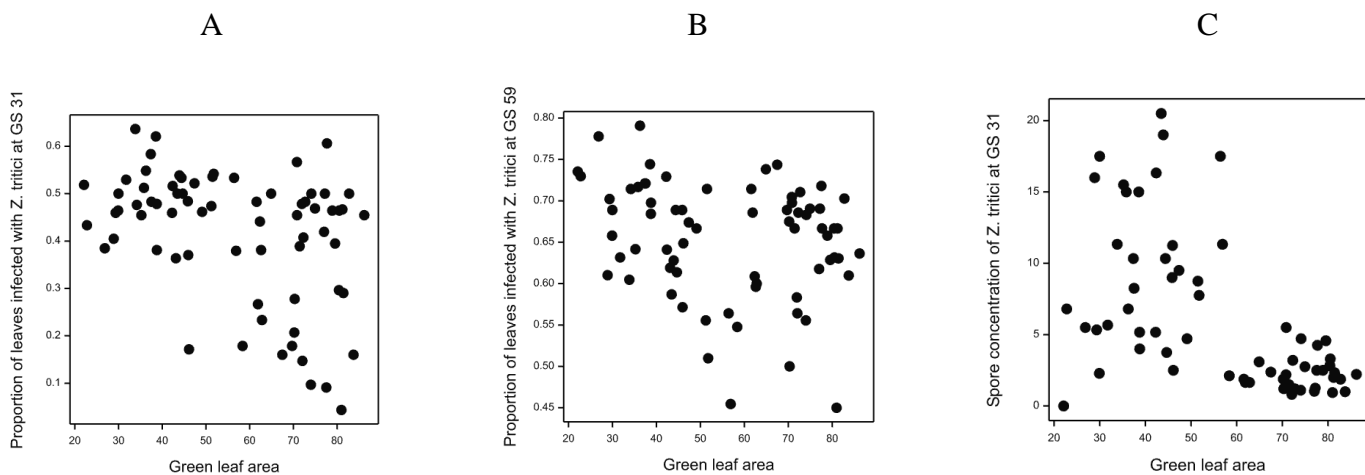
Figure 34. Relationship between green leaf area for the first and second flag leaf and (A) proportion of leaves infected with *Z. tritici* at growth stage 31 (r = − 0.4; P = 0.002)and (B) at growth stage 59 (r = − 0.3; P = 0.05), (C) spore concentration of *Z. tritici* at growth stage 31 (r = − 0.6; P = <0.001). In the 2013-2014 season.
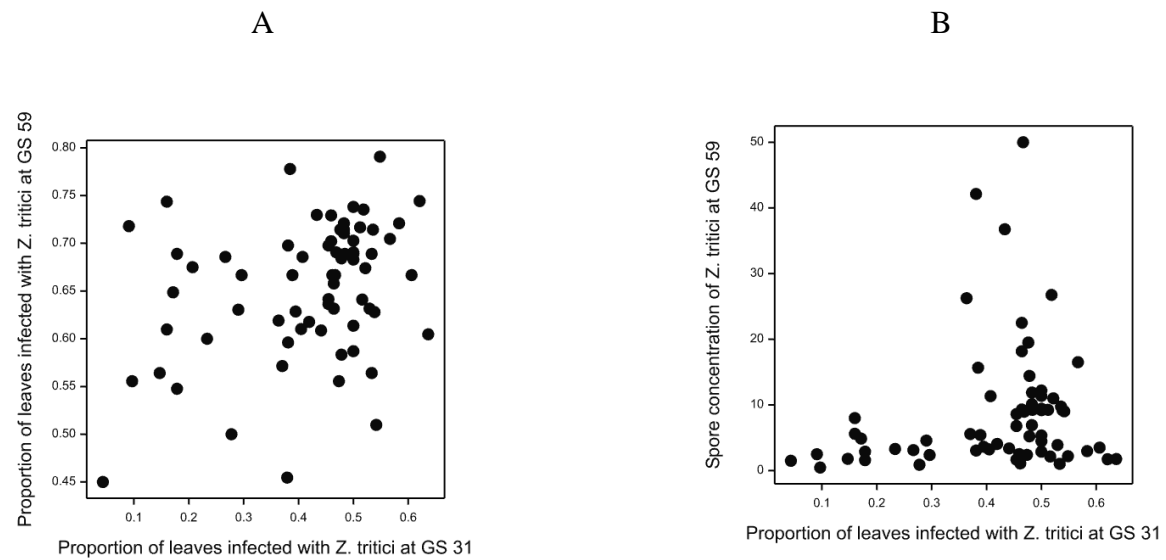
Figure 35. Relationship between disease measurements   in the 2013-2014, proportion of leaves infected with *Z. tritici* at growth stage 31 with,

(A) proportion of leaves infected with *Z. tritici* at growth stage 59 (r = 0.4; P = <0.001),

(B) spore concentration of *Z. tritici* at growth stage 59 (r = 0.2; P = 0.09).

4-3-3- 2014-2015 season (cv. M. Huntsman)

4-3-3-1- Growth stage 31, 2014-2015:

Proportion of leaves infected with *Z. tritici* was decreased from 22% to 14% (P = <0.001, Figure 36 A) with sulphur fertilizer compare with the plot did not received sulphur. Spore concentration of *Z. tritici* was about 70% lower in plots treated with sulphur fertilizer (Figure 36 B; P < 0.001). No other treatments had detectable effects. Please refer to Tables 2F-19, 2F-20 and A7, A8 (Appendix) for more details.
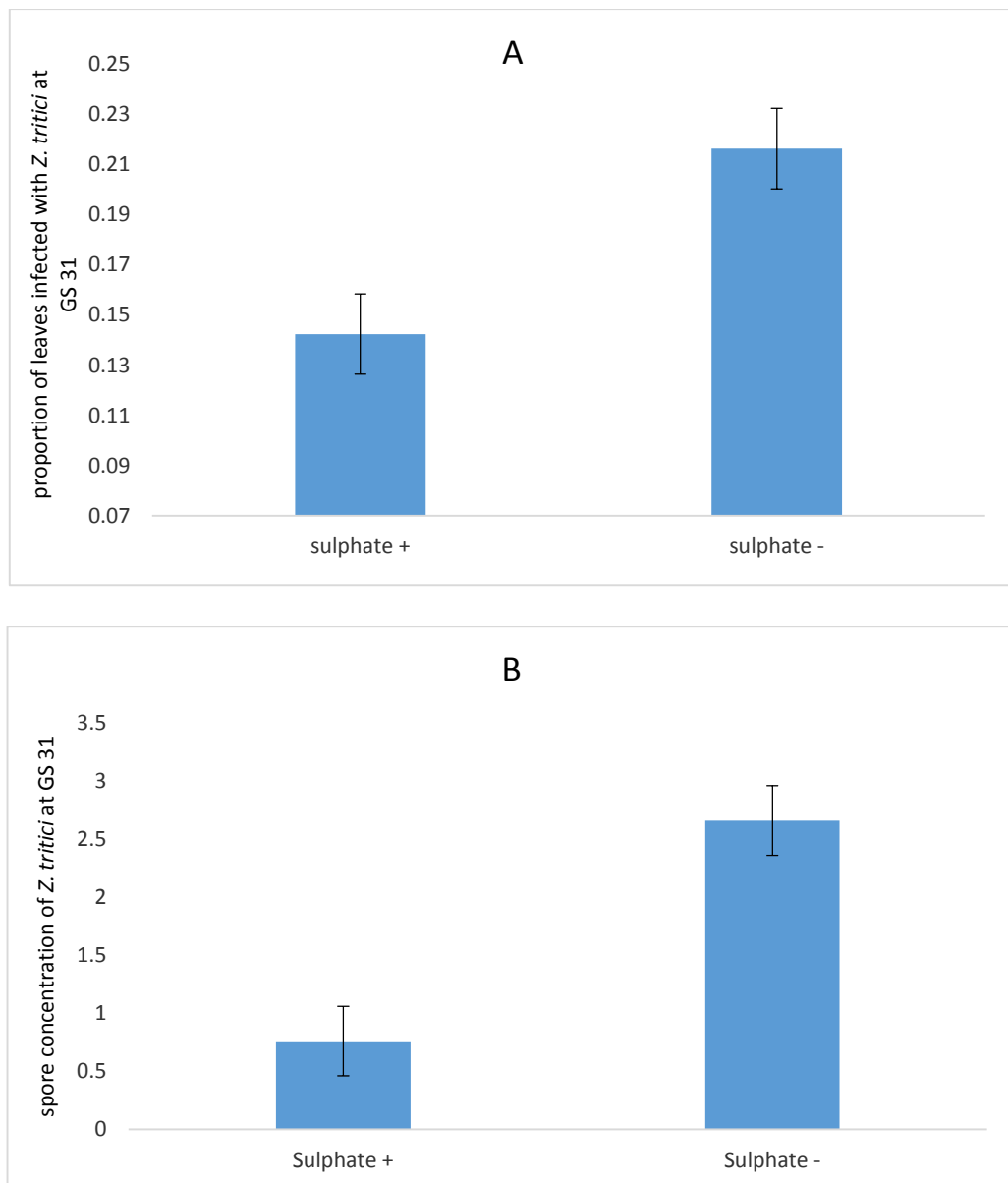
Figure 36. (A) Proportion of infected leaves of *Z. tritici* at growth stage 31 (2014-2015) treated with or without sulphur fertilizers as a factorial combination (*p.* <0.001, Error bar= ± S.E.D.). (B) Spore concentration 1×10^3 of *Z. tritici* at growth stage 31 treated with or without sulphur fertilizer as a factorial combination (*p.* <.001, Error bar= ± S.E.D., d.f. = 35).

The correlations between visual disease measurements were varied. The proportion of leaves infected with *Z. tritici* at GS 31 correlated poorly with that at GS 59 and disease severity of *Z. tritici* at GS 77 (r = 0.4; P = 0.007 and 0.3; P = 0.04 respectively) (Figure 37 A and C). The correlation with the proportion of leaves infected at GS 77 was slightly higher (r = 0.5; P = 0.002) (Figure 37 B), possibly because the proportion of infected leaves at GS 59 was low in comparison with GS 31 and 77. The proportion of leaves infected with *Z. tritici* correlated well with spore concentration of *Z. tritici* at GS 31 and 77 (r = 0.6; P = <0.001, 0.6; P = <0.001 respectively) (Figure 37; D and E).
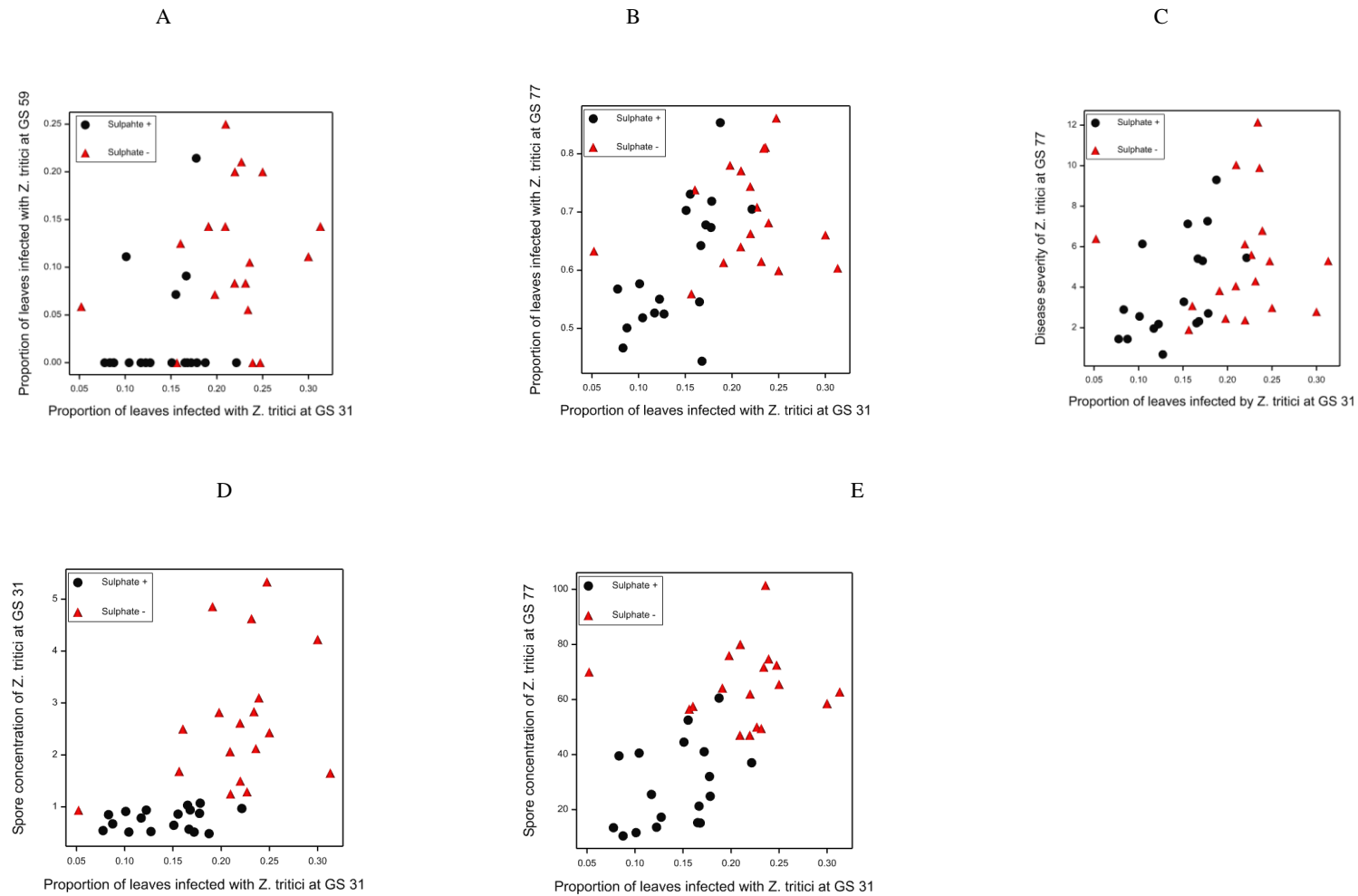
Figure 37. Relationship between proportions of leaves infected with *Z. tritici* at GS 31which treated with or without sulphur fertilization with each of (A) Proportion of leaves infected with *Z. tritici* at GS 59 (r = 0.4; P =0.007), (B) proportion of leaves infected with *Z. tritici* at GS 77 (r =0.5; P = 0.002), (C) disease severity of *Z. tritici* at GS 77 (r = 0.3; 0.04), (D) spore concentration of *Z. tritici* at GS 31 (r = 0.6; P = <0.001), (E) spore concentration of *Z. tritici* at GS 77 (r =0.6; P = <0.001).

4-3-3-2- Growth stage 59, 2014-2015:

Sulphur reduced proportion of leaves infected with *Z. tritici* from 11% to 3% compared to none sulphur (Figure 38; P = 0.014). Please refer to Tables 2F-21 and A9 (Appendix) for more detail.
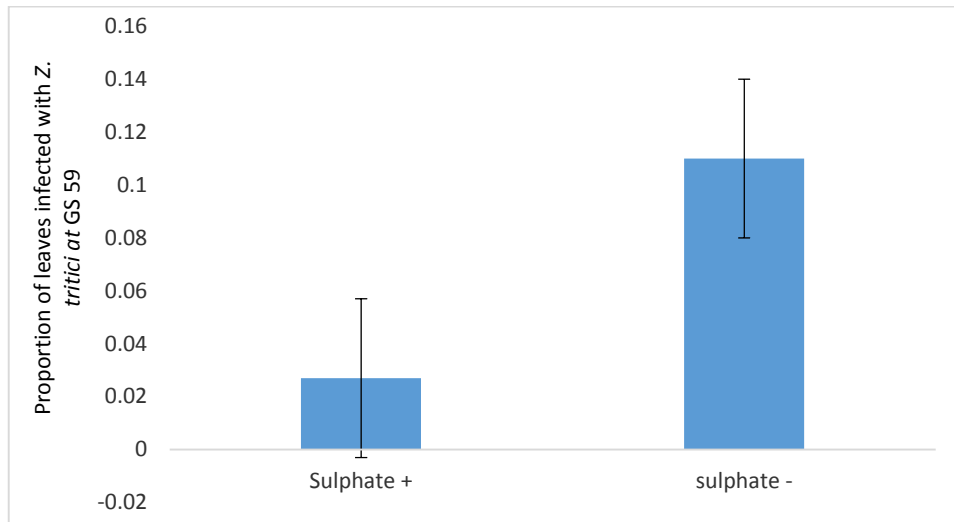
Figure 38. Proportion of leaves infected with *Z. tritici* at GS 59 (2014-2015) treated with or without sulphur fertilizer. (*P* value 0.014; Error bar = ± S.E.D., d.f. = 35).

The proportion of leaves infected with *Z. tritici* was increased in plots that had been sprayed with fungicide from 4% to 12%, but only in plots inoculated with *P. nodorum* (Figure 39; interaction P = 0.04).
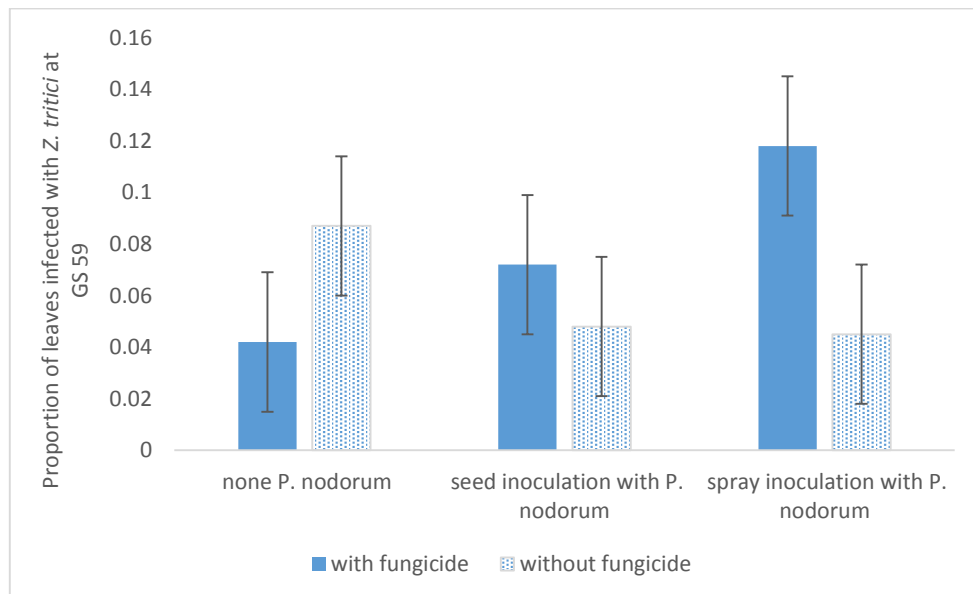
Figure 39. Interaction of fungicide (tebuconazole) and the inoculation of *P. nodorum* on proportion of leaves infected with *Z. tritici* at GS 59 (2014-2015). (P value 0.04; Error bar = ± S.E.D., d.f. = 35).

The correlation between proportions of leaves infected with *Z. tritici* at growth stage 59 with other disese measurement were poor. The proportion of leaves infected with *Z. tritici* at GS 59 related poorly with disease severity of *Z. tritici* at GS 77 (r = 0.3; P = 0.1; Figure 40 A). Also the correlation between the proportion of leaves with *Z. tritici* and spore concentration at GS 31, 59, 77 of *Z. tritici* were poor (r = 0.2; P = 0.2, r = 0.4; P = 0.008 and r = 0.4, P = 0.01 respectively) (Figure 40 B, C and D). This could be explained by low rainfall between the GS 31 and GS 59 periods.
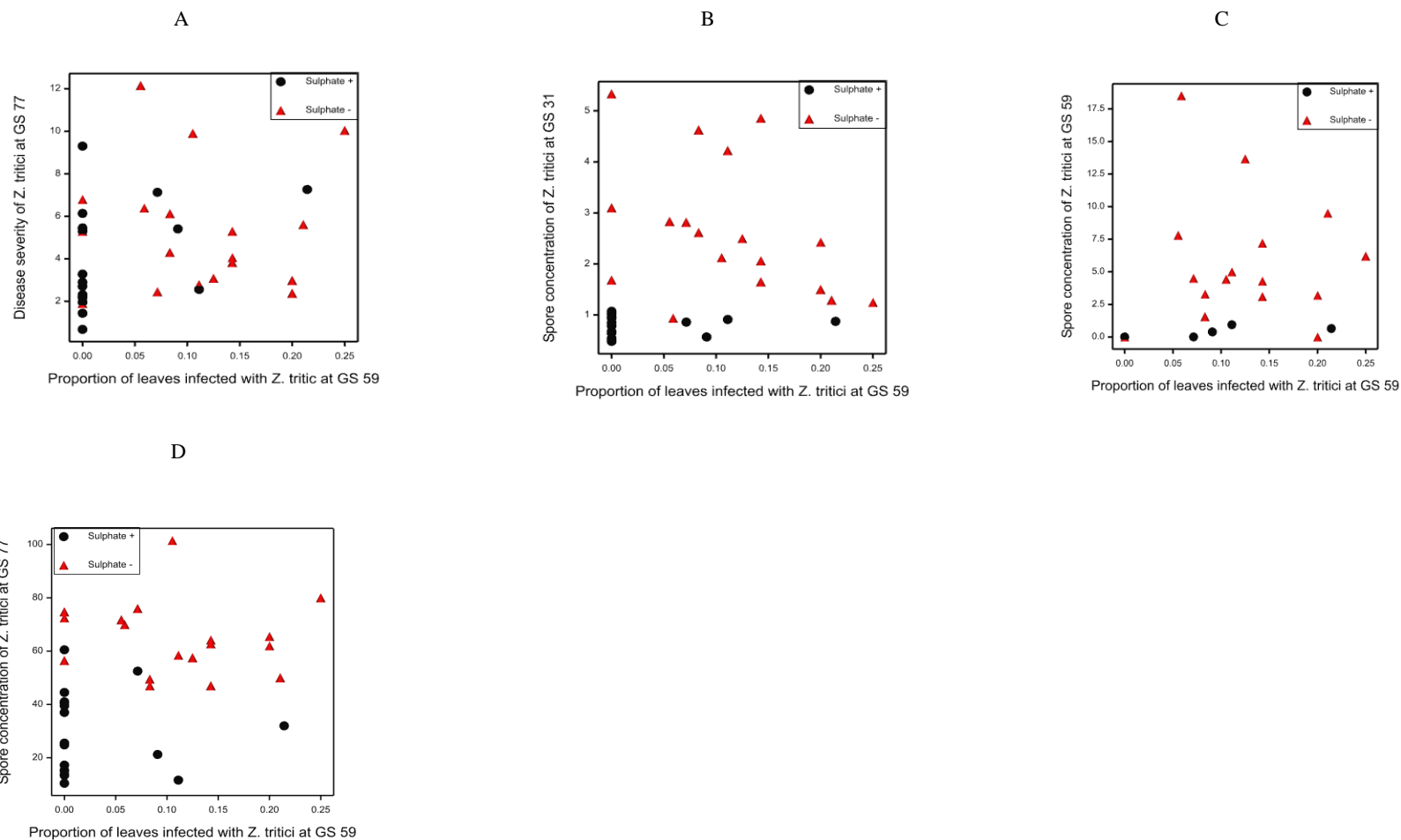
Figure 40. Relationship between proportions of leaves infected with *Z. tritici* at GS 59 with each one of (A) disease severity of *Z. tritici* at GS 77 (r =0.3; P = 0.1), (B) spore concentration of *Z. tritici* at GS 31 (r =0.2; P = 0.2), (C) spore concentration of *Z. tritici* at GS 59 (r =0.4; P = 0.008), and (D) spore concentration of *Z. tritici* at GS 77 (r =0.4; P = 0.01).

4-3-3-3- Growth stage 77, 2014-2015:

Sulphur fertilized plots had about 26% lower proportion of leaves infected with *Z. tritici* at late milk stage (GS77) (Table 13). Severity of *Z. tritici* was also reduced, from 5.3 % in potassium chloride treated plots to 3.9 with potassium sulphate treated plots. (P =0.01; Table 13). Spore concentration of *Z. tritici* was about 77% lower in plots treated with sulphur fertilizer than in plots did not treated with sulphur fertilizer. (Table 13). No other effects were greater than background variability, except that spore concentration was reduced by fungicide by about 20% but not significantly (Figure 41). Please refer to Tables 2F-22, 2F-23, 2F-24 and A10, A11, and A12 (Appendix) for more details.

Table 12. Disease measurements of *Z. tritici* at growth stage 77 in year 2014-2015 treated with or without sulphur fertilizer as a factorial combination.

measurements	% leaves infected	Spore concentration	Disease severity
treatment			
sulphur +	0.6	29	3.9
sulphur -	0.7	65	5.3
S.E.D.	0.02	3.5	0.5
P value	0.001	<0.001	0.01
d.f.	35	35	35
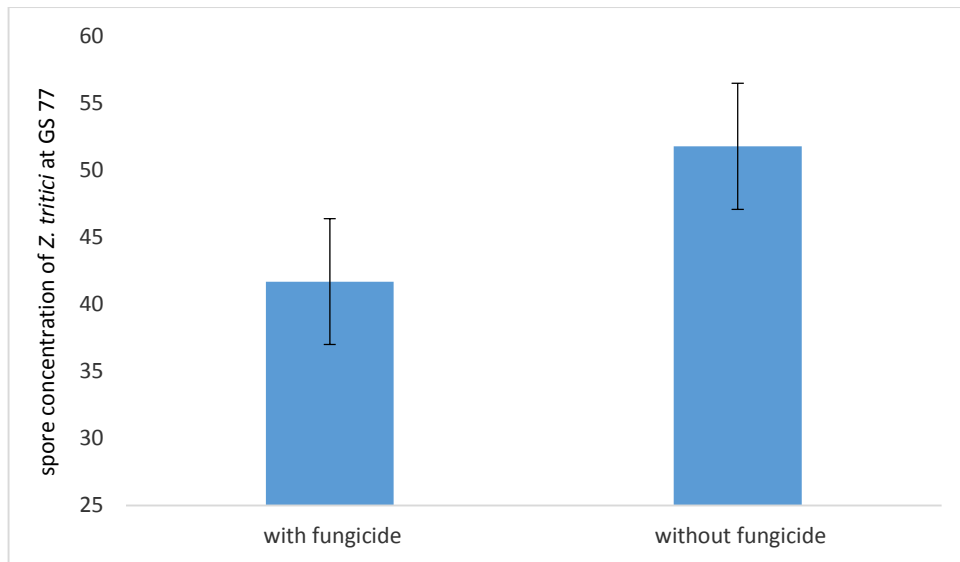
Figure 41. Spore concentration 1×10^3 of *Z. tritici* at growth stage 77 (2014-2015) treated either with or without tebuconazole as a factorial combination (*p.* 0.06, Error bar= ± S.E.D., d.f. = 35).

The proportion of leaves infected with *Z. tritici* at GS 77 related poorly with the proportion of leaves infected and spore concentration of *Z. tritici* at GS 59 and spore concentration at GS 31(r = 0.3, 0.3 and 0.3 respectively) (Figure 42 A, D and C). However, there was a good correlation with disease severity and spore concentration of *Z. tritici* at GS 77 (r = 0.7; P = <0.001 and 0.7; P = <0.001 respectively) (Figure 42 B and E). related poorly with spore concentration of *Z. tritici* at GS 59 (r = 0.3; P = 0.09) (Figure 42 G), but was found to be good between disease severity at GS 77 and spore concentration at GS 77 (r = 0.6; P = <0.001) (Figure 42 F).
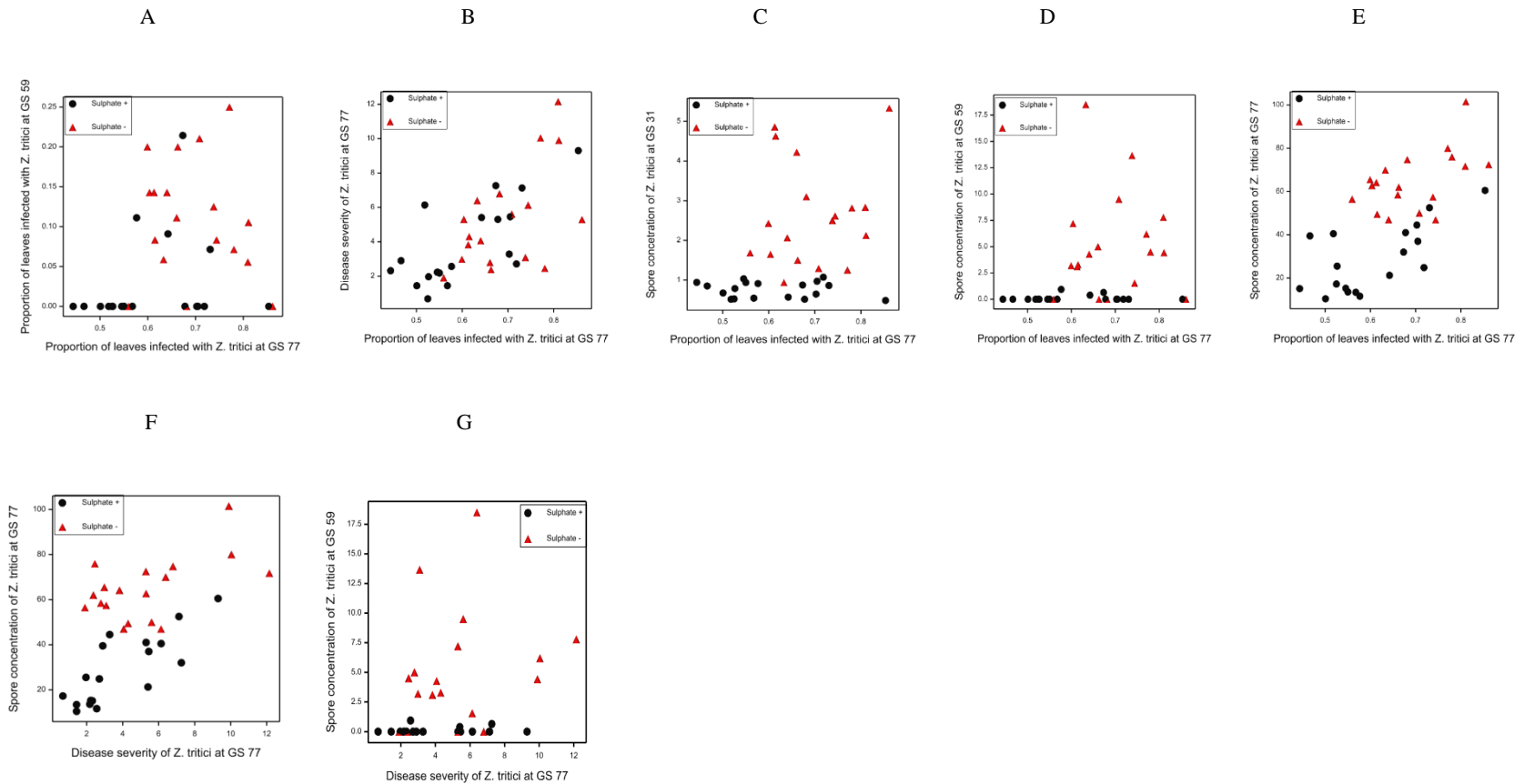
Figure 42. Relationship between proportions of leaves infected with *Z. tritici* at GS 77 with each of (A) proportion of leaves infected with *Z. tritici* at GS 59 (r =0.3; P = 0.14), (B) disease severity of *Z. tritici* at GS 77 (r =0.7; P = <0.001), (C) spore concentration of *Z. tritici* at GS 31 (r =0.3; P = 0.04), (D) spore concentration of *Z. tritici* at GS 59 (r =0.3; P = 0.1), (E) spore concentration of *Z. tritici* at GS 77 (r =0.7; P = <0.001). Relationship between disease severities of *Z. tritici* at GS 77 and (F) spore concentration of *Z. tritici* at GS 77 (r =0.6; P = <0.001), (G) spore concentration of *Z. tritici* at GS 59 (r =0.3; P = 0.09).

4-3-3-4- Growth stage 77, 2014-2015: *Parastagonospora nodorum*

A high proportion of leaves infected in plots spray inoculated with *P. nodorum* had symptoms of the disease. Otherwise it was almost absent. Similarly, no spores were counted in the plots which were not spray-inoculated. In spite of seed inoculation, no *P. nodorum* appeared at early growth-stage. In the plots where it was present the proportion of leaves infected with *P. nodorum* was reduced by sulphur fertilizer, but not statistically significantly (Figure 43 A; P = 0.07). In the plots treated with fungicide to against *Z. tritici*, the proportion of *P. nodorum* was increased by 18% in comparison to the plot not treated (Figure 43 B; P = 0.03). Please refer to Tables 2F-25, 2F-26, 2F-27 and A13 (Appendix) for more detail.
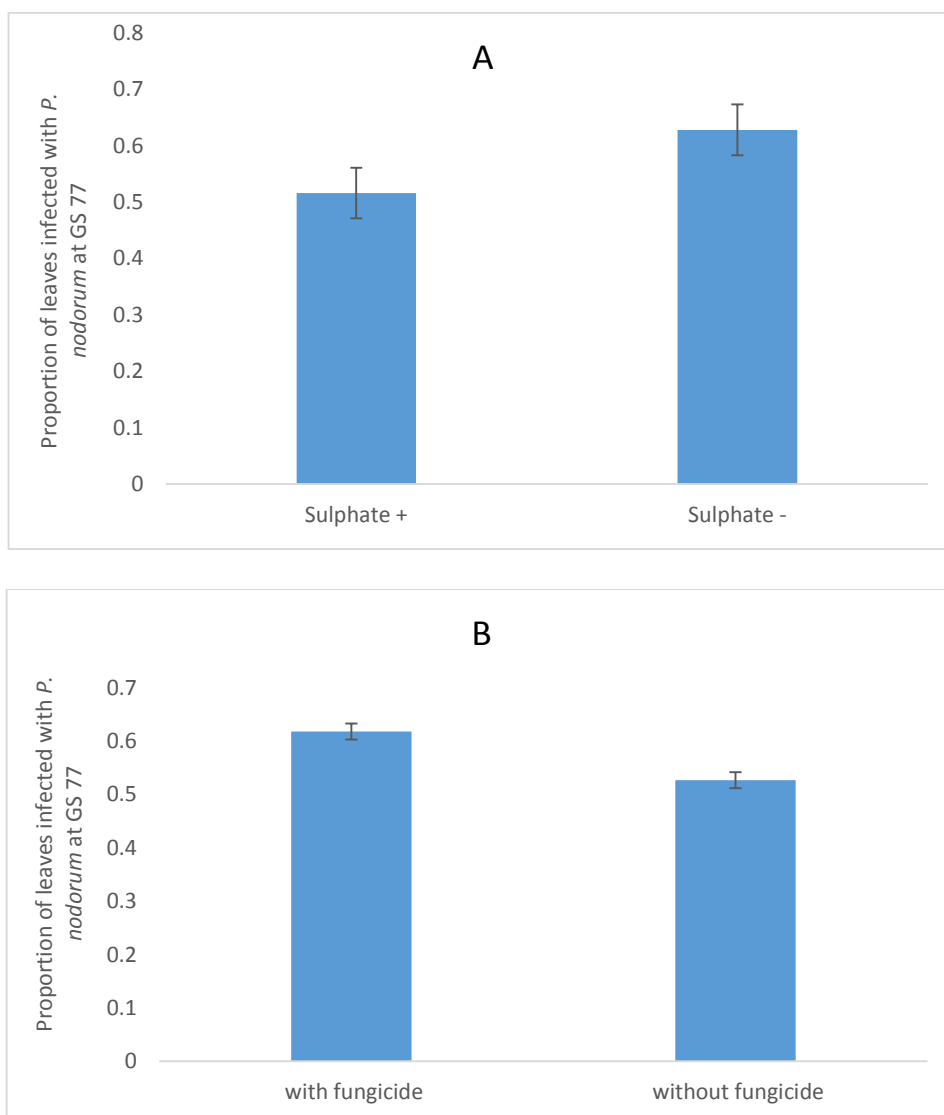
Figure 43. The proportion of leaves infected with with *Parastagonospora nodorum* of winter wheat cv. M. Huntsman at GS 77 (2014-2015), in plots which were treated (A) with sulphur fertilizer or not (B) with tebuconazole at GS77 or not. (Error bar = ±S.E.D.; P value A= 0.07; B =0.03, d.f. = 35).

The spore concentration of *P. nodorum* in washings was reduced by sulphur fertilization but not significantly; the mean spore concentration was reduced from 11 to 7.5×10^3 spore/ml. Please refer to Table A14 (Appendix) for more detail.

There was a definite correlation between the proportion of leaves infected with *P. nodorum* at GS 77 and both disease severity and spore concentration of *P. nodorum* at GS 77 (r = 0.7; P = <0.001 and 0.8; P = <0.001 respectively), (Figure 44 A and B). In spite of autumn inoculation, no *P. nodorum* appeared at the early stage even in inoculated plots, but the spring spray inoculation was successful.

A   B
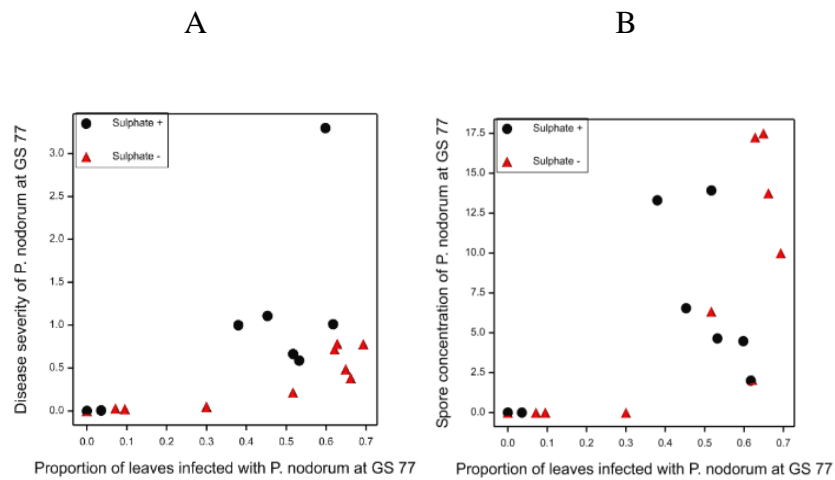
Figure 44. Relationship between proportions of leaves infected with *P. nodorum* at GS 77, 2014-2015 which were treated either with or without sulphur fertilizer, and each of (A) disease severity of *P. nodorum* at GS 77 (r =0.7; P = <0.001), and (B) spore concentration of *P. nodorum* at GS 77 (r =0.8; P = <0.001).

4-3-3-5- Correlation between whole-plant sulphur concentration at GS 31 and77, and disease measurements 2014-2015:

Whole-plant concentrations of sulphur at GS 31 and 77 were negatively correlated with visual disease measurements. Concentration of sulphur at GS 31 correlated negatively with proportion of leaves infected with *Z. tritici* at GS 31 and 59 (r = - 0.6, - 0.6 respectively) (Figure 45 A and B), but had little relation to the proportion of leaves infected or disease severity of *Z. tritici* at GS 77 (r = - 0.4, - 0.2 respectively) (Figure 45 C and D). Sulphur concentration was negatively related to spore concentration of *Z. tritici* at all three of GS 31, 59 and 77 (r = -0.6, -0.5, -0.6 respectively; Figure 45 E, F and G). The correlation of sulphur concentration at GS 77 with visual disease measurements at all stages was low. The correlation of sulphur concentration with *Z. tritici* at GS 31, 59 and 77 was r = -0.4, -0.3, -0.3 respectively (Figure 46 A, B and C). In addition, disease severity at GS 77 and spore concentration of *Z. tritici* at GS 31, 59 and 77 were negatively correlated (r = -0.3, -0.4, -0.3, -0.4 respectively) (Figure 46 D, E and F). This might be because the whole-plant concentration of sulphur was higher at GS 31 than at GS 77.
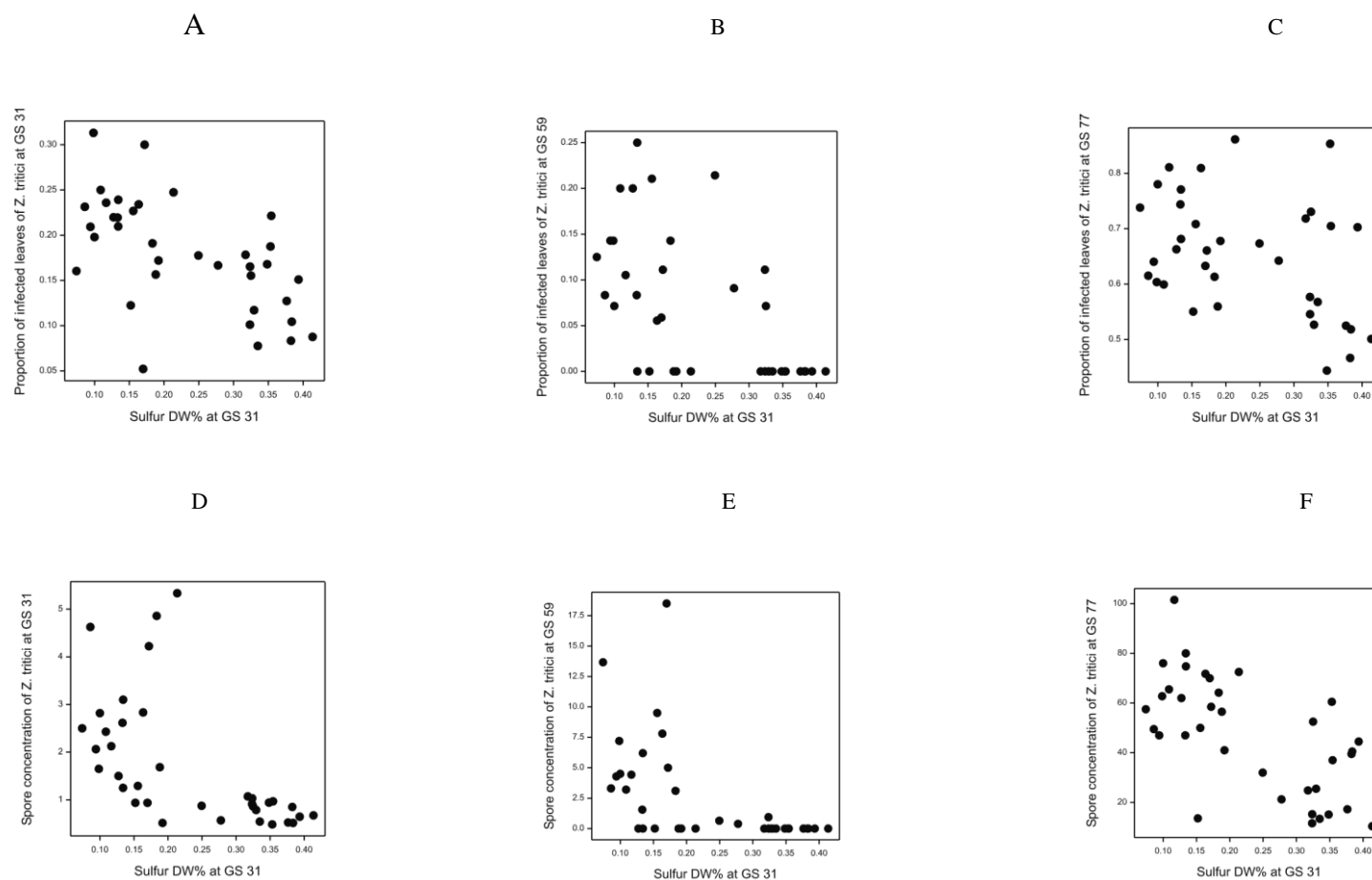
Figure 45. Relationship between concentrations of sulphur as dry weight in whole plant materials at GS 31 with each of (A) proportion of leaves infected with *Z. tritici* at GS 31 (r = − 0.6; P = <0.001), (B) proportion of leaves infected with *Z. tritici* at GS 59 (r = − 0.6; P = <0.001), (C) proportion of leaves infected with *Z. tritici* at GS 77 (r = − 0.4; P = 0.02), (D) spore concentration of *Z. tritici* at GS 31 (r = − 0.6; P = <0.001), (E) spore concentration of *Z. tritici* at GS 59 (r = − 0.5; P = <0.001), (F) spore concentration of *Z. tritici* at GS 77 (r = − 0.6; P = <0.001).
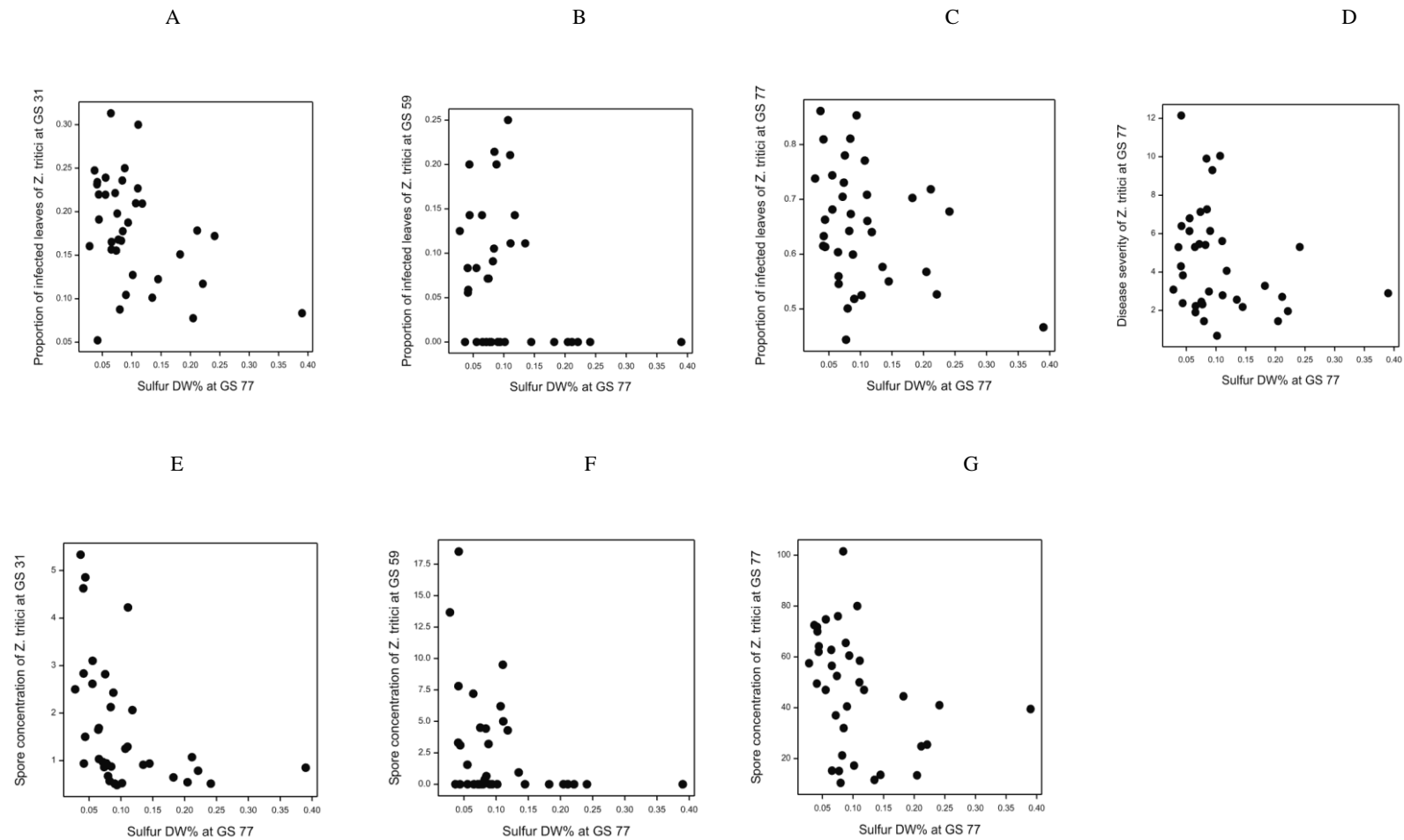
Figure 46. Relationship between concentration of sulphur as dry weight in whole plant materials at GS 77 with each of (A) proportion of leaves infected with *Z. tritici* at GS 31 (r = − 0.4; P = 0.01), (B) proportion of leaves infected with *Z. tritici* at GS 59 (r = − 0.3; P = 0.07), (C) proportion of leaves infected with *Z. tritici* at GS 77 (r = − 0.3; P = 0.05), (D) disease severity of *Z. tritici* at GS 77 (r = − 0.3; P = 0.1), (E) spore concentration of *Z. tritici* at GS 31 (r = − 0.4; P = 0.009), (F) spore concentration of *Z. tritici* at GS 59 (r = − 0.3; P = 0.05), (G) spore concentration of *Z. tritici* at GS 77 (r = − 0.4; P = 0.03).

4-4- Discussion:

The overall results showed that sulphur fertilizer reduced both pathogens. *Z. tritici* was reduced more than *P. nodorum*, while there was no effect of silicon fertilizer. Wheat cultivars varied in susceptibility; cv. Gallant was more susceptible to *Z. tritici* than M. Huntsman. There was clear evidence of interaction between these two pathogens or plant reactions to them, even though there were no *P. nodorum* symptoms in 2013-2014. Inoculation with *P. nodorum* reduced the proportion of leaves infedted with *Z. tritici*. In addition, when the seedlings were inoculated with *Z. tritici*, this was associated with reduction in *Z. tritici* later.

4-4-1- Effect of fertilizer on pathogens:

Later, in the summer 2013 season, fertilizers had significant effects on both pathogens. Fertilizers did not affect the proportion of leaves having visible symptoms or disease incidence at GS 31. This could be due to fertilizer being applied just before assessment. Consequently, either fertilizers might not have been absorbed or the seedlings might need more time to accumulate nutrients differentially and respond.

Sulphur as potassium sulphate reduced *P. nodorum* and *Z. tritici*, measured by either proportion of leaves infected or disease severity. The proportion of leaves infected and disease severity were both determined as less than expected for these diseases. This might be due to either (1) in a spring crop, there is less time for disease development or (2) low rain-fall and temperature over the growing season (Table 14), since *P. nodorum* and *Z. tritici* require high rainfall and moderate temperatures during growing season (Shaw 1991; Simón *et al*., 2003). In addition, rainfall plays an important role in spreading the spores (Shaw 1991; Shah & Bergstorm, 2000).

Khan & Kulshrestha (1991) found high concentrations of sulphur dioxide inhibit conidial germination of powdery mildew. However, in this experiment the proportion of infected leaves of powdery mildew at GS 59 was increased by sulphur fertilizer. This could be because better sulphur nutrition raised the proportion of protein in leaves, and if the protein increases, there is more supply of nutrients to biotrophic pathogens.

Potassium chloride and calcium silicate fertilizers affected the proportion of leaves infected with *Z. tritici* at GS 72. Rodgers-Gray & Shaw (2004) found infection by powdery mildew and *Z. tritici* were reduced by silicon fertilisation. Deliopoulos *et al*. (2010) reviewed the role of inorganic salts against fungal diseases. Potassium chloride was one of these salts, used as a foliar application to

against disease. The best control achieved by using these inorganic salts was of powdery mildew and *Z. tritici*.

In the season 2013-2014 sulphur fertilization significantly reduced the proportion of infected leaves of *Z. tritici* at GS 31, but the spore concentration was not affected by sulphur fertilisation. Most plots suffered flooding between December and January (please refer to Figure 13, picture 1), and this might have affected either the efficacy of sulphur fertilizer or caused leaching of it from the soil. Sulphur fertilization significantly reduced the proportion of leaves infected and spore concentration of *Z. tritici* at GS 59. This might be explained by reference to the role of glutathione. Glutathione is the most sulphur containing antioxidant in plants. In addition, it's essential in plant defence against abiotic and biotic stress conditions, because it is involved in the detoxification of reactive oxygen species, the modulation of defence gene expression, regulation of enzymatic activities and redox signalling (Zechmann, 2014).

Although there were very few symptoms of *P. nodorum*, there was some effect of *P. nodorum* inoculation on the proportion of infected leaves with *Z. tritici*. The low level of symptoms that occurred might be explained by the low temperature and the heavy rainfall at the beginning of the season. The infection process for *P. nodorum* requires high relative humidity and temperature between 22-25°C to succeed maximally (Scharen & Krupinsky, 1970). The latent period is shortest at 20°C with high humidity (Shearer & Zadoks, 1972).

Both wheat cultivars were infected by yellow rust (*Puccinia graminis*) at GS 59, but the proportion of infected leaves was much more with cv. Gallant than M. Huntsman. The proportion of leaves infected was increased by sulphur fertilizer in cv. Gallant, but there was no effect of sulphur with cv. M. Huntsman. Gallant is susceptible to yellow rust (*P. graminis*) with resistance grade 5 according to recommended list from AHDB (2013-2014), and treatment with sulphur fertilizer leads to increased concentration of protein in the leaves, which, as for mildew, may benefit biotrophic fungi.

Sulphur concentration (%DW) in plant tissue was negatively correlated with both spore concentration and proportion of infected leaf at growth stage 59. This might be because sulphur is required to produce glutathione, an antioxidant, essential for plant as defence against biotic and abiotic stress (Zhang *et al*., 2015; Zechmann 2014; Gallardo *et al*., 2014; Calmes *et al*., 2015). Hiruma *et al*. (2013) suggested that the synthesis of glutathione and tryptophan-derived metabolites during the hyper-sensitive response plays a crucial role in terminating the invasive growth of both nonadapted and adapted hemibiotrophs.

There was a negative correlation between spore concentration at growth stage 31 and green leaf area; that is, spores increased when there was less green leaf area. When there are fewer spores then the spread of pathogen is slower. On the other hand, the rate of growth of leaf area, rather than shortage of inoculum, will often be a limiting factor on the amount of disease (Shaw, 1999). There was a positive correlation between the proportion of leaves infected and spore concentration of *Z. tritici* at growth stage 31. That means both measurements are measuring the same thing and gives confidence that the data are not completely dominated by random noise.

In the season 2014-2015 fertilizers had significant and substantial effects on disease caused by *Z. tritici*. Sulphur fertilizer hugely reduced the proportion of leaves infected, disease severity and spore concentration. at growth stages 31, 59 and 77. This could again be through greater concentrations of glutathione making the plant more resistant.

Sulphur fertilizer had significant effects on the proportion of leaves infected with *P. nodorum* at growth stage 77, with a slight effect on spore concentration, but less than *Z. tritici*. There was no effect of sulphur on disease severity of *P. nodorum*. This might be due to two reasons. Firstly, *P. nodorum* did not show any symptoms at early growth stages. Secondly, the concentration of sulphur in dry matter at growth stage 77 was less than at growth stage 31.

In the 2014/15 experiments tebuconazole was used as fungicide in plots originally intended to investigate the interaction of *Z. tritici* with sulphur fertilizer with or without *P. nodorum* inoculation. There was no effect on *Z. tritici* in any measurement, except that spore concentration at GS 77 was slightly reduced. This was expected since the population of *Z. tritici* has evolved considerable resistance to the fungicide (Mavroeidi & Shaw, 2006; Curvers *et al*., 2015; Dooley *et al*., 2016). Although *Z. tritici* did not show much development at GS 59, the proportion of infected leaves was increased by tebuconazole only in plots that were inoculated with *P. nodorum*. That might be explained in two ways. Either the *P nodorum* inoculation had more effect in increasing *Z. tritici* than tebuconazole in reducing it, or tebuconazole had more effect on *P. nodorum* than on *Z. tritici*.

The proportion of leaves infected with *Z. tritici* at growth stage 31 had a positive relation with spore concentration at same growth stage. That means both disease measurements were related to the same underlying disease process. Measures of pathogen over time increased, except that at GS 59 the proportion of infected leaves with *Z. tritici* was equal to or less than the proportion of infected leaves at GS 31. This might be because there was little rainfall at that time (March and April; Table 71; chapter 5) which meant less development. Furthermore, the latent period of *Z.*

tritici infection on the crop leaves layer was closer to inoculum sources within the crop and that led to rise the infections. This occurred soon after a leaf layer started to emerge with more time for multiplication of disease within that layer (Shaw & Royle, 1993). Measures of pathogen population in the late season (GS77) were much higher than at GS 31. This is as expected. However, spore concentrations were unrelated to severity and incidence of symptoms. This might be because the pathogen had started to produce pseudothecia.

The concentrations of sulphur in whole plants, and disease measurements, were related negatively. The relationship was better at GS 31 than at GS 77. This could be explained in two ways. Either the efficacy of sulphur was only at the early stage of plant development, or there was more disease development late in the season, and by that time the level of sulphur available in the soil was less than at GS 31.

The correlation data with *P. nodorum* shows a positive correlation between proportion of infected leaves with each of disease severity and spore concentration at growth stage 77, which means that all measurements are giving some information about the underlying pathogen population.

4-4-2- Co-inoculation effect:

In the first two experiments, 2013 and 2013-2014, there was some effect of the inoculations on pathogen abundance. Bearchell *et al*. (2005) and Shaw *et al*. (2008) suggested that may there was a causal link between sulphur and both pathogens (*Z. tritici* and *P. nodorum*), so that *P. nodorum* was at a peak in the 1970s while *Z. tritici* has dominated from the 1980s till now. But Chandramohan & Shaw (2013) found that both pathogens were reduced by sulphur (as sulphurous acid). Hence, the reduction in one or both of these pathogens may be due either to competition between them when they attack the same host simultaneously, or to competition, when one pathogen attacks the host before another.

4-4-3- Effect of pathogens on wheat cultivars:

All wheat cultivars were susceptible to both pathogens. There was less disease development with cv. Paragon, but less disease appeared when the crop was fertilised with sulphur. Both wheat cultivars Gallant and M. Huntsman were affected by both pathogens, but cv. Gallant had greater incidence and severity. According to the AHDB recommended list of winter wheat cultivars Gallant is a cultivar susceptible to *Z. tritici* and *P. nodorum*, which agrees with this observation. Resistance of cv. Gallant did not appear to be affected by the fertilizers that were used in this experiment but cv. M. Huntsman had less disease and more green leaf area when fertilized with

sulphur. Therefore, cv. M. Huntsman may be a cultivar with good resistance to both pathogens but in which resistance is especially strong when sulphur fertilization is abundant.

CHAPTER FIVE

EFFECT OF SULPHUR AND SILICON FERTILIZERS ON YIELD AND QUALITY OF WHEAT.

5-1- Introduction:

In the previous chapter the effect of fertilizers on wheat diseases under field conditions was reported. There was a significant effect of sulphur fertilizer on disease. However, many studies have reported the influence of sulphur on wheat quality and yield (Griffiths *et al.*, 1995; AHDB 2002; Wieser *et al.*, 2004; Järvan *et al.*, 2008; Steinfurth *et al.*, 2012). These studies show the effect of sulphur fertilization without reporting interactions between pathogens and the host under field conditions. This chapter will cover the effect of sulphur fertilization on quality and yield of wheat under UK field conditions, as influenced by disease.

Deficiency of sulphur in the UK was first detected in oilseed rape then, after a few years, developed in other crops (Chalmers *et al.*, 1999). Deficiency has become common as a result of the decline in deposition of sulphur dioxide emission from atmosphere, and the decline in fertilizers containing S. Yield quality and protein content of crops cannot reach the potential maximum amounts without adequate amounts of S, which also enhances nitrogen utilization (Sahota, 2006).

Fertilizers often improve wheat quality and yield, depending on soil nutrient availability and reservoirs. Nitrogen, and often sulphur and potassium, can improve wheat quality and increase yield in the majority of soils (Zörb *et al.*, 2014; Steinfurth *et al.*, 2012; Duggan *et al.*, 2010; Järvan *et al.*, 2008; Wieser *et al.*, 2004; Griffiths *et al.*, 1995; Tea *et al.*, 2004). Sulphur deficiency in wheat grain can be diagnosed by the proportion of nitrogen to sulphur. A lack of sulphur in soils in many regions of the world has become increasingly apparent in surveys of soil. This had various causes, including the use of fertilizer alternatives containing no sulphur, particularly replacement of ammonium sulphate with ammonia or ammonium nitrate, and high production of wheat through the increased use of fertilizers containing nitrogen. Leaching of sulphur from the soil contributes to sulphur deficiency in soil. Ercoli *et al.* (2012) found in Italy that the average of sulphur leaching of the two seasons with durum wheat was 35 kg S per hectare.

In wheat, sulphur plays an important role in bread-making quality (Ryant & Hřivna, 2004) because of its relation to protein composition. It is well known that protein plays a key role in the process of bread-making. The mature grain contains 8-20% gluten protein. The gluten proteins, gliadins and

glutens constitute up to 80-85% of the total protein content in the flour. They add to the flour extensibility and elasticity, which are two of the characteristics necessary for good bread quality (Kuktaité 2004; Järvan *et al.*, 2008). Zhao *et al.* (1999) and McGrath (2003) reported that bread-making quality was more closely correlated to sulphur concentration than to nitrogen concentration. Sulphate is the main compound of sulphur present in each of the stem, leaves and ears at the stage of grain development, while glutathione is the most important during grain fill. S concentration differences become obvious at growth stage 77 comparing grain from plots with low or high sulphur fertilization (Steinfurth *et al.*, 2012).

Sulphur fertilisation can interact with disease. The main effect of foliar disease is to reduce the leaf area available to intercept solar radiation and consequently to reduce photosynthesis, especially in the flag leaf (Serrago *et al.*, 2009). Application of fungicides a short time after flag leaf emergence can greatly raise yield and mean grain weight in the temperate conditions of the UK (Cook & King 1984; Cook & Thomas 1990; Griffiths *et al.*, 1995). Although fungicides delay senescence, there are still concerns that levels of protein could be reduced by being diluted by increased carbohydrate production (Dimmock & Gooding 2002). Gooding *et al.* (1991) found in one of five years' experiments that there was reduction in S concentration when fungicide and urea were applied. Ruske *et al.* (2004) found (in the single winter wheat cultivar Malacca) that applied fungicides contributed to reduced sulphur concentrations which decreased protein.

As detailed in chapter 4 three field experiments were conducted in 2013-2015 to investigate the effect of sulphur fertilization on disease, yield and quality of wheat grown under UK field conditions. This chapter explains the yield and quality measurements made in these experiments. The previous chapter found that sulphur fertilization reduced both of *Z. tritici* and *P. nodorum*. The effects of disease are therefore somewhat confounded with direct effects of fertilization in these experiment.

5-2- Methodology:

See chapter two.

5-3-Results:

5-3-1- 2013 season (cv. Paragon):

The silicon concentration in the plants was unmeasurable with the assay used: samples gave negative means after correction of soil contamination using the titanium comparator (Table 14). The effect of fertilisation on soil silicic acid was undetectable (Table 14).

Table 13. Average soluble silicon concentration in plant and soil samples at growth stage 59, from spring cv. Paragon, treated with one of four alternative fertilizers under field conditions in 2013.

Fertilizers	Si conc. plant mg/g	Si conc. Soil mg/g
$Ca(OH)_2$	-22.2	477.4
$CaSiO_3$	-34.0	476.0
KCl	-25.5	482.4
K_2SO_4	-35.3	460.3
L.S.D.	15	27.6
P value	0.2	0.4

Sulphur fertilizer increased the concentration of sulphur by 0.05 percentage points and nitrogen of dry flour by 0.03 percentage points compared to potassium chloride, and reduced the N/S ratio (all $P < 0.001$) and increased each of SDS sedimentation ($P = 0.03$), and HFN ($P. = 0.03$). The potassium chloride treatment had a higher TGW, specific weight and HI ($P = 0.005$, 0.002 and 0.005 respectively) than other treatments (Table 16). Please refer to Tables 2F-28 - 2F-36 (Appendix) for more detail.

Table 14. Mean of rainfall (mm) and temperature (ºC) of three experiments. All data were provided from the weather station at Sonning Farm, University of Reading, Berkshire, UK.

		January	February	March	April	May	June	July	August	September	October	November	December
2013	Rainfall/ mm				16	43.5	18	34.6	21.6	51.6	89.2	53.4	99.4
	TemperatureºC				8	10.6	14.4	19.2	17.55	13.93	12.5	6.4	1.9
2014	Rainfall/ mm	141.2	110.8	23	63	76.6	63.2	16.8	83.6	8.4	94.6	95.5	41.6
	TemperatureºC	6.1	6.6	8.14	10.1	12.5	16	18.7	15.6	15.3	12.5	8	11.23
2015	Rainfall/ mm	62.2	103	23.2	12.2	44.8	24.7	60	77.7	45.4			
	TemperatureºC	4.5	3.9	6.9	9.7	11.5	15.3	16.8	16.3	12.64			

Table 15. Mean values of yield and quality parameters on spring wheat cv. Paragon grown in 2013 season under different fertilizer regimes. Sodium dodecyl sulphate SDS/ml volume, thousand grain weight TGW (g), specific weight (kg/hl), harvest index H.I, sulphur concentration in dry flour, nitrogen concentration in dry flour, ratio of nitrogen to sulphur N/S, hagberg falling number HFN (s), total yield (g/m^2) and moisture content in grain are shown. Statistical significance of differences between treatments is indicated by *P* value. Date of sowing 16th April 2013.

Fertilizer	SDS/ml	TGW/g	Spec. weight kg/hl	H.I.	S %DW	N %DW	N/S ratio	HFN	Total. yield (g /m^2)
Ca(OH)$_2$	88.8	26.8	63.56	0.44	0.14	2.80	19.40	458	176
CaSiO$_3$	88.8	26.4	62.13	0.44	0.15	2.87	19.13	466	171
KCl	88.2	28.6	65.59	0.45	0.14	2.82	20.26	466	187
K$_2$SO$_4$	90.9	25.7	60.52	0.42	0.19	3.14	16.48	486	168
L.S.D.	1.9	1.6	2.10	0.02	0.005	0.12	0.48	23	24
P value	0.03	0.005	0.002	0.005	<0.001	<0.001	<0.001	0.03	0.4

Yield was positively correlated to Thousand grain weight and specific weight (r = 0.6 and 0.44; *P*. <0.001 and 0.001 respectively; Figure 47 C and D). This is an expected relation because both of these measurements are determined by the size and shape of grain. Yield was strongly inversely correlated to nitrogen concentration in wheat flour (r = -0.63; *P*. <0.001), (Figure 47 G). Please refer to Table 2F-34 (Appendix) for more detail.
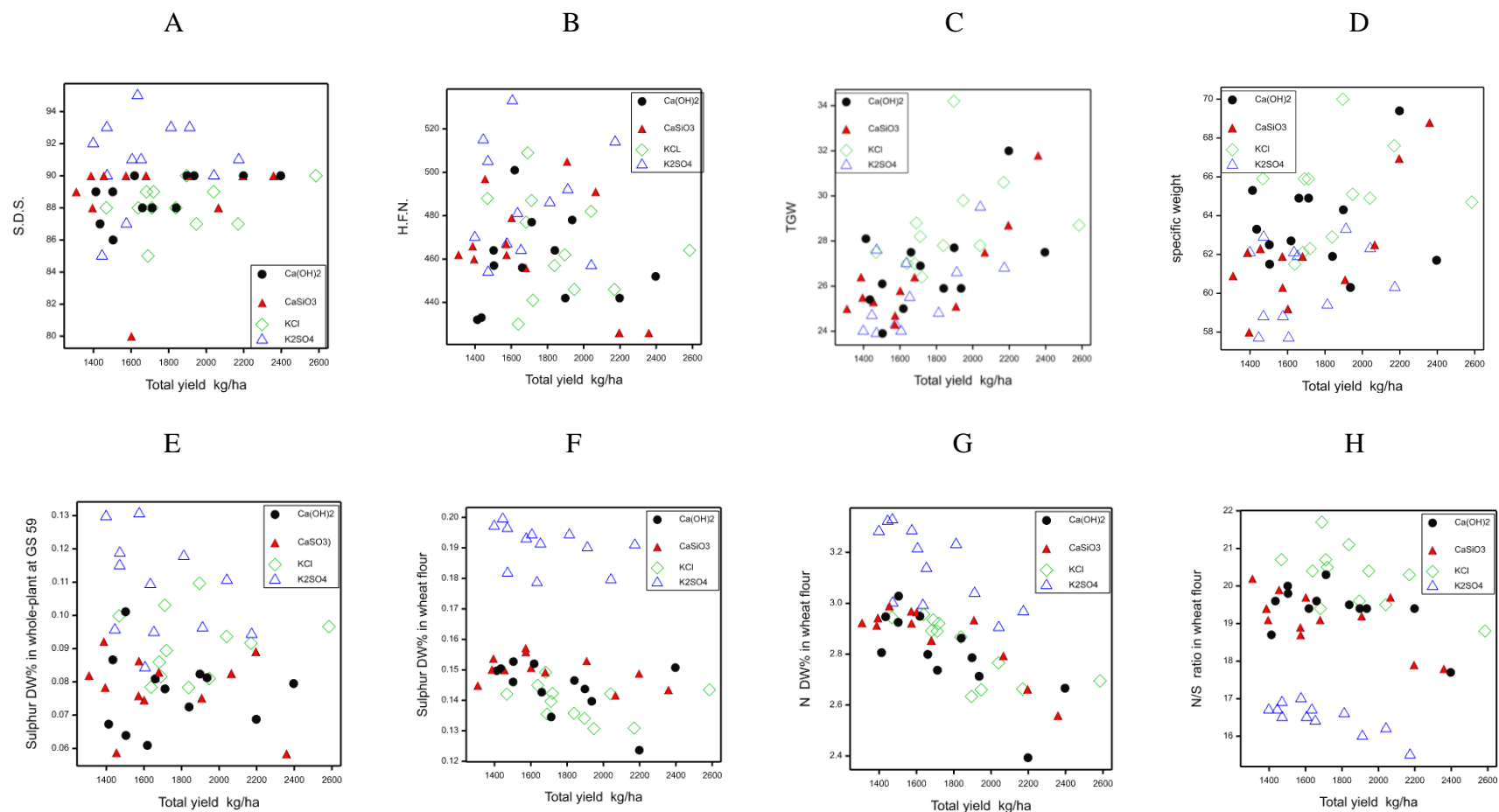
Figure 47. Relationship between total yield (kg/ha) of spring wheat cv. Paragon in 2013 season with (A) S.D.S sodium dodcyl sulphate (r = 0.13; P = 0.4), (B) H.F.N. hagberg falling number (r = 0.18; P = 0.2), (C) TGW thousand grain weight (r = 0.6; P = <0.001), (D) specific weight of wheat grain (r = 0.44; P = 0.0017), (E) sulphur concentrations in whole-plant at growth stage 59 (r = -0.11; P = 0.4), (F) Sulphur concentrations in wheat flour (r = -0.26; P = 0.06), (G) nitrogen concentration in wheat flour (r = -0.63; P = <0.001), and (H) N/S ratio in wheat flour (r = -0.08; P=0.5).

5-3-1-1- Effect of inoculation with *Z. tritici* 2013:

Total yield in cv. Paragon was slightly reduced in plots inoculated with *Z. tritici* but not significantly (Figure 48; *P* = 0.065).
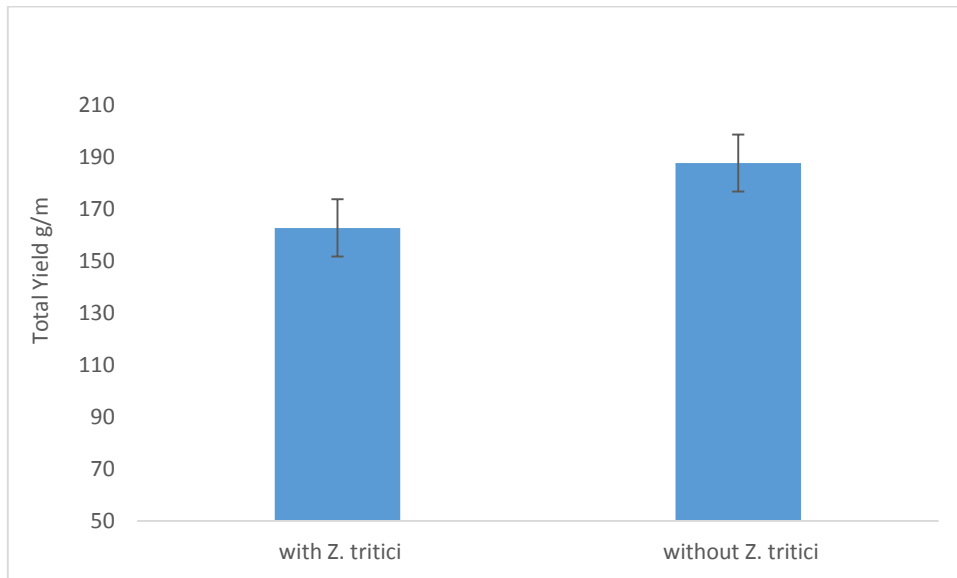
Figure 48. Effect of *Z. tritici* inoculation on total yield g/m^2 from cv. Paragon 2013 season averaged over four alternative fertilizers and inoculation with or without *P. nodorum*. (P. 0.065; Error bar = ± S.E.D., d.f. = 47).

Thousand grain weight was slightly affected in plots inoculated with *Z. tritici* (Figure 49). Please refer to Table 2F-35 (Appendix) for more detail.
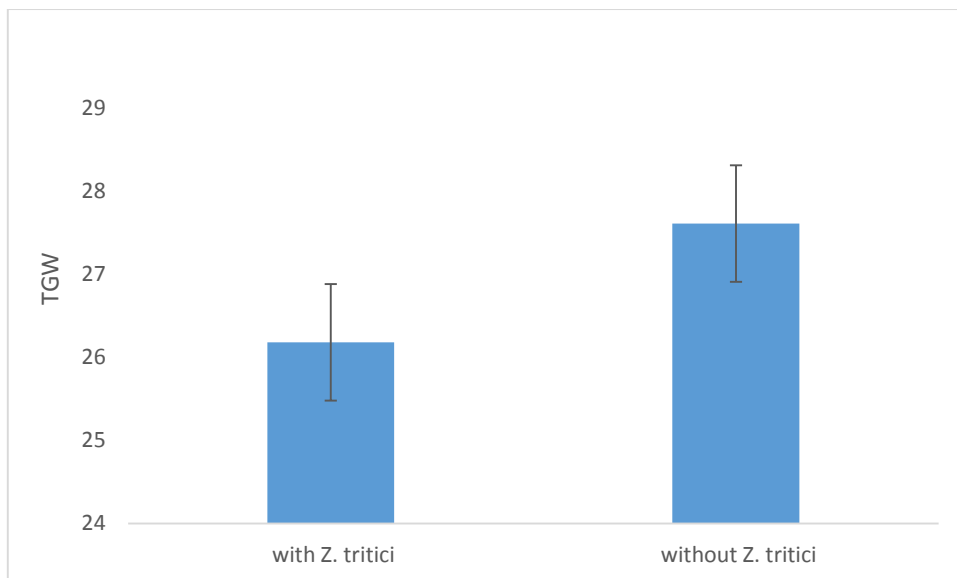
Figure 49. Effect of *Z. tritici* inoculation on total thousand grain weight from cv. Paragon 2012-2013 season inoculated with *Z. tritici* or not, averaged over other factors. (*P*. 0.08; Error bar = ± S.E.D, d.f. = 47).

Nitrogen concentration in wheat flour was slightly reduced in plots inoculated with *Z. tritici* (Figure 50). Please refer to Table 2F-36 (Appendix) for more detal.
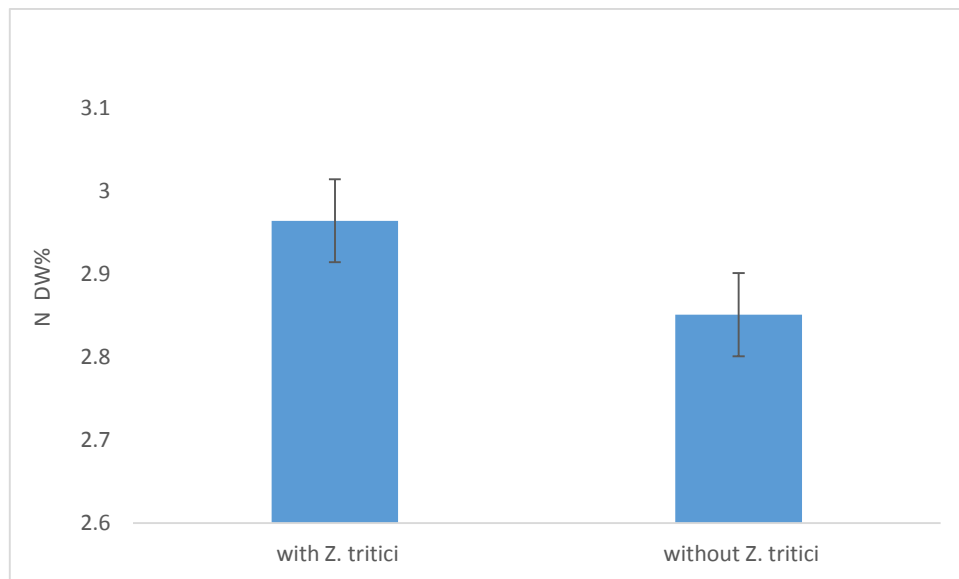
Figure 50. Effect of *Z. tritici* inoculation on Nitrogen concentration in flour from cv. Paragon 2012-2013 season treated with four alternative fertilizers and inoculation either with or without *P. nodorum* and *Z. tritici*. (*P*. 0.08; Error bar = ± S.E.D, d.f. = 47).

5-3-2- 2013-2014 season (cv. Gallant and M. Huntsman):

M. Huntsman yielded 33% more than Gallant, presumably because of the severe attack of yellow rust. Sulphur fertilizer increased significantly the concentration of sulphur in dry flour, and reduced both the concentration of nitrogen in dry flour, and N/S ratio (P <0.001), in both wheat cultivars. In addition, sulphur fertilizer increased total yield of both cultivars. Fertilizers did not affect Hagberg falling number or harvest index. There were slight differences in specific weight due to potassium fertilizers, larger in M. Huntsman than in Gallant. Sulphur fertilization reduced TGW by 10 and 11.5 % in comparison with potassium chloride and calcium silicate respectively with cv. Gallant but not with M. Huntsman; (Table 17). Please refer to Tables 2F-37 – 2F-46 (Appendix) for more detail.

Table 16. Mean values of yield and quality parameters in winter wheat grown in the 2013-2014 season under different fertilizer regimes. Sodium dodecyl sulphate (SDS)/ml volume, thousand grain weight (TGW/g), specific weight (kg/hl), harvest index H.I., sulphur in dry flour S (%), nitrogen in dry flour (%), ratio of nitrogen to sulphur N/S, hagberg falling number (HFN/s), and total yield/kg at 15% are shown. Statistical significance of differences between treatments is indicated by *P* value. Date of sowing 8th October 2013.

Cultivar	Fertilizer	SDS, ml	TGW, g	Spec. weight kg/hl	H.I.	S %DW	N %DW	N/S ratio	HFN	Total yield at 15% moisture t/ha
M. Huntsman	$CaSiO_3$	48.3	44.2	65.3	0.48	0.12	2.20	18.9	358.1	6
	$KCl+Ca(OH)_2$	49.0	44.2	65.4	0.50	0.12	2.17	18.3	351.8	6.3
	K_2SO_4	49.0	43.2	63.4	0.47	0.14	2.05	15.2	352.8	6.7
Gallant	$CaSiO3$	75.03	41.3	63.0	0.47	0.13	2.50	19.8	442.8	4.4
	$KCl+Ca(OH)_2$	77.4	40.6	62.2	0.46	0.13	2.50	19.6	458.6	4.3
	K_2SO_4	83.7	38.6	61.2	0.46	0.15	2.42	15.6	456.1	4.9
Cultivar L.S.D.		5.60	4.5	4.9	0.03	0.016	0.08	2.0	18.5	0.8
Fertilizer L.S.D.		3.70	1.2	1.6	0.02	0.006	0.03	1.3	20.5	0.3
Cultivars × Fertilizer L.S.D.		6.80	4.7	5.1	0.04	0.17	0.09	2.4	29	0.9
cultivar *P. value*		<.001	0.1	0.3	0.27	0.07	<0.001	0.37	<0.001	<.0001
Fertilizer *P. value*		0.04	0.01	0.05	0.29	<0.001	<0.001	<0.001	0.88	0.002

5-3-2-1-Green leaf area 2013-2014 season:

At growth stage 72, green leaf area of cv. M. Huntsman was 53% greater than cv. Gallant. There were no significant differences in green leaf area between the fertilizer treatments with cv. Gallant, but with cv. M. Huntsman there were slight differences between sulphate fertilized plots and the plots fertilized with calcium silicate or potassium chloride of 66, 76, and 73 mm^2 respectively. (Figure 51). Please refer to Table 2F-42 (Appendix) for more detail.
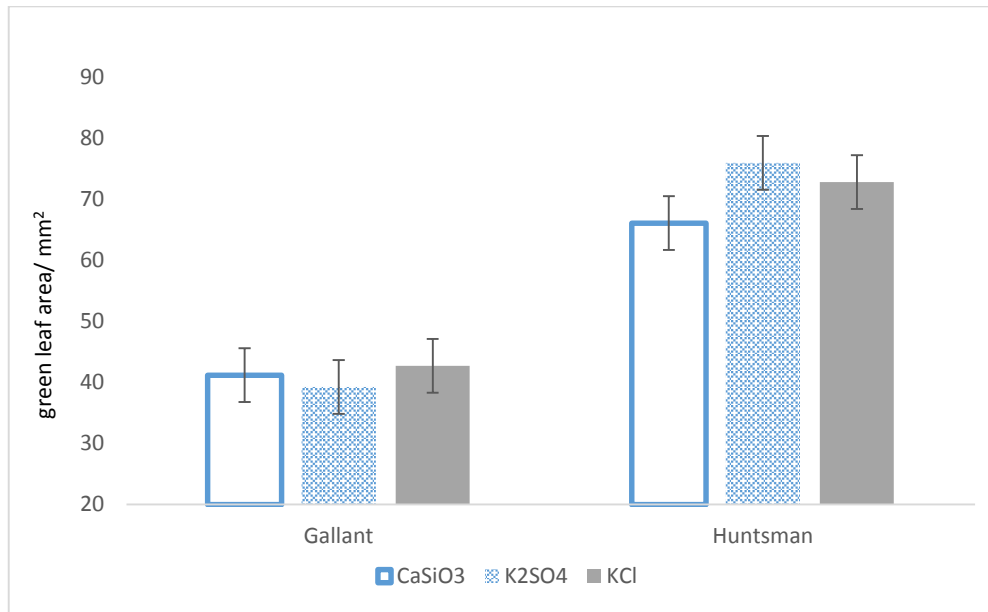
Figure 51. Green leaf area/ mm^2, in two wheat cultivars treated with three alternative fertilizers in factorial combination, in 2013-2014 season. (P= 0.04), (Error bar = ± S.E. D., d.f. = 71)

5-3-2-2- Yield:

Cultivar M. Huntsman achieved a higher yield than Gallant ($P. <0.001$). cv. M. Huntsman had a higher sulphur concentration than Gallant (Figure 52).
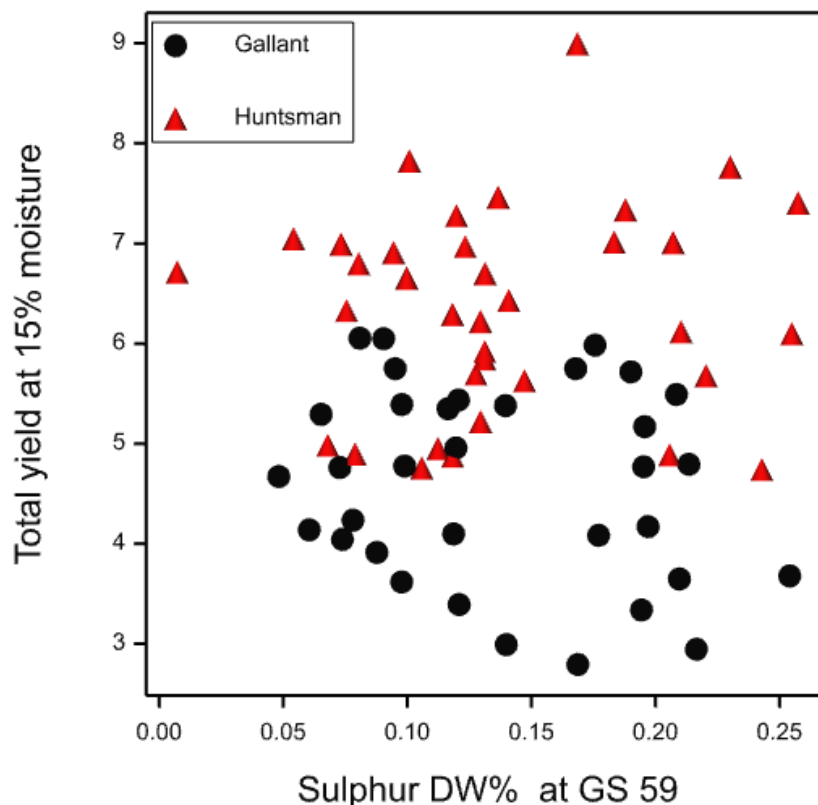
Figure 52. Relationships between whole-plant sulphur concentration at growth stage 59 and total yield at 15 % moisture in winter wheat cultivars M. Huntsman and Gallant in 2013-2014 season.

The correlation of yield with other quantity and quality measurements in 2013-2014 season was low in both cultivars. There were differences between the two cultivars: Gallant outperformed M. Huntsman. Thousand-grain weight and specific weight ($r = 0.57$ and 0.67; $P. <0.001$ and <0.001 respectively), (Figure 54 C and D) were higher in Gallant than in M. Huntsman ($r = 0.4$, 0.46 respectively), (Figure 53, C and D). Nitrogen concentration in grain flour was negatively related to total yield in both cultivars, but more in cv. Gallant ($r = -0.77$), (Figure 54 H) than M. Huntsman ($r = -0.6$), (Figure 53 H).
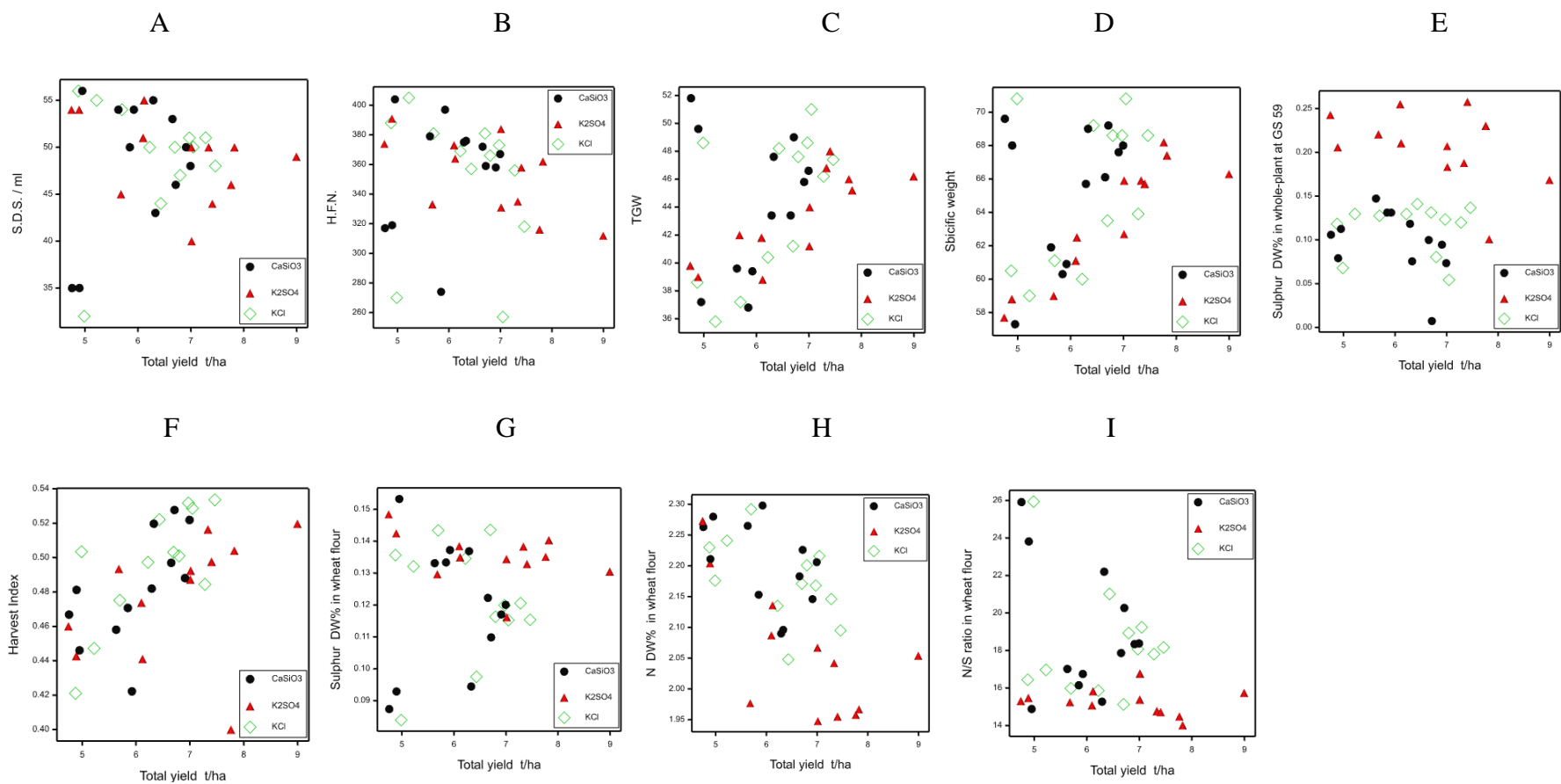
Figure 53. Relationship between total yield T/ha of winter wheat cv. M. Huntsman for 2013-2014 season with each of (A) S.D.S sodium dodecyl sulphate (r = 0.02; P = 0.9), (B) H.F.N. hagberg falling number (r = -0.2; P = 0.2), (C) TGW thousand grain weight (r = 0.4; P = 0.015), (D) specific weight of wheat grain (r = 0.46; P = 0.04), (E) sulphur concentrations in whole-plant at growth stage 59 (r = 0.05; P = 0.8), (F) Harvest Index (= 0.3; P = <0.001), (G) Sulphur concentrations in wheat flour (r = 0.05; P = 0.8), (H) nitrogen concentration in wheat flour (r = -0.6; P= <0.001), and (I) N/S ratio in wheat flour (r = -0.3; P = 0.07).
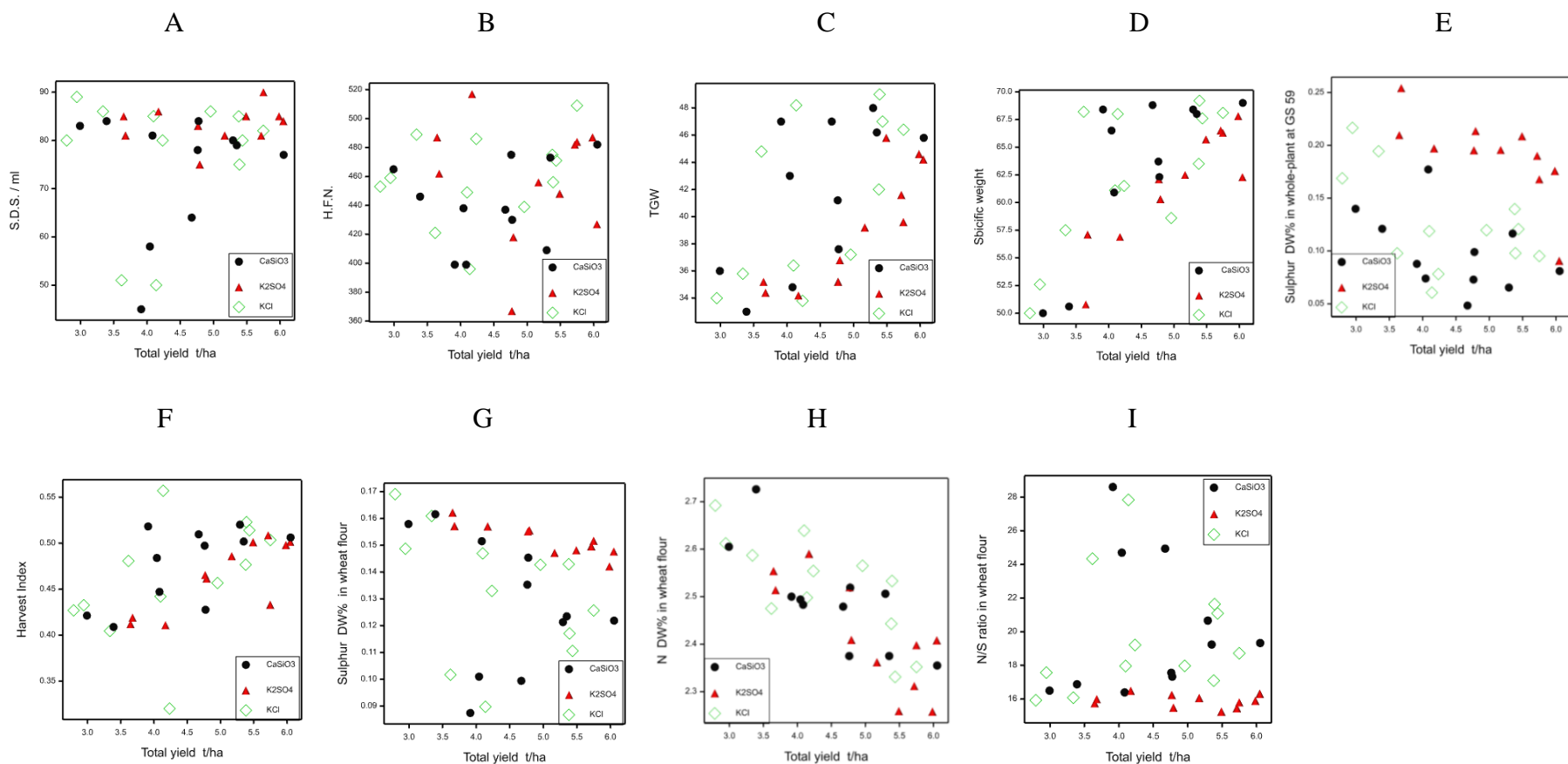
Figure 54. Relationship between total yield T/ha for winter wheat cv. Gallant for 2013-2014 season with each one of (A) S.D.S sodium dodecyl sulphate (r = 0.2; P = 0.3), (B) H.F.N. hagberg falling number (r = 0.15; P = 0.4), (C) TGW thousand grain weight (r = 0.57; P = <0.001), (D) specific weight of wheat grain (r = 0.67; P = <0.001), (E) sulphur concentrations in whole-plant at growth stage 59 (r = -0.15; P = 0.3), (F) Harvest Index (r = 0.53; P = 0.0014), (G) Sulphur concentrations in wheat flour (r = -0.12; P = 0.5), (H) nitrogen concentration in wheat flour (r = -0.77; P = <0.001), and (I) N/S ratio in wheat flour (r = -0.15; P = 0.4).

5-3-2-3- Interactions between fertilizer and disease on yield and quality parameters:

Sulphur fertilizer decreased concentration of nitrogen independently of *P. nodorum* or *Z. tritici* inoculation, except when inoculated with both pathogens. Any effects of silicon fertilization were too small to detect against plot-plot variation (Figure 55). Please refer to Table 2F-44 (Appendix) for more detail.
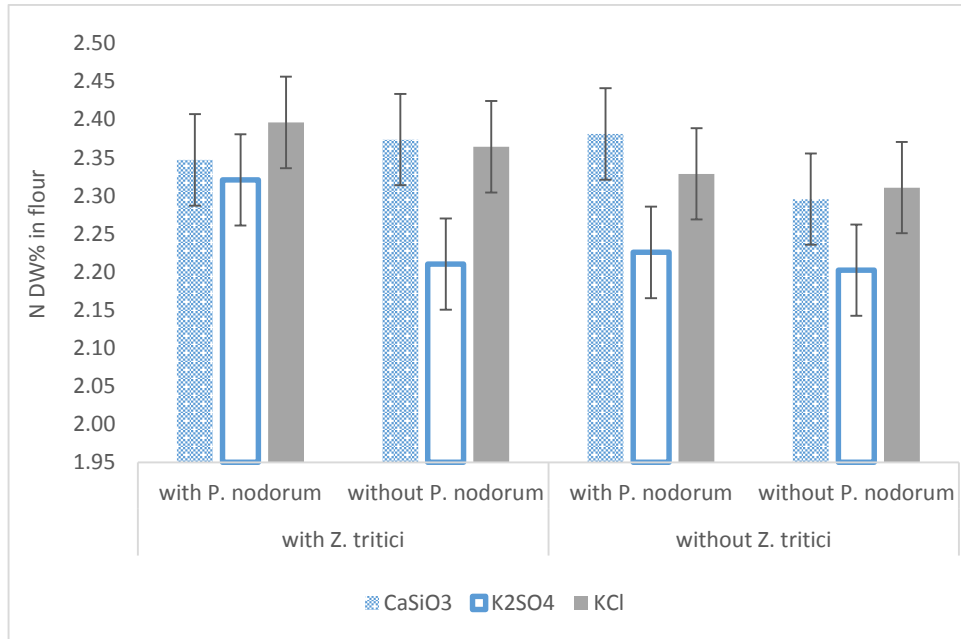
Figure 55. Nitrogen concentration in wheat flour from both cultivars M. Huntsman and Gallant (2013-2014) treated with one of three fertilizers and inoculated either with or without *P. nodorum* and *Z. tritici* in a factorial combination. (*P*. 0.014), (Error bar = ± S.E.D, d.f. = 71).

There were no statistically significant differences in N/S ratio between plots treated with the different fertilizers when the wheat was inoculated with both pathogens. Otherwise, sulphur fertilization decreased N/S ratio, irrespective of inoculation, by 21% in comparison with the other fertilizers (Figure 56). Please refer to Table 2F-45 (Appendix) for more detail.
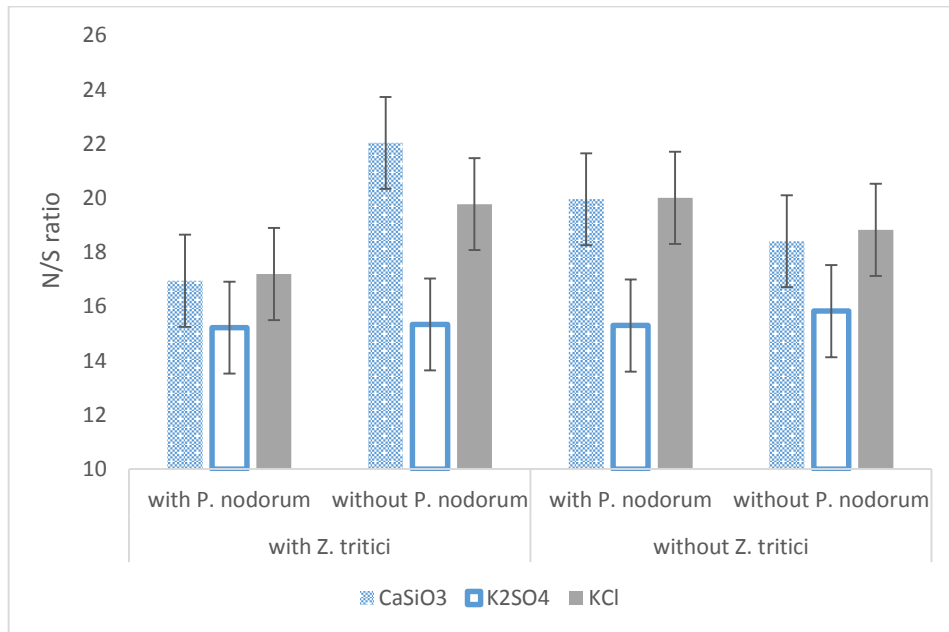
Figure 56. N/S ratio in wheat flour from cultivars M. Huntsman and Gallant (2013-2014) treated with one of three fertilizers and inoculated either with or without *P. nodorum* and *Z. tritici* in a factorial combination. (*P.* = 0.038), (Error bar = ± S.E.D, d.f. = 71).

There was a slight interaction between inoculation with *Z. tritici* and *P. nodorum* in their effect on S.D.S. S.D.S was decreased by 13% when plots were inoculated with *Z. tritici* as well as *P. nodorum* but in plots without *P. nodorum Z. tritici* inoculation reduced S.D.S. (Figure 57). Please refer to Table 2F-46 (Appendix) for more detail.
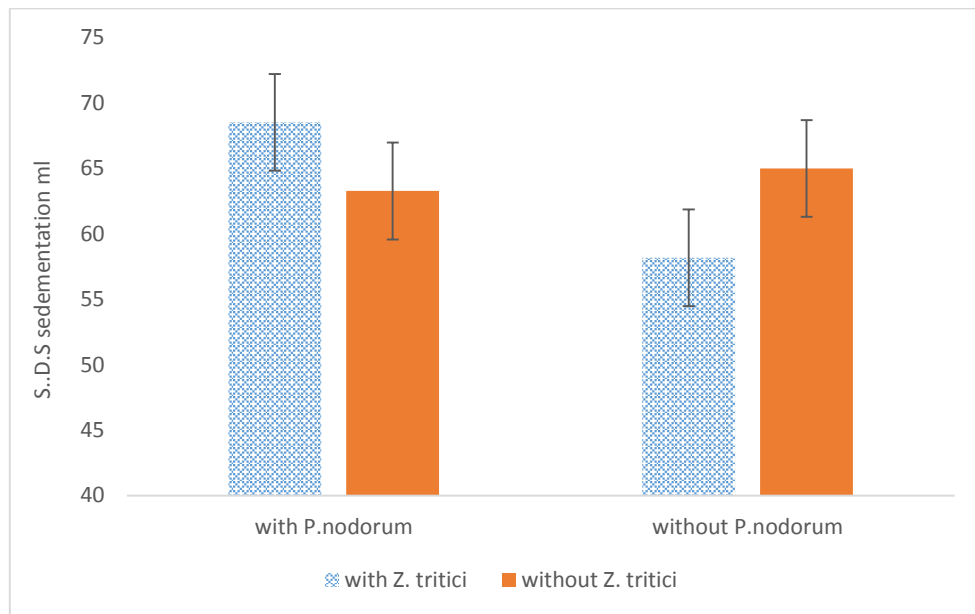
Figure 57. S.D.S. in wheat flour from 2013-2014 plots inoculated either with or without *P. nodorum* and *Z. tritici* in a factorial combination averaged over cultivars M. Huntsman and Gallant. (*P*. 0.04), (Error bar = ± S.E.D, d.f. = 71).

5-3-3- 2014-2015 season (cv. M. Huntsman):

Sulphur fertilizer increased both sodium dodecyl sulphate sedimentation and the concentration of sulphur, and reduced the N/S ratio (all *P.* <0.001). It reduced thousand-grain weight, nitrogen concentration and specific weight (*P.* <0.001, <0.001 and 0.005 respectively; Tables 18). Please refer to Tables 2F-47 – 2F-56 (Appendix) for more detail.

Table 17. Effect of fertilizers on each of sodium dodecyl sulphate SDS volume/ml, thousand grain weight TGW/g, specific weight, low sulphur in dry flour S%DM, nitrogen in dry flour N%DM, ratio of nitrogen upon sulphur N/S, hagberg falling number HFN, and total yield at 15% t/ha. Statistical significance of difference between treatments is indicated by *P.* value. cv. M. Huntsman; date of sowing 1st October 2014.

fertilizer	S.D.S (ml)	H.F.N.(s)	T.G.W. (g)	specific weight (kg/hl)	N (%)	S (%)	N/S	Total yield at 15% (t/ha)
sulphur -	44.4	294	55	74	2.3	0.10	23.2	4.3
sulphur +	55.2	310	51	73	2.1	0.13	16.1	6.2
L.S.D.	2.7	32	0.5	0.5	0.04	0.006	1.2	0.47
P value	<0.001	0.3	<0.001	0.005	<0.001	<0.001	<0.001	<0.001

5-3-4- Relationship of yield to sulphur concentration at growth stages 31 and 77 and quality parameters:

Sulphur concentration at growth stage 31 was 78% higher than concentration of sulphur at growth stage 77, but there was little correlation between the two sets of data. (Figure 58 A). Please refer to Tables 2F-50 and 2F-51 (Appendix) for more detail.

Yield was strongly correlated with most other measurements, either positively or negatively. Sulphur concentration in whole plants at GS 31 and 77 was positively correlated with yield (r = 0.65 and 0.44; *P*. <0.001 and 0.007 respectively), (Figure 59 B and C). Hence, sulphur concentration in whole plants at GS 31 was a better predictor of total yield than concentration at GS 77. Please refer to Tables 2F-50, 2F-51 and 2F-56.

Yield was strongly correlated with the quality parameters. It was positively correlated with S.D.S and sulphur concentration in flour (r = 0.85; P<0.001, r = 0.76; P = <0.001 respectively; Figure 59 A and E). It was negatively correlated with nitrogen concentration in flour and N/S ratio (r= -0.9; P = <0.001, r = -0.9; P = <0.001) (Figure 59 F and G). Please refer to Tables 2F-47, 2F-49 and 2F-56 (Appendix) for more detail.
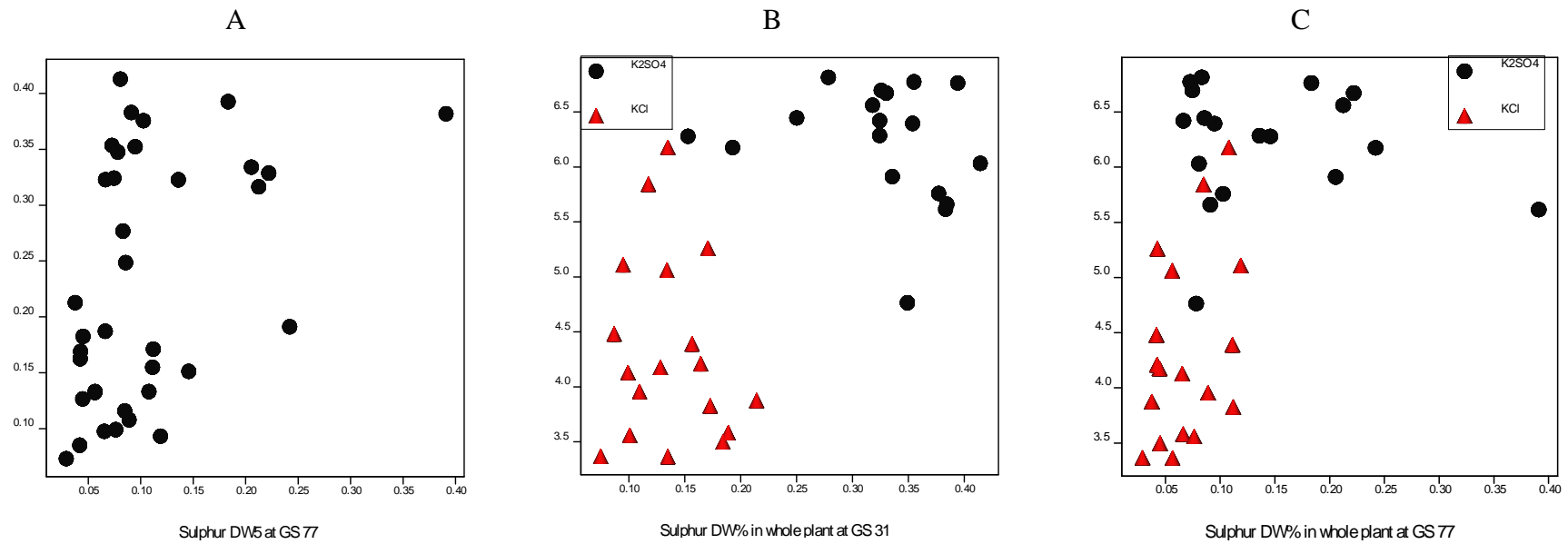
Figure 58. (A) Relationship between whole-plant sulphur concentrations at growth stage 31 and 77 (r = 0.42; P = 0.01). (B) relationship between yield t/ha and sulphur concentration in whole plant at growth stage 31(r= 0.65; P = <0.001). (C) relationship between total yield t/ha and sulphur concentration at growth stage 77 (r = 0.44; P = 0.007).
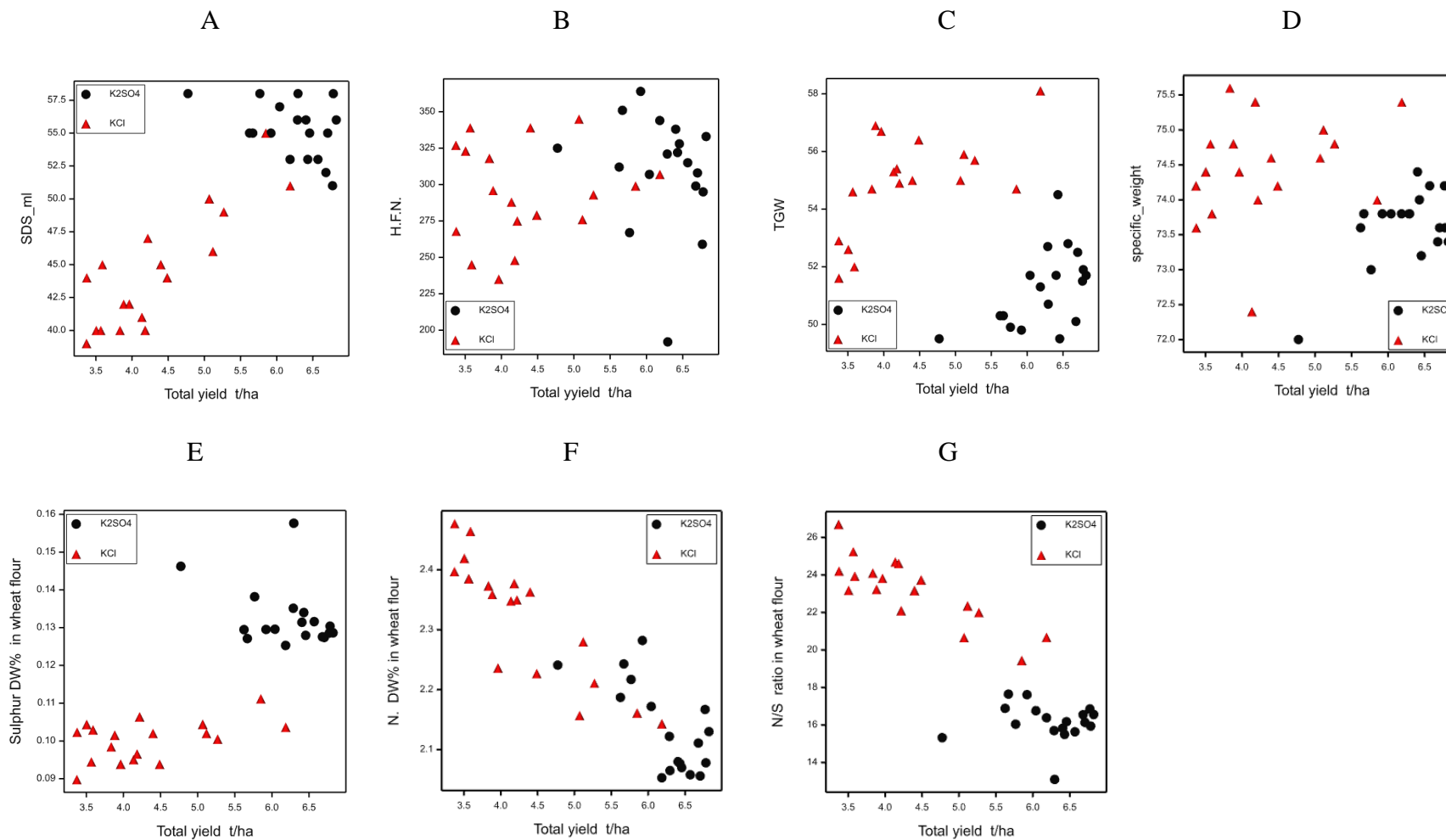
Figure 59. Relationship between total yield (T/ha) of winter wheat cv. M. Huntsman in the 2014-2015 season with (A) S.D.S sodium dodcyl sulphate (r = 0.85; P = <0.001), (B) H.F.N. hagberg falling number (r = 0.2; P = 0.4), (C) TGW thousand grain weight (r = -0.4; P = 0.013), (D) specific wieth of wheat grain (r = -0.25; P = 0.13), (E) Sulphur concentration in wheat flour (r = 0.76; P = <0.001), (F) nitrogen concentration in wheat flour (r = -0.9; P = <0.001), and (G) N/S ratio in wheat flour (r = -0.9; P = <0.001).

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5-3-5- Effect of *P. nodorum* inoculation on yield and quality parameters:

Spray inoculation with *P. nodorum* slightly increased Hagberg falling number, by 7% and 6% in comparison with seed inoculation or no inoculation respectively (Figure 60). Please refer to Table 2F-52 (Appendix) for more detail.
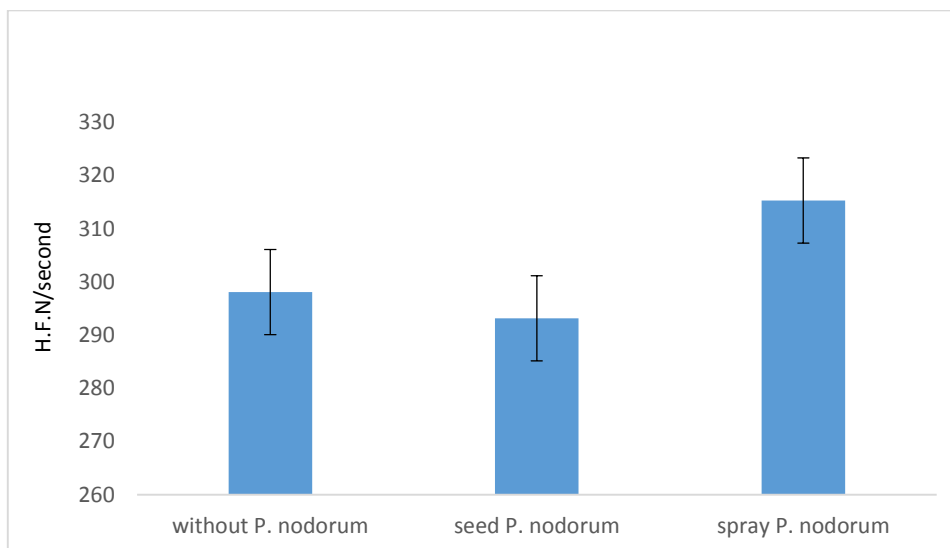
Figure 60. Hagberg falling number in winter wheat cv. M. Huntsman (2014-2015) inoculated either with two different ways of inoculated or without inoculated by *P. nodorum*. (*P.* 0.055; Error bar = ± S.E.D, d.f. = 35).

Spray inoculation with *P. nodorum* increased nitrogen concentrations in wheat flour by 3% and 5% in comparison with either seed inoculation or no inoculation (Figure 61). Please refer to Table 2F-53 (Appendix) for more detail.
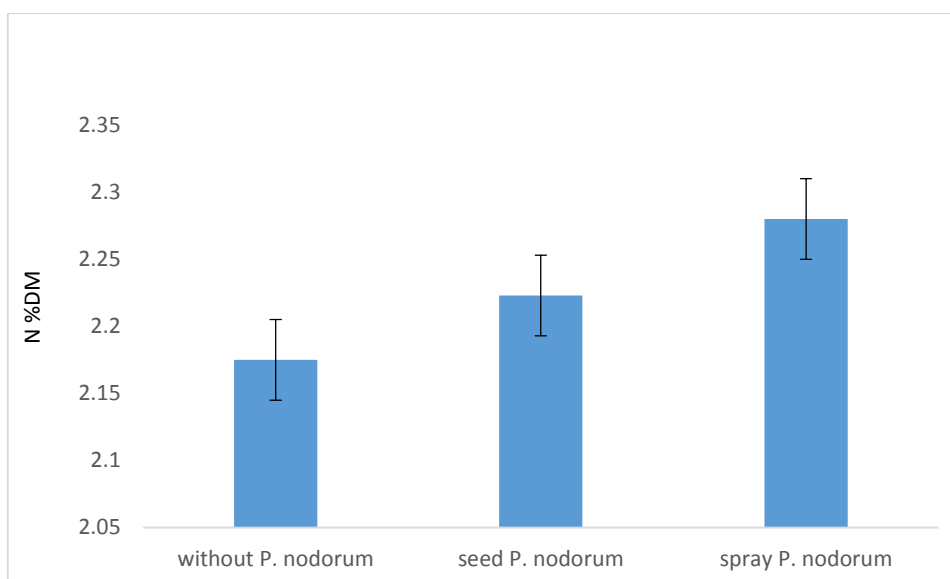
Figure 61. Nitrogen concentrations in flour from cv. M. Huntsman (2014-2015) inoculated either with two different ways of inoculated or without inoculated by *P. nodorum*. (*P.* 0.026; Error bar = ± S.E.D., d.f. = 35).

Spray inoculation with *P. nodorum* decreased TGW by 2.5% and 5% in comparison with seed inoculation and no inoculation respectively (Figure 62). Please refer to Table 2F-54 (Appendix) for more detail.
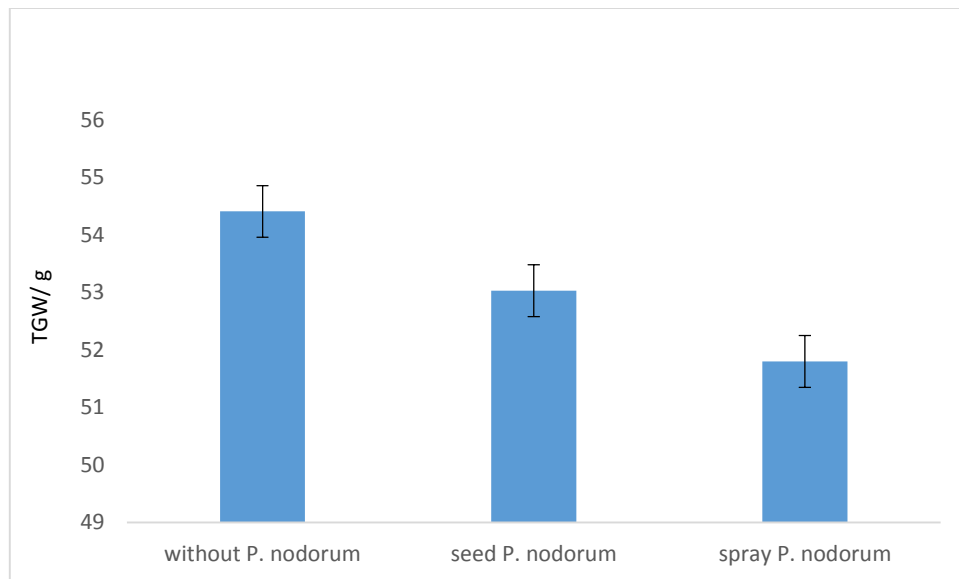
Figure 62. Hagberg falling number in winter wheat cv. M. Huntsman (2014-2015) inoculated in one of two ways with *P. nodorum*. ($P.$ <0.001; Error bar = ± S.E.D., d.f. = 35).

In plots fertilized with potassium sulphate, spray inoculation with *P. nodorum*, and to a lesser extent seed inoculation, decreased thousand-grain weight regardless of the fungicide spray (Figure 63, A). In plots fertilized with potassium chloride and sprayed with fungicide inoculation with *P. nodorum* did not affect TGW. In plots fertilized with potassium chloride spray or seed inoculation with *P. nodorum* reduced TGW, spray more than seed (Figure 63, B). Please refer to Table 2F-54 (Appendix) for more detail.
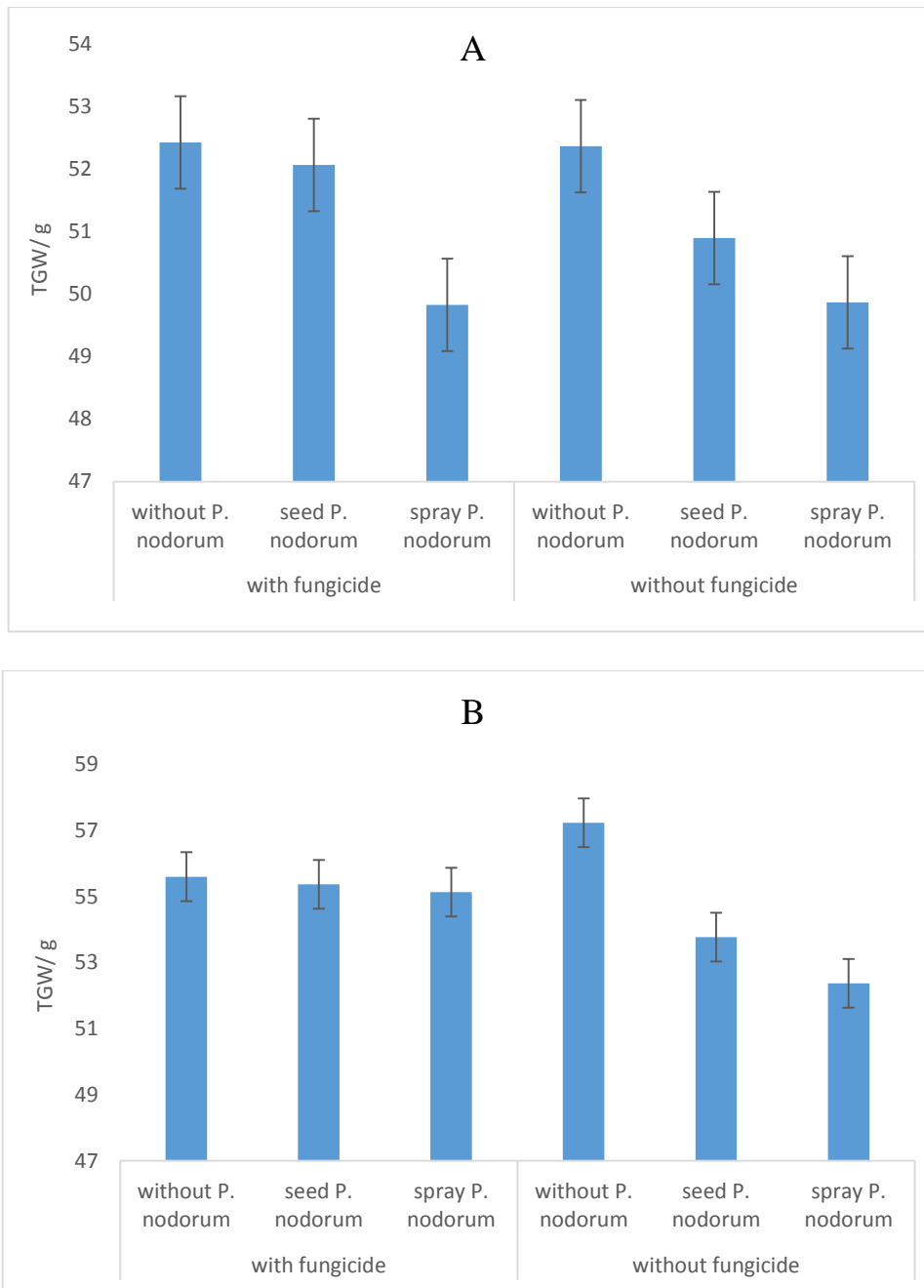
Figure 63. TGW of wheat grain from cv. M. Huntsman (2014-2015) treated with A: K_2SO_4; B: KCl fertilizer, fungicide and inoculated either with or without *P. nodorum* as a factorial combination (*P*. 0.005; Error bar = $\pm$ S.E.D, d.f. = 35).

Seed or spray inoculation of *P. nodorum* decreased specific weight, more or less equally. (Figure 64). Please refer to Table 2F-55 (Appendix) for more detail.
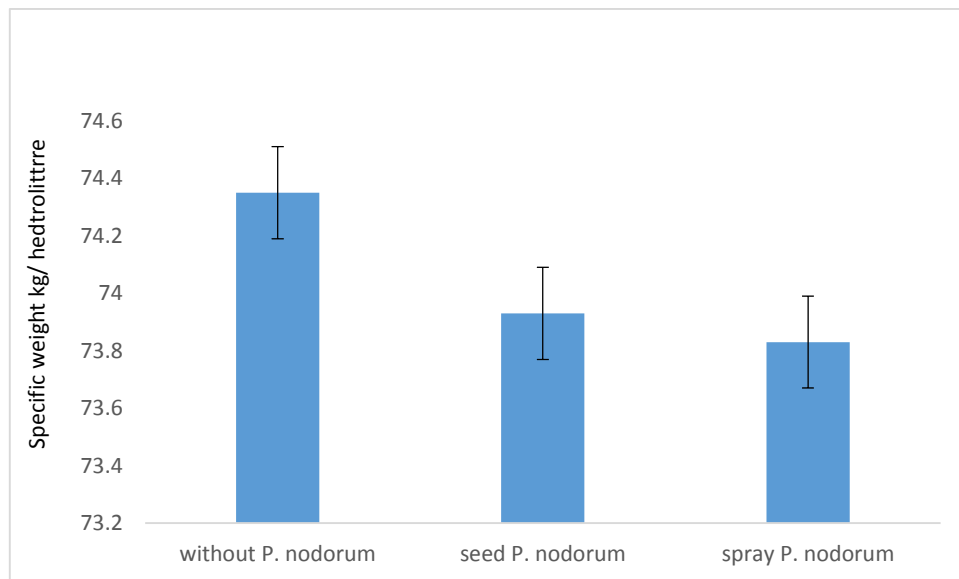
Figure 64. Specific weight of wheat grain from cv. M. Huntsman (2014-2015) inoculated either with two different ways of inoculated or without *P. nodorum* as a factorial combination (*P*. 0.023; Error bar = ± S.E.D, d.f. = 35).

Spray inoculation with *P. nodorum* decreased total yield by 10% in comparison with non-inoculation (Figure 65; P = 0.05). Again seed inoculated plots were intermediate. Please refer to Table 2F-56 (Appendix) for more detail.
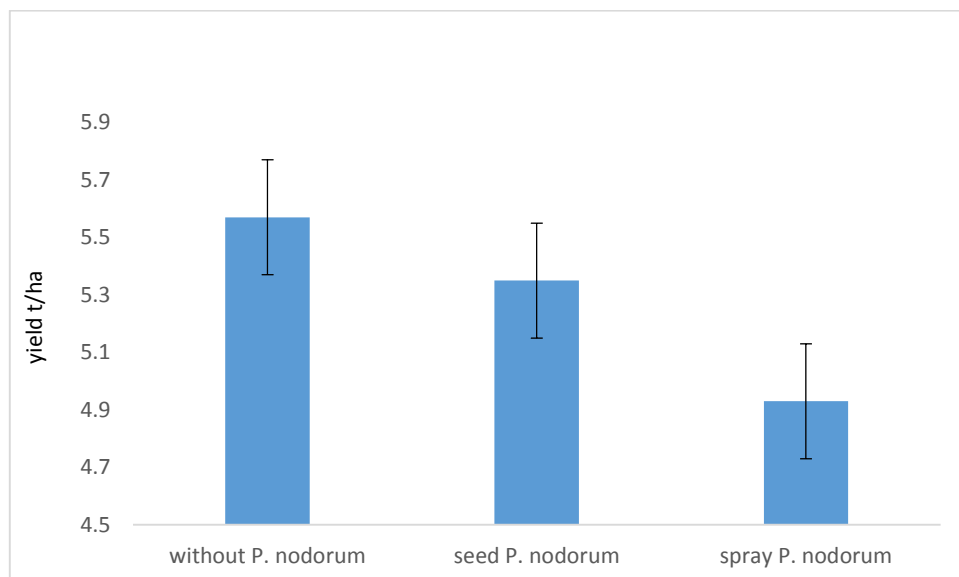
Figure 65. Total yield of wheat grain (t/ha) from cv. M. Huntsman (2014-2015) inoculated with (in one of two ways) or without *P. nodorum* averaged over fertiliser and fungicide treatments (*P*. 0.05; Error bar = ± S.E.D, d.f. = 35).

5-3-6- Correlations between quality and yield variables:

Indicator measurements of wheat bread making quality are S.D.S and N/S ratio. Both of these measurements were correlated more highly with sulphur concentration at GS 31 than at GS 77 (S.D.S at GS 31 r = 0.73, at GS77 r = 0.37), (Figure 67 D and E) and grain N/S ratio at GS31 r = 0.79, at GS77 r = 0.46), (Figure 66 D and H).
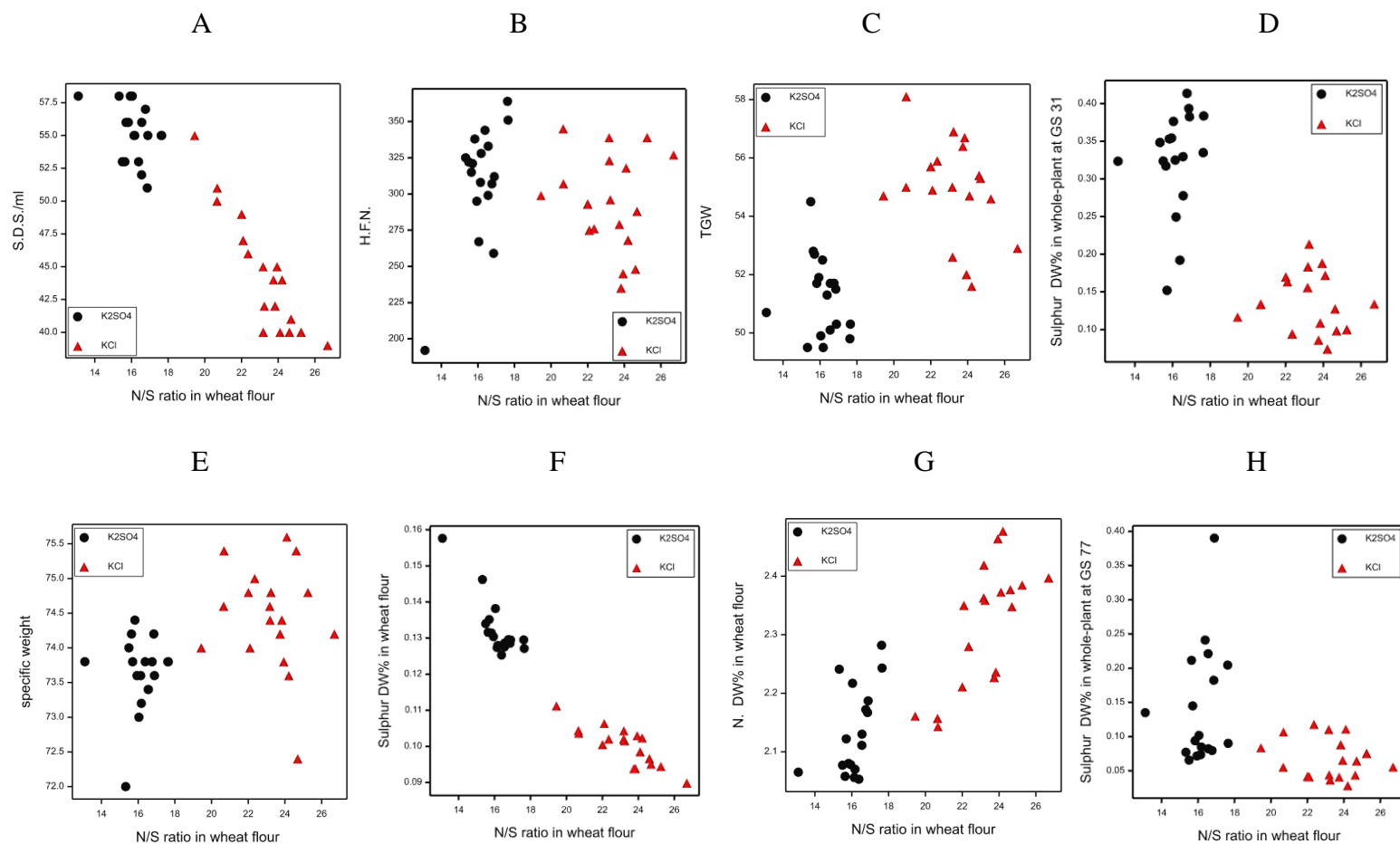
Figure 66. Relationship between N/S ratio in flour from winter wheat cv. M. Huntsman in 2014-2015 season with each one of (A) S.D.S sodium dodecyl sulphate (r = -0.94; P = <0.001), (B) H.F.N. hagberg falling number (r = -0.12; P = 0.5), (C) TGW thousand grain weight (r = 0.64; P = <0.001), (D) sulphur concentrations in whole-plant at growth stage 31 (r = -0.79; P = <0.001), (E) specific weight of wheat grain (r = 0.47; P = 0.004), (F) Sulphur concentrations in wheat flour (r = -0.96; P = <0.001), (G) nitrogen concentration in wheat flour (r = -0.83; P = <0.001), and (H) sulphur concentrations of whole-plant at growth stage 77 (r = -0.46; P = 0.005).
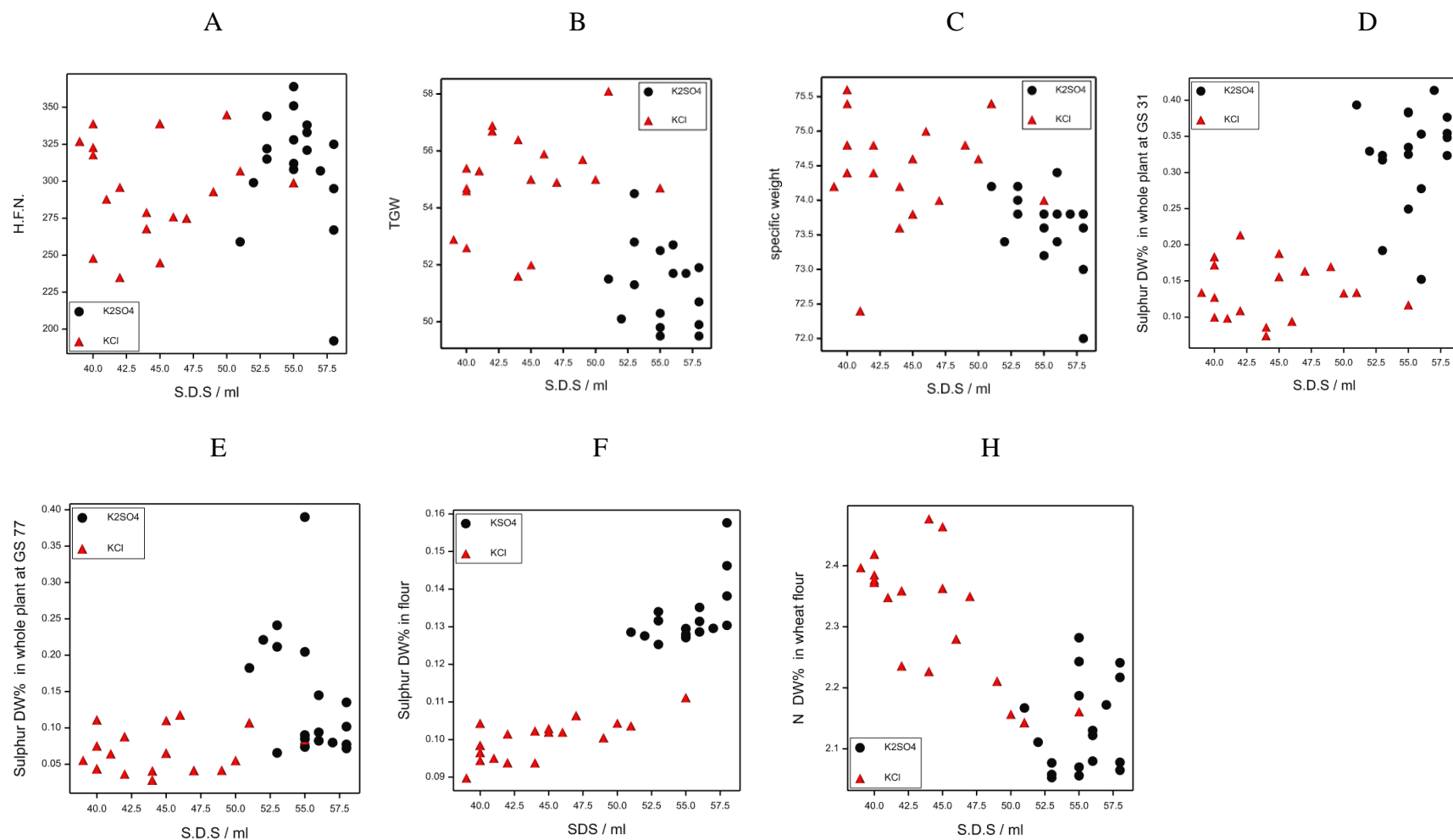
Figure 67. Relationship between Sulphur Dodecyl Sedementation in flour from winter wheat cv. M. Huntsman in 2014-2015 season with (A) H.F.N., hagberg falling number (r = -0.15; P = 0.4), (B) TGW, thousand grain weight (r = -0.61; P = <0.001), (C) specific weight of grain (r = -0.49; P = 0.002), (D) sulphur concentrations in whole-plant at growth stage 31 (r = 0.73; P = <0.001), (E) sulphur concentrations of whole-plant at growth stage 77 (r = 0.37; P = 0.02), (F) Sulphur concentrations in flour (r = 0.89; P = <0.001), and (G) nitrogen concentration in flour (r = -0.78; P = <0.001).

5-4 Discussion:

5-4-1 Effect of fertilizers on yield and quality:

Over all, the results showed that quality and yield were improved by sulphur fertilization, while there was no effect of silicon fertilization on quality and yield. The quality of wheat in 2013 (cv. Paragon) was significantly improved by sulphur fertilization, but there was no effect on yield. This might have been because the rainfall in this season was lower than normal in the period between June and July (Table 15), which is important for grain filling (Liam, 1997). Furthermore, the glasshouse experiment (chapter 3) showed that cv. Paragon had a higher percentage of dead leaves than other varieties. Thus, the failure of yield to respond to sulphur fertilisation may be due to rapid maturation and limited green leaf area.

In 2013-2014, there were differences between cultivars in the quality and yield parameters. Quality parameters (SDS, HFN, S DW% and N DW% in flour) were higher in cv. Gallant. Gallant is a modern bread making variety, while cv. M. Huntsman was a bread making variety bred about 50 years ago (1970). Although, S DW% was increased and N DW% and N/S ratio decreased with sulphur fertilization, SDS and HFN were not affected by sulphur fertilization. Although most of the plots suffered from flooding (Figure 13, picture 1) which might have led to leaching of sulphur fertilizer, sulphur fertilizer was applied as split dressing, and caused clear differences in yield. Yield of both cultivars was improved by sulphur fertilization, but the yield was much higher in cv. M. Huntsman than Gallant. This might be because yellow rust infected cv. Gallant very severely. Moreover, green leaf area at GS 77 for M. Huntsman was higher than Gallant indicating an extended flag leaf life and consequently increased yield.

In 2014-2015, all quality and yield measurements were considerably affected by sulphur fertilization. That reflected the role of sulphur in improving the quality characteristic of wheat especially in relation to bread making (Ryant & Hřivna 2004; Kuktaité 2004; Steinfurth *et al.*, 2012; Ercoli 2012). The concentration of sulphur in whole plants was higher at growth stage 31 than at growth stage 77. Furthermore, yield was significantly correlated with sulphur concentration at growth stage 31but not at growth stage 77. Likewise, sulphur concentration in whole plants was correlated with S.D.S., and N/S ratio more highly at growth stage 31 than at growth stage 77.

The use of tebuconazole to reduce disease in the second third of April had no effect on quality or yield measurements. That might be because *Z. tritici* is substantially resistant (Mavroeidi & Shaw, 2006; Curvers *et al.*, 2015). Pepler *et al.* (2005), Ruske *et al.* (2004) and Gooding *et al.* (1991) all reported that utilization of fungicide sprays against disease contributes to decreased sulphur

concentration in grain, but within this study the effect was not found, which is consistent with the lack of disease control. In the earlier work use of this fungicide in commercial programmes gave reductions of 90% in disease.

Although sulphur fertilization increased most of the parameters, thousand-grain weight was reduced with sulphur. This might be because the ratio of protein to starch was higher.

5-4-2 Effect of disease on yield and yield quality:

Many studies have investigated the effect of septoria diseases on yield (Eyal *et al.*, 1987; Shaw and Royle 1989; Serrago 2009). In my work, yield and TGW were reduced by *Z. tritici* inoculation by 14.3% and 5% respectively in spring wheat grown in 2013. The concentration of nitrogen in flour was increased by *Z. tritici* inoculation by 4% in that season. Although cv. Paragon is a fairly resistant cultivar (AHDB 2013), the glasshouse experiment (chapter 3) showed that it was susceptible when inoculated with *Z. tritici*. Reduction in the flag leaf area influences grain filling. The concentration of nitrogen in flour was increased and TGW decreased in plots inoculated with *Z. tritici*. That indicates an increase in the ratio of protein to starch in grain. However, there was no effect of *Z. tritici* inoculation on yield in the other two seasons. There was a reduction in yield of 10% caused by spray inoculation of *P. nodorum* in 2014-2015. This inoculation also reduced both TGW and specific weight in 2014-2015, but increased the percentage of nitrogen in flour by 4%, and slightly increased H.F.N. This may be explained by the control of yellow rust and a sufficient amount of sulphur fertilizer. Half the plots received fungicide (tebuconazole). This might have led to less *P. nodorum* on the upper leaf (flag leaf).

TGW, S.D.S, N/S ratio, and the percentage of nitrogen in flour measured in different seasons were affected by the interaction between fertilizer treatments and pathogens. In 2014-2015 TGW was increased in a way which depended on an interaction between fertilizers, fungicide and spray inoculation of *P. nodorum.* This might be explained by the role of potassium in grain, through increased content of starch. In 2013 sulphur as potassium sulphate significantly decreased N/S ratio, with or without pathogen inoculation. Many studies have shown that sulphur stimulates nitrogen uptake (Serrago *et al.*, 2009), consequently it increased the concentration of nitrogen.

Despite sulphur fertilization improving the quality measurements over three experiments, the effects on yield varied. This depended upon the wheat cultivar, disease severity, and the time of applied sulphur fertilization. The results from the previous chapter and this chapter showed that *Z. tritici* and improved quality and yield parameters were significantly correlated with the sulphur concentration at growth stage 31. Many studies have shown a positive effect of sulphur on some or

most quality and yield measurements without referring to the effect on diseases as part of the mechanism. The novelty of this chapter was to investigate the effect of sulphur on the quality and yield of wheat crops under disease pressure. Sulphur fertilization reduced both pathogens (but *Z. tritici* was reduced more than *P. nodorum),* and when the disease severity was reduced, a consequent improvement in quality and yield occurred.

CHAPTER SIX:

6-1- General Discussion:

This thesis investigated the effect of sulphur and silicon fertilization on septoria diseases, yield and quality of wheat under field conditions without any chemical treatment against these diseases, except in the final experiment which used tebuconazole in specific plots. The abundance of both pathogens was affected by the supply of sulphur fertilizer. Bearchell *et al.* (2005) found, in a long period study, that there might be a causal link between air pollution by SO_2 and the abundance of both pathogens. Shaw *et al.* (2008) therefore argued that we should be cautious about forecasts of plant disease severity based on mechanistic models, because in short-term experiments the abundance of the two pathogens would not be linked to SO_2.

Several hypotheses were tested within this thesis. Firstly, sulphur fertilizer was expected to reduce the abundance of both *Z. tritici* and *P. nodorum* under field conditions. This study showed that both pathogens were reduced by sulphur fertilizer. Furthermore, sulphur fertilizer reduced the combined abundance of both pathogens. The second hypothesis was also accepted because the co-inoculation of both pathogens reduced the individual abundance compared to inoculation with a single pathogen. The third hypothesis was that sulphur fertilizer would improve wheat yield and quality under disease pressure, whereas silicon would not. This was broadly confirmed. Fourthly, sulphur fertilizer did not increase the proportion of P. nodorum with mixed or single infections. The final hypothesis was that, silicon fertilizer would reduce both pathogens and improve yield and quality under field conditions. This hypothesis was largely rejected.

6-1-1- Effect of sulphur fertilization on disease and yield and quality of wheat:

The glasshouse experiment showed that all cultivars were adapted to all isolates, and at the same time that the proportion of leaves infected was affected by sulphur fertilization. However, the conditions in a glasshouse are different from field conditions. Thus, the effect of pathogens may not be same under field conditions. Šíp *et al.* (2015) concluded that host resistance was strongly related to environment factors rather than being only a fixed reaction of cultivars to isolates.

The results from field experiments showed that *Z. tritici* and *P. nodorum* were reduced by sulphur fertilization. Though *P. nodorum* inoculation did not succeed in autumn, the spring spray in 2014-2015 was successful. Chandramohan (2010) found that *P. nodorum,* with a high level of sulphur fertilisation, induced resistance to *Z. tritici*. She also investigated the possible modes of action of sulphur on these pathogens: the percentage of *P. nodorum* conidia germinating was reduced more

strongly than that of *Z. tritici* in sulphurous acid at pH4. Also, at a high level of sulphur, the percentage of coverage by symptoms of *Z. tritici* on the second leaf was reduced much more than that of *P. nodorum*. Hence, due to a lesser effect of sulphur fertilization on *P. nodorum*, and greater on *Z. tritici*, it is plausible that the competition between these pathogens under sulphur fertilization leads to more reduction in *Z. tritici*. Therefore, this glasshouse result suggested it would be worthwhile to find the effect of applied sulphur fertilization under medium-scale field conditions. The results obtained from the experiments in this thesis showed that both pathogens were reduced by sulphur fertilization, but *Z. tritici* more than *P. nodorum*. Thus, the variation in abundance of both pathogens in the historical record might be explained in two different ways. First, the large effect of sulphur on *Z. tritici,* with less on *P. nodorum,* would make the abundance of *P. nodorum* more than *Z. tritici*. Second, with stable and adequate level of sulphur, *P. nodorum* in competition with *Z. tritici* would dominate *Z. tritici*.

Foliar diseases lead to reduced photosynthetic capacity of the plant through destruction of leaf tissue. This reduction causes loss in yield and grain quality of crops. Sulphur insufficiency in crops not only limits the growth of crops and subsequent grain yield, but also leads to poor quality of the product because of its relation to protein composition. It is well known that protein plays a key role in the process of bread-making. Pepler *et al.* (2005) mentioned that there is little documentation about the effect of fungicides on S and S/N ratio. Dimmock & Gooding (2002) reported that use of fungicides against foliar diseases can reduce or increase the grain protein concentration. For instance, control of yellow rust at flag leaf emergence led to increased grain yield and protein concentration, whilst controlling *Septoria* spp. often reduced the grain protein concentration. Pepler *et al.* (2005) found that susceptible cultivars (Malacca) infected with *Z. tritici* and treated with fungicide led to delays in flag leaf senescence but decreased the N and S in grain. Overall, the results in chapter six showed that sulphur fertilizer improves the quality and yield in both winter wheat cultivars, but not in the spring wheat experiment in 2013 (cv. Paragon). That may be because in the later experiments an adequate amount of sulphur fertilizer was divided and applied over the growth season, whereas in the first experiment the sulphur fertilizer was applied all at one time. In all experiments the sulphur fertilizer was applied to the soil, which might lead to leaching from the soil. Thus, more investigation is required to test the effect of applying the oxidised sulphur compounds as a foliar application to the leaves.

In the introductory chapter it was explained that deficiency of sulphur could be due to various reasons. One of these is the use of fertilizer alternatives containing no sulphur, such as the use of ammonium nitrate instead of ammonium sulphate. Thus, increasing the use of ammonium sulphate

might contribute to reducing the abundance of the both pathogens. Hence, more investigation is required to test this idea. Moreover, it would be desirable to investigate the interaction that may happen if both urea and ammonium sulphate were used; it could be beneficial to the farmer to use only one compound and applying at a single time.

Specific weight and TGW were reduced with sulphur fertilization in all experiments. Kifkafi *et al.* (2001) mentioned that potassium can increase the ratio betwen protein and starch in grain. However, comparison was made between plots receiving the same amount of potassium, in which the proportion of starch in the grain should be at the same level. Therefore, the increased ratio of protein to starch but reduced TGW and specific weight in grain from plots treated with sulphur fertilizer was a sulphur effect.

Fixen (1993) reported the role of chloride in suppressing numerous diseases on a variety of crop species. The real mechanism is unknown, but there are two suggestions. One is through suppression of disease and the other through increased tolerance of the crop. The Fixen study also reported that the mechanism was not directly on the pathogen. It also found that application of either NaCl or KCl reduced the severity of yellow rust in wheat, but the effect was greater when these two were applied together. Thus, more investigation is required to test the effect of chloride on decreasing or increasing the two pathogens studied in this research. Furthermore, by replacing the potassium sulpahte with ammonium sulphate to test the effect of sulphur and using urea in the control treatment would enable the effect sulphur to be investigated in absence of chloride.

The relationships between the variables measured in chapters five and six showed that the disease at growth stage 31 was correlated well with the sulphur concentration in the grain. Yield and quality measurements also correlated well with the sulphur concentration at growth stage 31. Both showed that adequate amounts of sulphur fertilizer throughout the growing season resulted in less disease and increased yield and quality of wheat grain. Hence, by replacing ammonium sulphate with ammonium nitrate or applying early (winter and autumn) may lead to inadequate amounts of sulphur. If inorganic sulphur is applied at one time it may leaching from the soil due to its high solubility. Thus, these results suggest that application of sulphur fertilizer throughout the growing season of wheat - for instance, dividing the amount of sulphur-containing fertilizer (as ammonium sulphate or as potassium sulphate) between four applications could lead to higher yields and improved quality of wheat grain. In addition, the sulphur supply to the leaves would be improved by a foliar application which might reduce the proportion of leaves infected by septoria diseases.

6-2- Conclusion:

The results from this thesis suggested that both *Z. tritici* and *P. nodorum* were reduced by sulphur fertilization. This suggested the change in relative prevalence of two pathogens are not due simply to opposite reactions to sulphur deposition, which confirms the work reported in Chandramohan (2010), and Chandramohan & Shaw (2013). In addition, the results suggested that the application of sulphur fertilizer throughout the growing season of wheat (ie, total application divided into four parts) could provide a supply of sulphur to wheat adequate to reduce *Z. tritici* and *P. nodorum* and improve yield and quality of grain.

6-3- Future work:

This study was done in soil that suffered sulphur deficiency, due to the reasons which were mentioned in chapter one. Thus, further investigations are required to test the effect of sulphur fertilization in different types of soil, in the area outside northern Europe, that have higher or lower sulphur concentrations in the soil. Investigation of the dose-related efficacy of sulphur fertilization in reducing either disease, and on quality and quantity of wheat. Further research could also be done using urea as a control for ammonium sulphate to remove the possibility of the chloride ion reducing disease as in this research. More biochemical investigations are needed to identify the way in which sulphur fertilisation modifies plant defences, perhaps focussing on the role and form of sulphur involved in producing glutathione. A more rapid way of detecting the pathogen from large field sampling would be useful, and an initial qPCR study was carried out and this is reported in appendix 1.

CHAPTER SEVEN:

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APPENDIX:

1- RAPID SAMPLING METHOD FOR ASSESSMENT OF DISEASE IN FIELD EXPERIMENTS BY QUANTITATIVE PCR.

1-1- Introduction:

Different techniques can be used to detect and quantify plant disease. These techniques work either by direct measurement of symptoms or by indirect detection using techniques such as enzyme-linked immunosorbent assay (ELISA), polymerase chain reaction (PCR) and Immunofluorescence (IF) (Prithiviraj *et al.*, 2004; Das, 2004; Li *et al.*, 2006; Saponari *et al.*, 2008; Ruiz-Ruiz *et al.*, 2009; Yvon *et al.*, 2009).

Diverse studies have been done to diagnose plant disease with the polymerase chain reaction (PCR) and real-time PCR. Most of these studies have used quite expensive materials, and used one or a small number of small samples from plants. The latter is a serious problem. Disease epidemics under field conditions can be affected by many factors, including rainfall, temperature, host, and agronomic treatments. These contribute to large variation between samples within the field and within specific plots. Therefore, a rapid method to quantify pathogens with substantial samples capable of characterising a whole plot is required. Low-cost reagents are required because it is necessary to use large amounts of field sample.

This appendix covers investigations of buffers, buffer volumes and storage conditions for the sample prior to extraction of DNA and different PCR methods to quantify pathogens in large field samples. The aim of these experiments was to obtain a small but representative amount of plant materials from hundreds of grams of fresh plant materials, so as to be able to use PCR inexpensively and quickly in field experiments.

1-1-1-Introduction: PCR and real-time PCR:

A very important invention in science was PCR by Mullis in 1984 (Mullis, 1987). This is the basis of many advanced techniques of plant disease detection (Schaad & Frederick, 2002). The sensitivity, specificity and quality of PCR or real-time PCR depends on primers which bind uniquely to specific sequences in the sample. Small amounts of microorganism can be detected because the technique is very sensitive. Extracted DNA (genetic material) is purified and amplified by PCR, before performing gel electrophoresis, in which the presence of specific bands shows the presence of pathogen in the plant (Sankaran *et al.*, 2010).

Detection of plant pathogens by classical PCR was described more than 20 years ago (Rasmussen & Wulff, 1991) in plant and in infected seeds (Prosen *et al.*, 1991). There were many reasons why classical PCR was not adopted by plant disease researchers to quantify pathogens. The labour and time required to identify, then confirm, a PCR product are considerable, because simple presence of a band in an agarose gel does not prove the result, even if the molecular weight of the DNA band is correct. Although many commercial kits are now available and pathogens can be rapidly and easily sequenced, only small numbers of samples can be used within this technique. Another reason for slow adoption was that the sensitivity of PCR was initially less than other techniques such as ELISA (Wang *et al.*, 1999; Schaad & Frederic, 2002). However, all these concerns are reduced with qPCR (Livak *et al.*, 1995).

The standard technique in most laboratories used for various applications in basic research is real time or quantitative polymerase-chain reaction (qPCR) (Pabinger *et al.*, 2014). This allows amplification and simultaneous quantification of a targeted DNA molecule. The ability to measure the amplification of DNA as the reaction progresses in real time is the advance over the classical PCR method (Higuchi *et al.*,1992). The number of amplification cycles required for the response curves to reach a particular threshold fluorescence signal level provides a quantification of the initial concentration of template molecules (Kubista *et al.*, 2006). Few PCR cycles are needed before the threshold is reached when more copies of the DNA template are present at the beginning of an experiment (Pabinger *et al.*, 2014).

1-1-1-1- SYBR-green and Taq-man reagents:

There are different chemical reagents can be used with qPCR to detect specific amplicons, but in my experiments only two reagents were used: SYBR-green and Taq-man. Both of these reagents can provide rapid absolute quantification of template copy number, but have specific strengths and disadvantages.

SYBR-green is a reagent used to detect double-stranded DNA product during the cycling phase of PCR. It is a dye that binds to double-stranded DNA and upon excitation emit light. A problem with SYBR-green is that it is sensitive to the formation of non-specific product (Wittwer *et al*., 1997). The characteristics encouraging use of SYBR-green are that it is simple and inexpensive (Donia *et al.*, 2010), but again this depends upon the specificity of the primer to the target organism (Schaad & Frederick, 2002). Taq-man reagents are more specific, due to the use of a specific probe, but Taq-man is more expensive than SYBR-green. (Table 72).

1-1-2- Comparison of cost-effectiveness of materials:

Most researchers are looking for rapid methods to detect plant disease which can be widely used. Plant diseases led to major losses of production worldwide, and detection and quantification of diseases in plants is critical for sustainable agriculture (Sankaran *et al.*, 2010). For this, inexpensive materials are required. qPCR amplification is affected by the quality, purity and the quantity of DNA sample used (Demeke & Jenkins, 2010). But standard extraction methods such as CTAB, Sodium dodecyl sulphate, polyvinylpyrrolidone methods, and commercial kits can only be used with small amounts of plant material. In addition, some of these methods used hazardous chemicals such as phenol and chloroform, and are time consuming. Inexpensive materials are therefore a further requirement to extract DNA from large field samples. In the experiments in this chapter two buffers were compared: phosphate buffered saline and CTAB buffer (a mixture of sorbitol, ethylene diamine tetra acetic acid and sodium bisulphate). Phosphate buffer is more than 20 times cheaper than CTAB. In addition, phosphate buffer can be prepared and used easily in comparison with CTAB buffer, and incurs no disposal costs, especially important when large volumes of buffer are needed.

After field sampling there are different methods to extract DNA and estimate the concentration of pathogen DNA in large samples. It may not be possible to process material immediately. Different storage times and conditions were therefore tested. The concentration of DNA may change due to different factors such as the plant age; some reagents, such as EDTA, may influence the DNA quality (Khosravinia & Ramesha, 2007); some seeds contain high levels of protein and polyphenols and through fermentation during storage ethanol is produced all of which influence DNA recovery and quality (Ramos *et al.*, 2014).

It is also necessary to determine if there is any fungus development or destruction of DNA during storage. Other variables affecting choice of method include the cost of each method and the availability of equipment. Here three different methods of DNA extraction were compared: air-drying in an oven, lyophilisation and liquidization.

Liquidization, oven drying and milling and lyophilizing and milling are three different methods that can be used with plant samples to make fine suspensions. A big oven is needed to dry large volume of plant samples, and similarly for lyophilisation. Liquidization can easily be done in a kitchen or industrial blender. The other two methods are expensive in comparison with liquidization, especially in a kitchen blender. Both also require more time, both to dry the sample, then to grind

the sample to make a powder. With the liquidization method, after liquidization, the extraction can either be started directly, or samples can be frozen at -20ºC till use.

In summary, the experiments reported in this chapter investigate the effect of different buffers, different buffer volumes, different storage conditions, and different methods of DNA extraction on the estimate of pathogen DNA in large field samples of wheat. The purpose of these experiments was to find a rapid and cost-effective method to extract DNA from large numbers of large field samples.

Table 18. Advantages and limitations of Taq-man and SYBR-green measurement technique in qPCR with description. (Manual of step-one plus 2.3 version, Invitrogen, UK).

Reagent Type	Description	Advantage	Limitation
Taq-Man reagents	TaqMan reagents use a fluorogenic probe to enable detection of a specific PCR product as it accumulates during PCR cycles.	•Provides increased specificity with the addition of a fluorogenic probe. •Provides multiplex capability. •Includes preformulated assays, optimized to run under universal thermal cycling conditions. • can be use either 1or 2 step for RT-PCR	Requires synthesis of a target specific fluorogenic probe as well as primers.
SYBR-Green reagents	SYBR Green reagents use SYBR® Green I dye, a doublestranded DNA binding dye, to detect PCR products as they accumulate during PCR cycles.	• Is economical (no DNA probe or DNA modification needed). •Allows for melt curve analysis to measure the Tm of all PCR products. • Can be used for either 1- or 2-step RT-PCR.	Binds non-specifically to all double-stranded DNA sequences. To avoid false positive signals, product must be checked for nonspecific product formation using melt curve or gel analysis.

1-2- Methodology:

Experiments were done to find an effective and easy to dispose of buffer, compare methods of homogenization before subsampling, determine the effect of storing samples before homogenization or drying, and to determine the volume of buffer needed to get stable results. Finally, the chosen protocol was used to quantify the abundance of *Z. tritici* measured by this method with other assessment methods. This was done using samples taken at growth stage 31 (first node detectable), and growth stage 80 (dough development) from a subset of the plots in the the 2014-2015 experiment described in chapter 4.

1-2-1- Liquidization to homogenize:

Several experiments were done to test the homogeneity of the liquidised sample; test the two different buffers that were used to liquidize the sample, using three ratios of buffer to material; test the storage conditions before extraction; test three different methods of preparing samples (Liquidized, oven dried, and lyophilized); and to compare results from the large field samples with other measures. Most of these experiments used liquidized samples and measured the *Z. tritici* DNA amount by SYBR-green. Experiments were also done to investigate the differences between two different reagents SYBR-green and Taq-man which used oven dried samples.

To test the homogeneity of liquidized samples three samples were collected at growth stage 15. Each sample DNA was extracted twice, and each sample was repeated three time in the qPCR plate. Two different buffers (phosphate and CTAB) and three different ratios of buffer to tissue wet weight (2ml/g, 6ml/g, and 20ml/g) were used. Plants were either liquidized directly, or kept overnight or for 48 hours at room temperature, or kept in a fridge (4^0C) overnight or for 48 hours, or kept in a -20^0C freezer overnight. All of these were done with two field replicates.

1-2-2- Preparation of buffer:

Stock phosphate buffer was prepared at $10\times$ final concentration then diluted before use. The stock contained 14.19g of Na_2HPO_4, 2.44g of KH_2PO_4, 80.06g of NaCl, and 2.01 of KCl per litre, adjusted to 7.4 pH by added either Sodium hydroxide or hydrochloric acid. The final concentration of $1\times$ was 10mM PO_4, 137mM NaCl, and 2.7 mM KCl.

CTAB buffer was prepared by dissolving sorbitol 6.37g, Tris 1.21g, EDTA (ethylene diamine tetra acetic acid) 0.0005M 1ml and sodium bisulphate 0.2g in 100 ml DNase-free water (Ristaino *et al.*, 2001).

1-2-3- Collection of plants:

All the samples were collected at different growth stages from the field experiment in 2014-2015 detailed in chapter 4-6. In the test of homogenization by liquidization, samples were collected at growth stage 15, then liquidised with phosphate buffer by homogenizer system (T25, IKA LaborteLink, Janke & Kunkel, GMBH & CO. KG, D 7813, Staufeni. Br, Germany). In the experiment to test the buffers, storage conditions, and buffer volume, samples were collected at growth stage 15 then liquidised. All roots were removed from the plant. For the large field samples, to test the different methods of preparation sample, samples were collected at growth stage 23 then liquidised with phosphate buffer, or for dried method samples, dried in an oven at 80°C for 48 hours then milled (Fritzch, serial no. 14.102/2114, Germany). Lyophilized samples were kept in a freeze-dryer for 48 hours then ground in a milling machine (Fritzch, serial no. 14.102/2114, Germany). Samples collected at two growth stages: one at GS31 and another one at growth GS80, were liquidized by using a kitchen blender (Kenwood-1400 W, BLM800WH, from Lakeland, UK) after removing leaves that showed more than 50% senescence. To test if there any differences between two chemical reagents that used to detect *Z. tiritici* (SYBR-green and Taq-man), samples were collected at growth stage 31, then dried in an oven at 80°C for 48 hours then ground by (Glen Creston P15 knife mill, serial number 011007GC) to make fine powder.

1-2-4- DNA extraction:

For liquidized samples a 700µl subsample was taken. In all liquidized samples the subsamples were centrifuged for 5 minute at 14000rpm. The supernatant was discarded and then DNA extracted from the precipitate to concentrate the DNA. In the final experiment the large field samples at GS 31 and 80 were DNA extracted directly from the liquidised subsample without centrifuging. For dried and lyophilized samples 20mg was weighed then ground again in tissue-lyser for 40 second at 350 HZ, then DNA extracted from. All DNA samples were extracted using a DNeasy Plant Mini Kit (QIAGEN) according to manufacturer's instructions. All samples were eluted into 50µl elution buffer. DNA concentration and quality was measured by NANO-drop (Thermos scientific/ UK), then diluted to 5ng/µl and stored at -20°C until use

1-2-5- qPCR assay and quantification of pathogens:

Pure fungus isolated from 100mg of pure culture scraped from agar was used to obtain a standard DNA concentration-amplification curve. SYBR-green and Taq-man reagents were used to quantify pathogens.

Two different primers were used to measure *Z. tritici* DNA, depending on assay type. For the SYBR-green assay the primer was designed by Selim (2009), and for the Taq-man assay the primer and probe were designed by Bearchell *et al.* (2005), (Table 73).

For the SYBR-green assay a 20µl final reaction was used to quantify amount of pathogen, containing 10µl Hi-Rox SYBR-green, 0.4µl each of forward and reverse primer, 5.2µl DNase-free water and 4µl of sample DNA. For Taq-man a 20µl final reaction was used, containing 10µl Taq-man master mix, 2µl Taq-man probe, 0.4µl each of forward and reverse primer, 3.2µl DNase-free water, and 4µl of sample DNA.

The quantity of each fungus in the sample was quantified by real-time PCR as pg in 20ng of wheat DNA extracted. For the SYBR-green assay thermal stage was set up as 95°C for 4 minutes. Followed by 40 cycles of melting at 95°C for 15 sec. then drop to 60°C for 1 minute. Each assay plate include was standard DNA for each fungus and a no-template sample (NTC). All data were obtained by STEP-one Plus, 2.3 version software.

For Taq-man, thermal stage was started at 95°C for 1 minute, followed by 35 cycles of 94°C for 30 second, 58°C for 20 seconds then 72°C for 20 seconds. The program finished with a final stage at 72°C for 5 minutes.

Table 19. Sequences of primers and probes used to detect pathogens *Z. tritici*. in samples sub-sampled from wheat plants collected from field crops.

Primer	Sequences (5'-3')	Gen-Bank number	Amplicon size	References
Syber-green *Z. tritici* forward	CCTCGCCGAACTTACGATCT	EF418622	60	Selim 2009
reverse	CGCGGACTTCCTTTCCTTG	-	-	
Taq-man *Z. tritici* forward	GCCTTCCTACCCCACCATGT	AY547264	63	Bearchell *et al.*, 2005
Reverse	CCTGAATCGCGCATCGTTA	-	-	
Z. tritici probe	FAM-TTACGCCAAGACATTC-MGB			

1-2-6- Data analysis:

All data were analysed by GenStat 16th edition using general analysis of variance with a fully balanced randomized block design. For homogenate the samples through treatment structure involved sample name multiply by technical replicate and block structure involved the extract. For buffers, storage, and buffer volume REML regression was used due to unbalanced design. For large field samples general analysis of variance was used with a fully balanced randomized block design and two treatments of fertilizer and one fungicide treatment. The DNA concentrations in growth stage 31 and 80 which were liquidised with phosphate buffer or dried were transformed to Log_{10} to get a normal distribution of the residuals.

1-3-Results:

The amount and quality of DNA varied between all the samples that were used in these experiments (Tables 21-25). Please refer to Tables A15-A22 for more details.

Table 20. The concentration and quality of DNA, in three samples collected from the same field plot at growth stage 15. Samples were liquidised with phosphate buffer (A 5ml/g, B 5ml/g, and C 7ml/g) and each sample was extracted twice.

Field sample	extraction	DNA Conc.	260/280	260/230
A	1	77.8	1.8	2.45
A	2	95	1.82	2.38
B	1	10.5	1.73	4.12
B	2	26.6	1.77	1.82
C	1	44.6	1.76	2.68
C	2	48.8	1.73	2.29

Table 21. Concentration and quality of DNA in samples collected from two field plots at growth stage 23, from two replicate plots which treated either with or without sulphur fertilization).

Sample ID	Replicate	Fertilizer	DNA Conc.	260/280	260/230
Liquidized	1	S +	6.8	1.51	2.98
Liquidized	2	S +	5.4	1.66	8.22
Liquidized	1	S -	5.9	1.55	2.24
Liquidized	2	S -	5	1.66	2.29
Lyophilized	1	S +	59.2	1.82	3.02
Lyophilized	2	S +	84.8	1.81	2.73
Lyophilized	1	S -	77.4	1.82	2.93
Lyophilized	2	S -	72	1.83	2.53
Dried	1	S +	27.9	1.79	3.55
Dried	2	S +	41.7	1.81	3.62
Dried	1	S -	30	1.8	4.24
Dried	2	S -	23.6	1.84	4.87

Table 22. The concentration and quality of DNA that were obtained from samples liquidised with two different buffers, all samples from field experiment 2014-2015 season at growth stage 15. Buffer 1 is phosphate buffer, buffer 2 is CTAB buffer.

storage	buffer	volume ml/g	DNA conc.	260/280	260/230
Fresh	1	2	101.4	1.8	1.86
Fresh	1	2	51.1	1.77	1.81
Fresh	2	2	117.6	1.82	2.1
Fresh	1	6	18.1	1.62	1.03
Fresh	2	6	41.5	1.83	2.68
Fresh	2	20	20.8	1.8	1.86
Fresh	2	20	22.3	1.86	2.61
Fresh	2	6	12.8	1.69	1.21
Fresh	1	20	24.3	1.76	2.25
Fresh	1	20	29.8	1.77	1.99
Fresh	2	2	175.6	1.82	2.07
Fresh	1	6	27.3	1.78	1.83
room 24hours	1	20	2.1	3.07	0.59
room 24hours	1	6	5.5	2.05	1.45
room 24hours	1	20	1.8	5.67	0.23
fridge 24hours	1	6	5.3	2.17	5.18
fridge 24hours	1	2	9.5	1.84	1.03
room 24hours	1	2	7.9	1.95	1.21
room 24hours	1	2	4.3	2.69	2.99
fridge 24hours	1	20	2.1	15.59	5.15
fridge 24hours	1	2	7.5	1.99	4.35
fridge 24hours	1	6	3.1	2.5	0.96
room 24hours	1	6	2.2	4.32	0.62
fridge 24hours	1	20	2.7	3.13	0.95
fridge 24hours	2	2	7.2	2.19	32.55
room 24hours	2	20	2.2	4.16	8.42
room 24hours	2	6	6.6	2.15	4.63
room 24hours	2	20	3.6	3.13	6.98
fridge 24hours	2	6	6.1	2.14	22.98
room 24hours	2	2	6.7	1.97	6.92
fridge 24hours	2	2	10.3	1.89	2.68
room 24hours	2	2	13.5	1.81	1.25
fridge 24hours	2	6	5.2	2.82	1.65
fridge 24hours	2	20	2.1	3.92	0.48

room 24hours	2	6	5.4	1.96	1
fridge 24hours	2	20	3.8	2.2	0.87
fridge 2 days	1	20	10.8	1.89	4.41
room 2 days	1	6	3.6	1.66	-3.07
fridge 2 days	1	2	10.1	1.65	3.89
room 2 days	1	2	5.6	1.78	-2.66
room 2 days	1	2	2.4	1.46	-0.3
fridge 2 days	1	6	14.2	1.67	2.72
fridge 2 days	1	6	10.8	1.84	15.71
fridge 2 days	1	2	8.7	1.72	1.91
room 2 days	1	20	6.9	1.82	3.81
room 2 days	1	20	3.9	1.75	1.45
fridge 2 days	1	20	8.2	1.75	3.67
room 2 days	1	6	5.8	1.21	34.52
fridge 2 days	2	2	8.9	1.68	17.23
room 2 days	2	2	9	1.61	1.07
room 2 days	2	6	9	1.49	6.37
room 2 days	2	6	15.3	1.59	1.99
fridge 2 days	2	6	12.9	1.71	7.82
fridge 2 days	2	20	32.8	1.87	2.54
room 2 days	2	2	14.4	1.66	1.94
room 2 days	2	20	4.1	1.66	4.28
room 2 days	2	20	2.1	1.29	0.37
fridge 2 days	2	6	7	1.79	17.13
fridge 2 days	2	20	3.9	1.94	1.06
fridge 2 days	2	2	11.5	1.92	6.18
Freezer 24 hours	2	2	61.4	1.83	1.96
Freezer 24 hours	1	20	50.8	1.83	2.71
Freezer 24 hours	2	6	51.7	1.84	2.55
Freezer 24 hours	2	20	32	1.85	2.13
Freezer 24 hours	1	20	33.3	1.87	3.26
Freezer 24 hours	2	6	32.1	1.8	1.57
Freezer 24 hours	1	6	58.9	1.85	2.08
Freezer 24 hours	2	20	57.2	1.8	2.17
Freezer 24 hours	1	6	37.3	1.68	0.99
Freezer 24 hours	1	2	46.9	1.77	1.77
Freezer 24 hours	2	2	45.3	1.8	2.41
Freezer 24 hours	1	2	51.3	1.8	2.21

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Table 23. The concentration and quality of DNA of wheat at growth stages 31, all samples from field experiment 2014/2015 season, which dried an oven. This is for Comparison of Taq-man and SYBR-green as detection reagents.

Sample ID	DNA Conc.	260/280	260/230
A1	75.9	1.78	2.34
B1	135.9	1.79	2.28
C1	110.6	1.78	2.27
D1	84.3	1.78	2.35
E1	46.9	1.77	2.68
F1	110.1	1.8	2.39
F2	90.2	1.76	2.28
E2	62.2	1.76	2.35
D2	91	1.78	2.11
C2	121.5	1.79	2.37
B2	114.6	1.8	2.29
A2	77.9	1.78	2.45
A3	115	1.79	2.43
B3	86.5	1.76	2.45
C3	87.8	1.76	2.28
D3	89.6	1.75	1.95
E3	127.3	1.8	2.41
F3	78.2	1.73	1.73
F4	92	1.78	2.43
E4	69.4	1.79	2.46
D4	70.8	1.77	2.29
C4	100.4	1.8	2.23
B4	98.3	1.78	2.33
A4	98.2	1.81	2.5
A5	122	1.79	2.27
B5	80.8	1.79	2.5
C5	76.6	1.77	2.3
D5	83.4	1.79	2.34
E5	24.7	1.66	2.52
F5	98.8	1.8	2.32
F6	54.9	1.73	2.57
E 6	74.9	1.79	2.51
D6	35.9	1.71	1.98
C6	40	1.74	2.56
B6	103.8	1.78	2.2
A6	36.7	1.67	2.04

Table 24. The concentration and quality of DNA of wheat at two different growth stages 31 and 80, all samples from field experiment 2014/2015 season, which liquidised with phosphate buffer.

Field sample	GS 31			GS 80		
	DNA Conc.	260/280	260/230	DNA Conc.	260/280	260/230
A 1	26	1.89	12.43	10	1.36	1.26
B 1	8.6	1.82	3.93	8.4	1.63	1.36
C1	5.4	1.83	0.92	6.6	1.53	1.43
D 1	23.9	1.77	5.52	5.3	1.47	10.55
E 1	18.2	1.8	23.95	8.8	1.48	1.51
F 1	14.9	1.86	3.24	7.9	1.41	1.34
F 2	5	1.61	0.84	14	1.73	1.94
E 2	15.3	1.83	11.99	23.6	1.75	2.13
D 2	18.8	1.88	8.73	4.7	1.51	3.54
C 2	4.2	1.6	0.6	6.2	1.53	2.65
B 2	4	1.75	1.28	10.2	1.5	1.35
A 2	19	1.8	5.63	10.3	1.53	1.19
A 3	4.5	1.92	0.68	7.8	1.58	1.45
B 3	19.6	1.81	4.03	8.6	1.43	1.59
C 3	4.7	1.88	0.64	6	1.36	13.93
D 3	5	1.58	0.9	3.8	1.49	16.7
E 3	24.3	1.55	0.93	6.1	1.58	1.37
F 3	3.3	1.71	0.4	6	1.5	3.3
F 4	15.6	1.56	1.23	6.1	1.35	3.37
E 4	7.8	1.78	2.81	5.8	1.5	1.66
D 4	4.1	1.73	1.09	4.3	1.4	1.85
C 4	7.9	1.55	4.56	4.8	1.58	5.2
B 4	10.2	1.76	45.71	9.9	1.58	1.9
A 4	15.2	1.61	1.66	7.7	1.56	1.48
A 5	9.2	1.54	5.79	6	1.5	3.36
B 5	12.1	2.02	4.24	10.4	1.49	1.47
C 5	18.6	1.77	2.23	6.1	1.61	8.88
D 5	11.4	1.73	16.16	4.5	1.5	1.78
E 5	22.1	1.73	1.4	5.4	1.39	5.45
F 5	7.6	1.72	3.81	5.6	1.4	5.45
F 6	33.9	1.72	1.81	7.5	1.4	1.13
E 6	10	1.54	1.78	6.5	1.45	0.98
D 6	17.2	1.75	2.84	6.1	1.46	4.29
C 6	4.3	1.3	0.87	5.4	1.46	2.91
B 6	24.1	1.63	1.13	7.6	1.52	1.23
A 6	2.7	1.45	0.34	5	1.49	9.4

1-3-1- Homogeneity of sample, and comparison methods:

There were no differences between the technical replicates of the same sample on one plate. There were slight differences between the second and third sample that was used to test the homogeneity (Table 25; Figure 65 A, B and C).

Table 25. Homogeneity of samples that were liquidized with phosphate buffer (A 5ml/g, B 5ml/g, and C 7ml/g). Each sample was extracted twice then run with qPCR in three technical replicates to detect amount of *Z. tritici* DNA. A, B, and C three different field samples were collected from cv. M. Huntsman as winter wheat, and approximately each plant of each sample has same visual disease symptoms. Degree of freedom d.f= 17; L.S.D. = 309.

Technical Rep. sample	1	2	3
A	184	184	184
B	88	88	88
C	298	298	298

Table 26. Summary table for the general ANOVA to check the homogeneity of the first three samples, and the interaction between the samples and technical replicate that was used to test consistency of qPCR with SYBR-green reagent. d.f. degree of freedom, s.s. sum of square, v.r. variance ratio, F.pr. *F*-test probability associated with variance ratio.

Source of variation	d.f.	s.s.	v.r.	F pr.
sample	2	132734	3.69	0.073
Technical reps.	2	0	0	1
Sample × technical reps.	4	0	0	1
Residual	8	144054		
Total	17	359126		

A

B

C
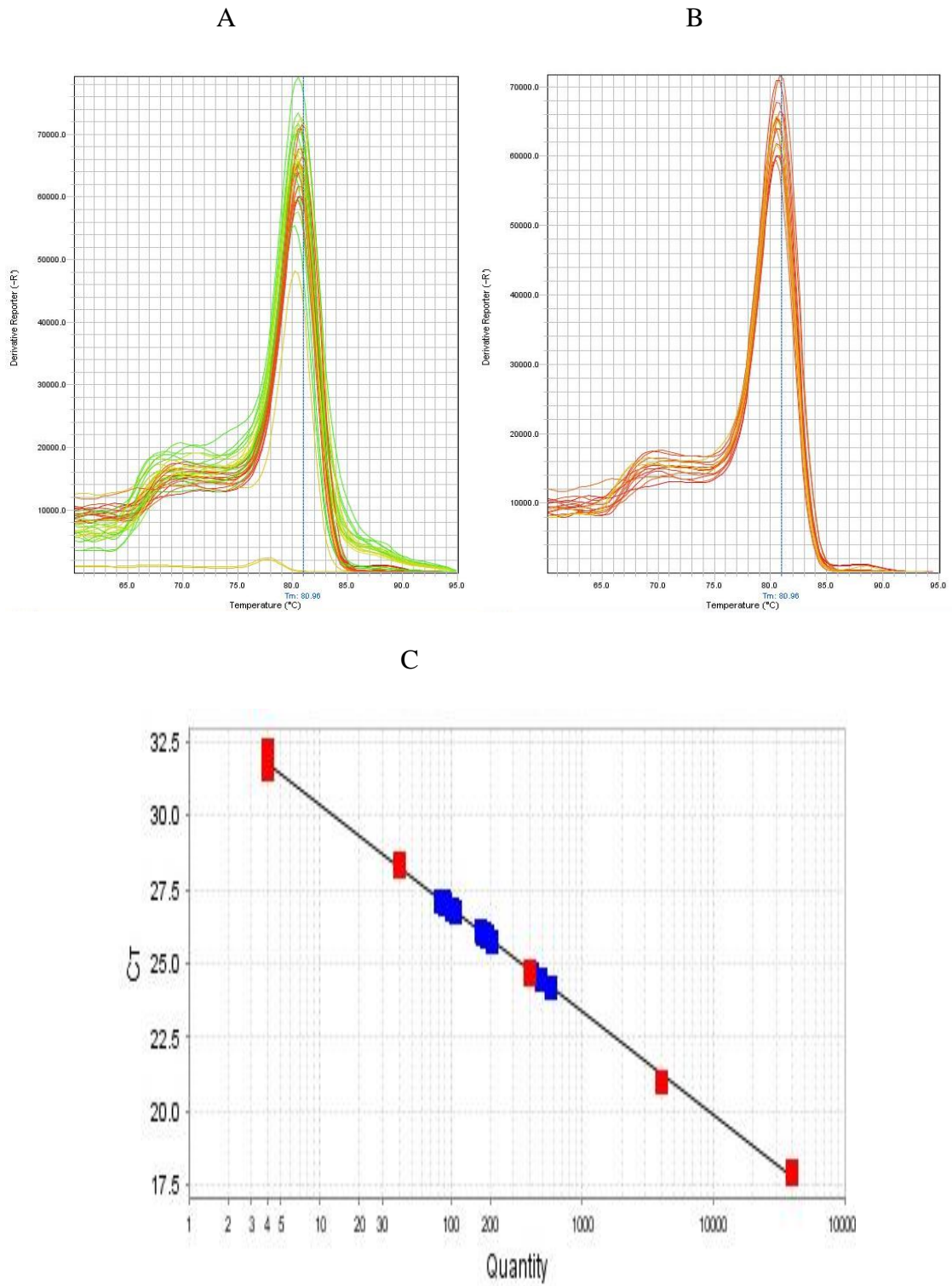
Figure 68. (A) shows the melt curve of *Z. tritici* with samples to test the sample homogeneity, (B) show the melt curve of *Z. tritici* only. (C) Standard curve of *Z. tritici* with unknown samples to test the sample homogeneity efficiency= 92.7, R^2= 0.998.

1-3-2- Buffer, storage conditions and ratio of buffer volume:

Buffer types showed significant differences (Figure 72). Concentration of *Z. tritici* DNA was higher with CTAB buffer by 55% in comparison with phosphate buffer (Figure 66; P=0.002).

Concentration of *Z. tritici* DNA was 47% greater when extracted with 6ml buffer per g fresh weight of tissue than with 2ml or 20ml/g, but this difference was not statistically significant (*P*. 0.17; Figure 67). The concentration of *Z. tritici* DNA was affected by storage conditions. It was increased in samples kept at room temperature or in a fridge for 48 hours by 58% and 44% respectively in comparison with samples liquidized fresh (Figure 68 A; Table 27), but there were no statistical differences in the repeat of this experiment (Figure 68 B).
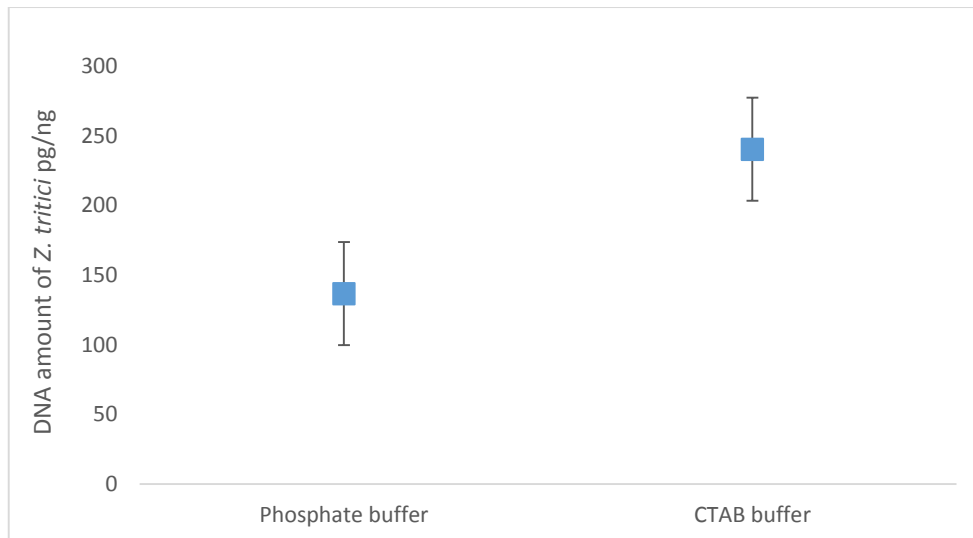
Figure 69. Effect of buffer type on the DNA amount of *Z. tiritici* pg /ng of wheat DNA extracted (Error bar = ± S.E.D, *P.* = 0.002).
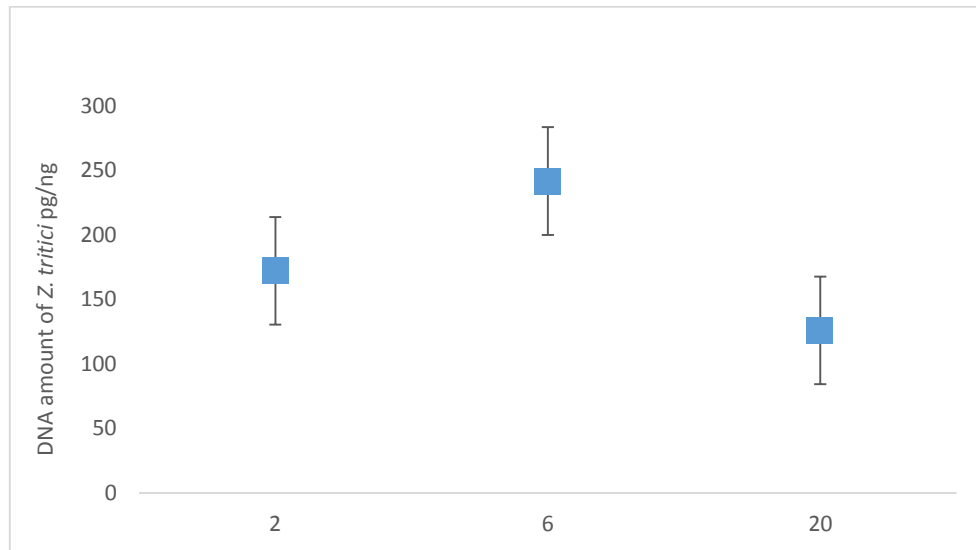
Figure 70. Effect of buffer volume on concentration of *Z. tritici* DNA pg/ng of wheat DNA, (error bar = ± S.E.D in two replicate experiments, *P.* =0.17).
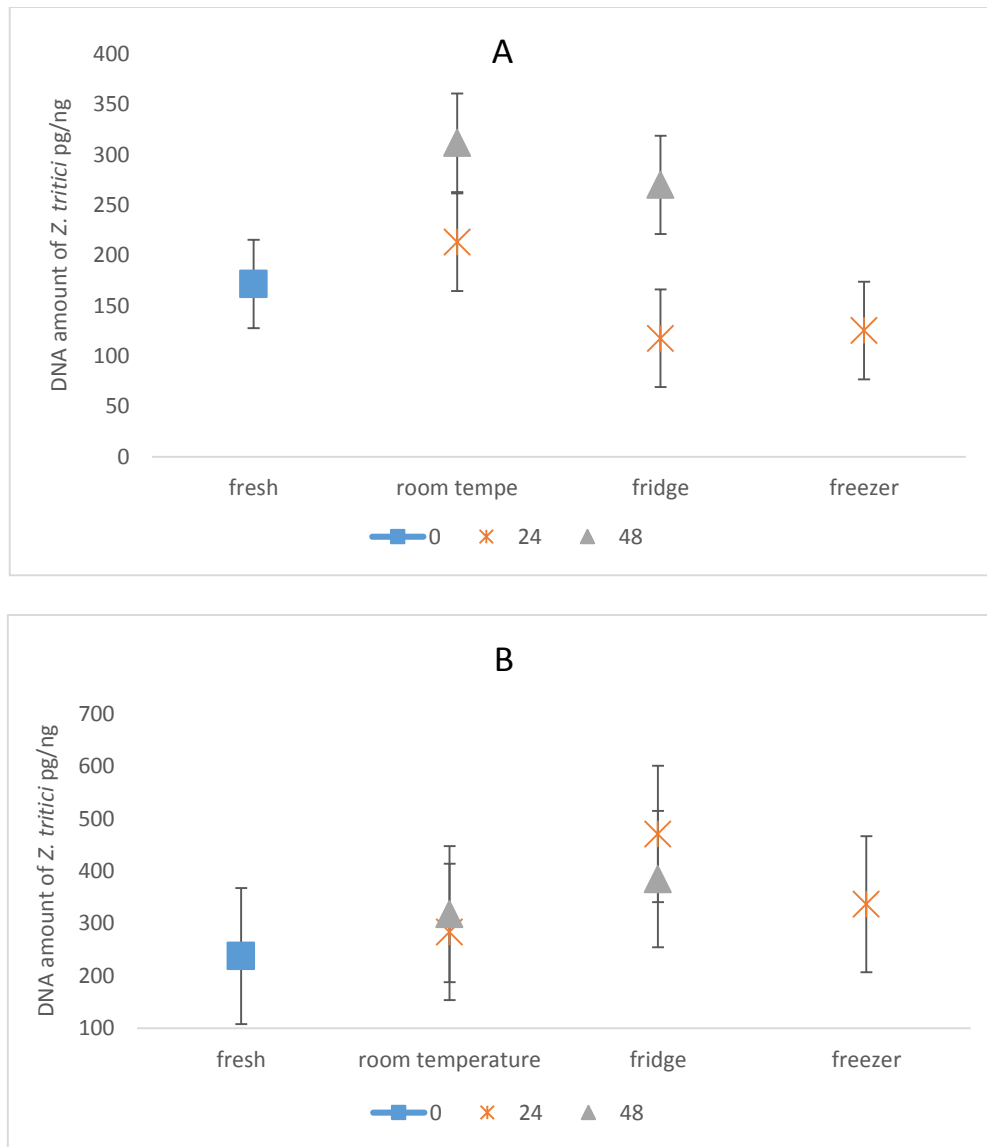
Figure 71. (A)Storage condition effect on DNA amount of *Z. tritici* pg/ng of wheat DNA, first experiment (error bar = ± S.E.D in two replicates, *P.* = 0.04). (B) Storage condition effect on DNA amount of *Z. tritici* pg/ng of wheat DNA, second experiment (error bar = ± S.E.D in two different replicate, *P.* = 0.6).

Concentration of *Z. tritici* DNA varied with storage conditions and buffer type. Samples stored at room temperature for 24 or 48 hours and liquidized with phosphate buffer did not show major differences (P. = 0.08; Figure 69). With CTAB buffer there were differences; the concentration of *Z. tritici* increased by 47% when samples were stored for 48 hours rather than 24 hours.

The samples kept at fridge for 24 and 48 hours when liquidized either with phosphate or CTAB buffer showed some differences. The concentration of *Z. tritici* increased by 136% when samples were liquidized with phosphate buffer and by 55% with CTAB buffer (Figure 69).
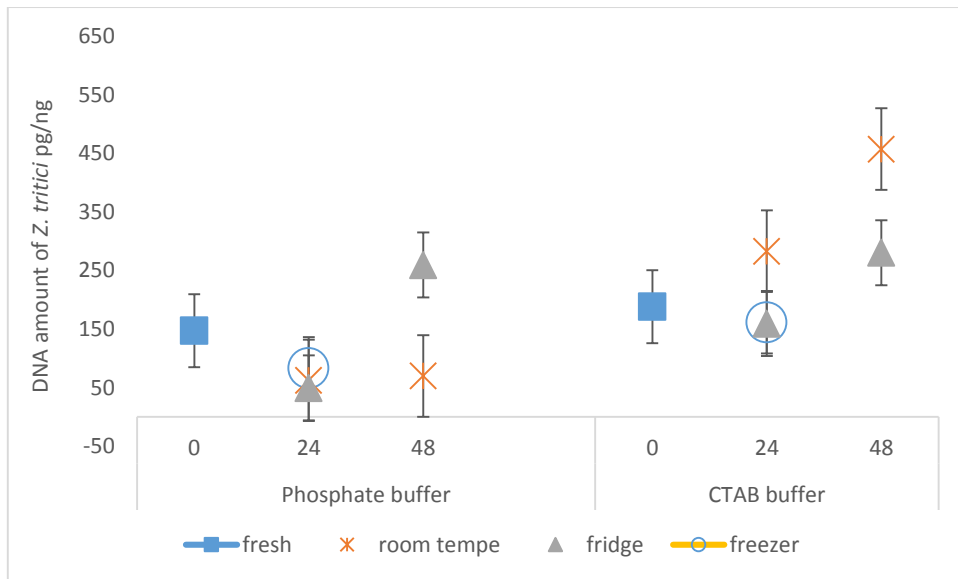
Figure 72. DNA amount of Z. *tritici* pg/ng of wheat DNA when samples stored either in room temperature or fridge for 24 and 48 hours and liquidize either with phosphate or CTAB buffer, (Error bar = ± S.E.D, *P.* = 0.08).

Table 27. Summary of ANOVA table in case of investigated each of buffers type, storage condition, buffer volume and the interaction of these factors that was analysed with SYBR-green as a factorial combination, with two replicate of field samples. Statistical analysis was done by Analysis of variance by ANOVA, REML or regression, due to unbalanced data. All the sample from winter wheat cv. M. Huntsman. d.f. degree of freedom, s.s. sum of square, v.r. variance ratio, F.pr. *F*-test probability associated with variance ratio.

Factor	d.f.	s.s.	v.r.	F pr.
Field sample	1	9306	0.57	0.46
buffer	1	201455	12.37	0.002
storage	5	233361	2.87	0.04
volume	2	61858	1.9	0.17
Buffer × storage	5	188380	2.31	0.08
Buffer × volume	2	35238	1.08	0.36
Storage × volume	8	198386	1.52	0.21
Buffer × storage × volume	6	25441	0.26	0.95
Residual	21	341997		
Total	51	1295422		

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No differences were found between samples prepared by drying, liquidised with phosphate buffer and lyophilisation (Figures 770; P = 0.14; Table 28).
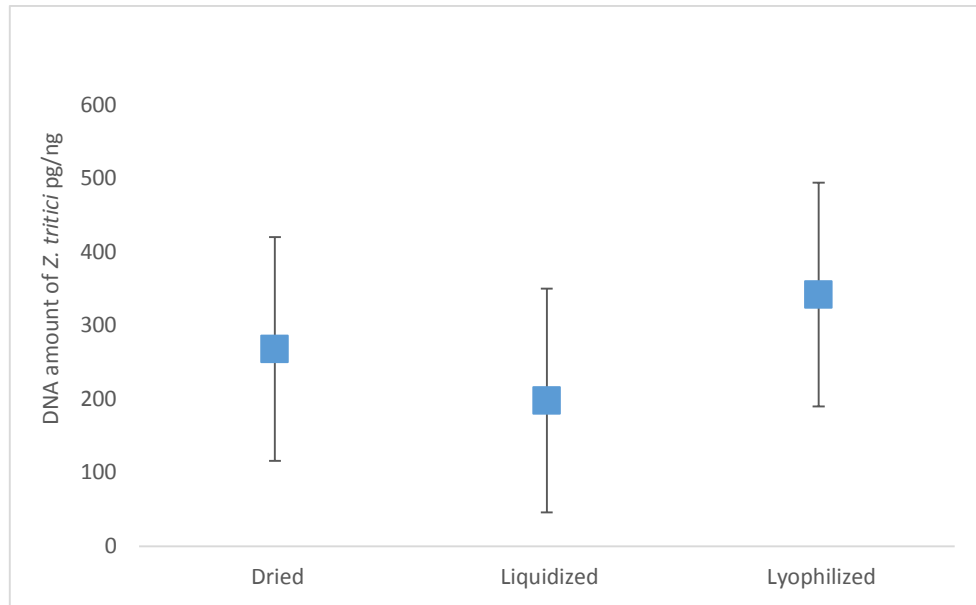
Figure 73. DNA amount of *Z. tritici* pg/ng of wheat DNA at growth stage 23, in different technique methods of field samples, (error bar = ± L.S.D in two different replicates, *P*. = 0.14).

Fertilizers showed no effect on concentration of *Z. tritici* DNA at growth stage 23, (Figure 71; P = 0.4).
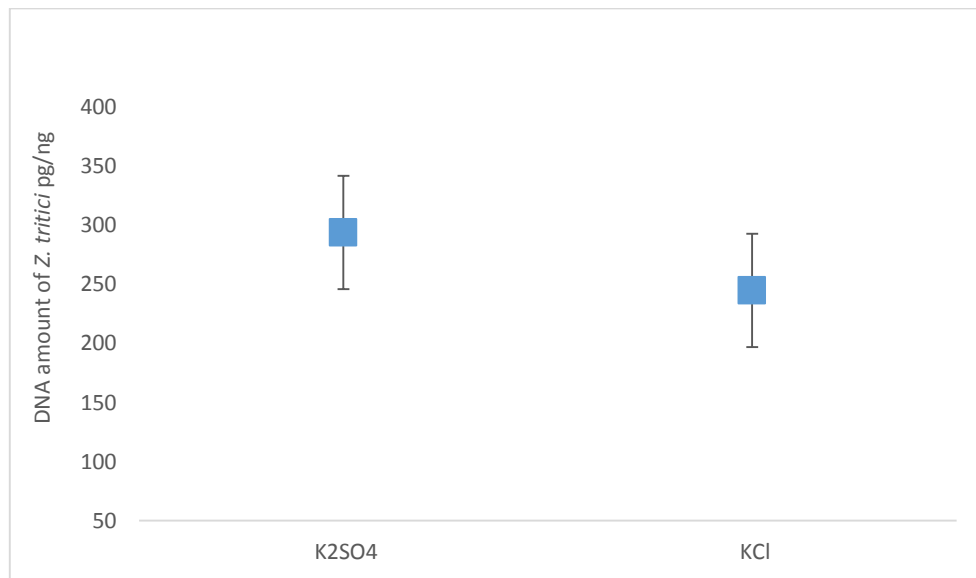
Figure 74. Effect of fertilizer form on DNA amount of *Z. tritici* estimated by extraction from liquidised replicated field samples at growth stage 23 from cv. M. Huntsman 2014-2015. (Error bar = ± S.E.D. in two different replicate, P = 0.4).

Table 28. Summary of ANOVA table for an investigation of samples preparation in different methods, from two different field replicates which treated with alternative fertilizers, as a factorial combination. All sample collected at growth stage 23 from winter wheat cv. M. Huntsman. d.f. degree of freedom, s.s. sum of square, v.r. variance ratio, F.pr. *F*-test probability associated with variance ratio.

Source of variation	d.f.	s.s.	v.r.	F pr.
method	2	41851	2.99	0.14
fertilizers	1	7045	1.01	0.362
Method × fertilizers	2	25054	1.79	0.259
Residual	5	34967		
Total	11	112073		

1-3-3- Comparison of Taq-man and SYBR-green as detection reagents:

The estimated concentration of *Z. tritici* DNA was similar using either SYBR-green or Taq-man (Figures 74 A and B; 75). There was a high correlation between concentration measured by Taq-man and SYBR-green (r = 0.91; Figure 73).

The estimated DNA concentration increase due to sulphate fertilization was 29% with SYBR-green (P = 0.04) and 32% with Taq-man (P = 0.05; Figure 72 A and B; Tables 29 and 30).
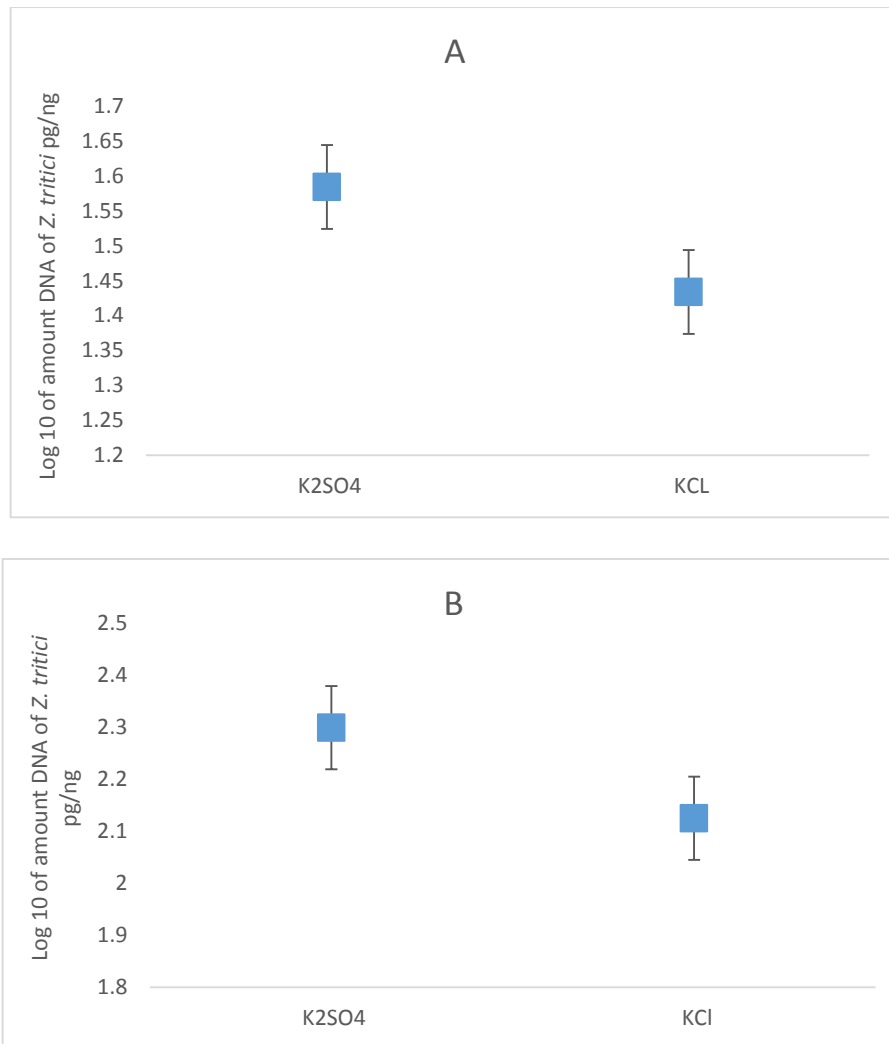
Figure 75. (A)Fertilizer effect measured using dried method on *Z. tritici* DNA at growth stage 31 with SYBR-green from field samples cv. M. Huntsman as a factorial combination. (Error bar = ± S.E.D. in two different replicate, P. = 0.04). (B)Fertilizer effect measured using the dried method on *Z. tritici* DNA at growth stage 31 wih Taq-man from field samples cv. M. Huntsman as a factorial combination. (Error bar = ± S.E.D. in two different replicate, P. = 0.05).
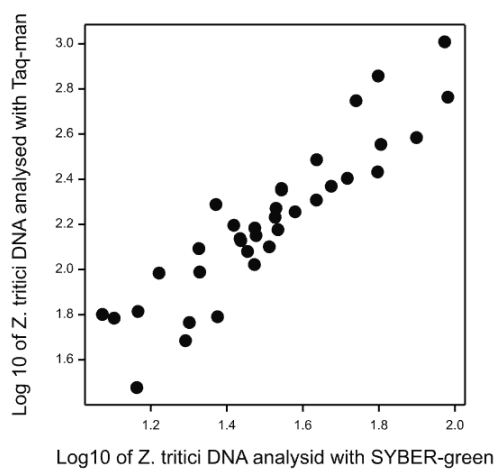
Figure 76. Correlation between the SYBR-green and Taq-man estimates of *Z. tritici* concentration at GS 31 with heat-dried samples (r= 0.91).

A
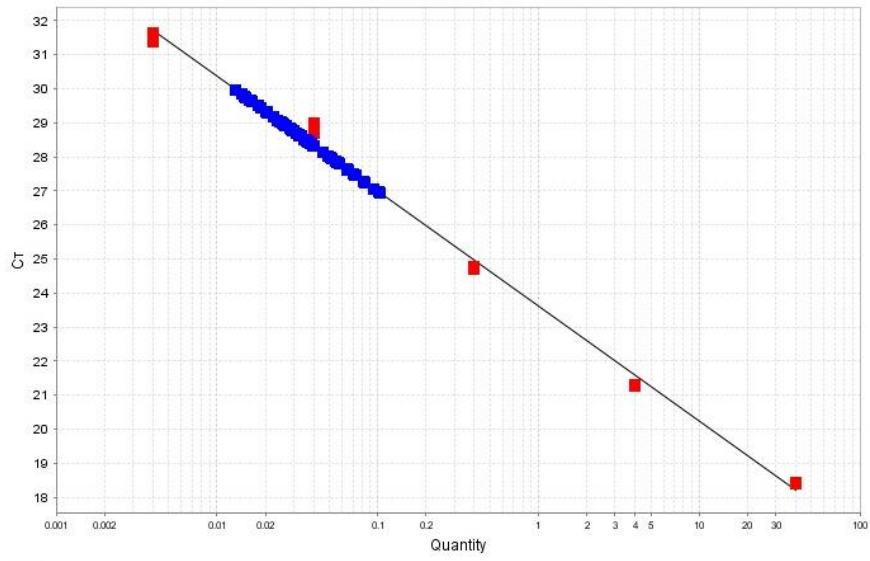
B
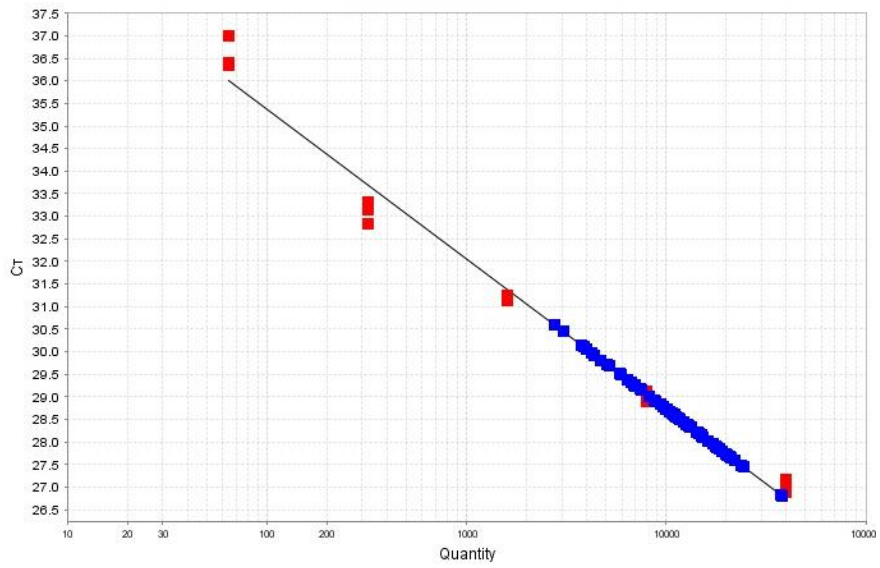
Figure 77. Standard curve of *Z. tritici* with unknown samples from heat-dried field samples at growth stage 31, 2014-2015 (A) with SYBR-green efficiency= 101.4, R^2= 0.995 (B) with Taq-man. efficiency=100.15, R^2= 0.983.
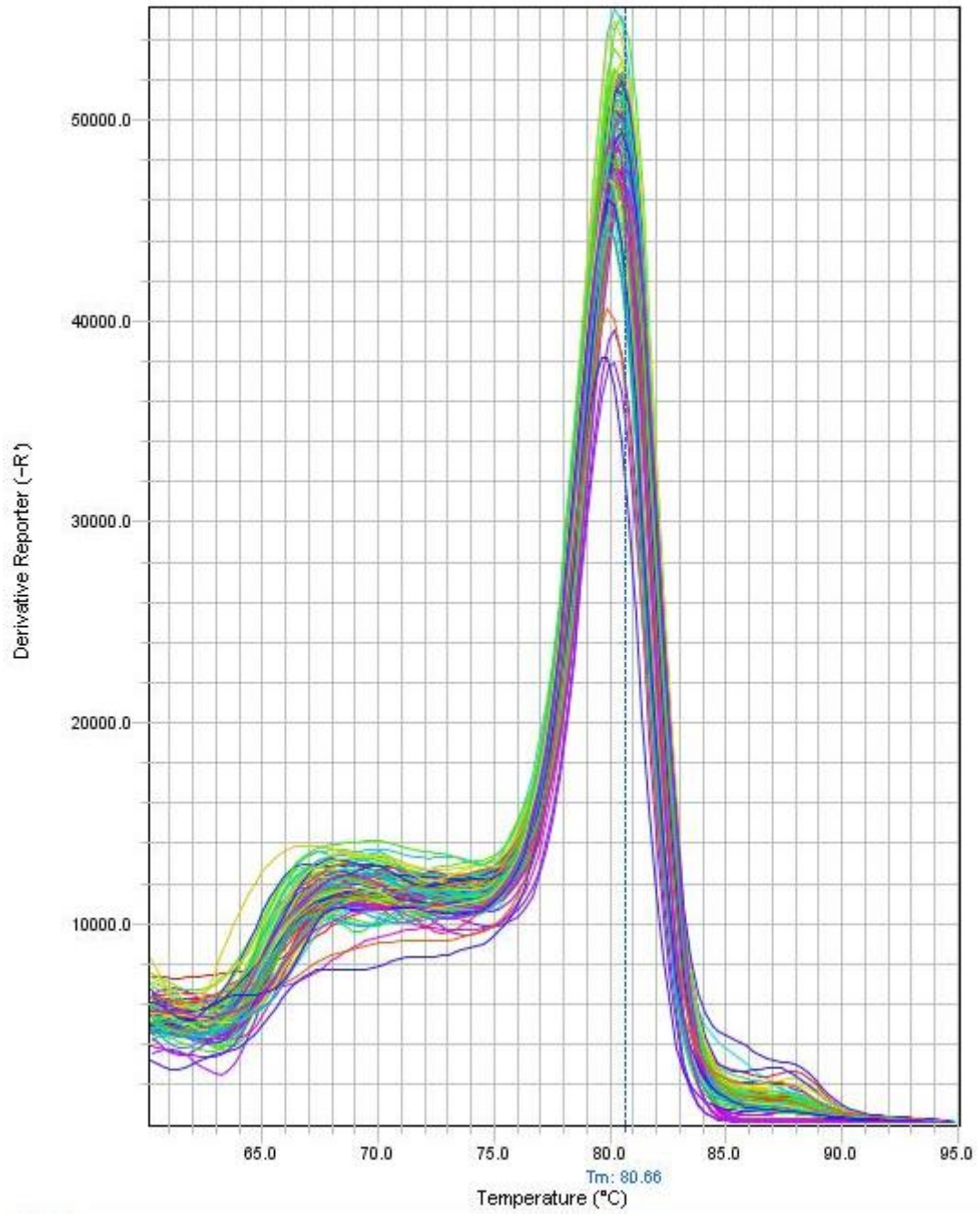
Figure 78. Melt curve of *Z. graminicola* with unknown samples in qPCR assay with SYBR-green, in heat-dried samples taken at growth stage 31 from the field experiment in 2014-2015 (ch 4-5).

Table 29. Summary of ANOVA table for heat-dried material sampled at GS 31 and analysed with SYBR-green to detect Z. *tritic*i, (all data transformed to Log$_{10}$) from three field plot which were treated with alternative fertilizers and inoculated or fungicide treated in factorial combination. All samples are from winter wheat cv. M. Huntsman. d.f. degree of freedom, s.s. sum of square, v.r. variance ratio, F.pr. *F*-test probability associated with variance ratio.

Source of variation	d.f.	s.s.	v.r.	F pr.
block stratum	2	951.7	1.15	
Block /main plot				
Fungicide	1	728.5	1.77	0.213
P. nodorum	2	2626.5	3.18	0.085
Fungicide × *P. nodorum*	2	212	0.26	0.778
Residual	10	4126.8	1.46	
Block / main plot / subplot				
fertilizer	1	1536.8	5.45	0.038
Fertilizer × Fungicide	1	36.7	0.13	0.724
Fertilizer × *P. nodorum*	2	749	1.33	0.301
Fertilizer × Fungicide × *P. nodorum*	2	1591.5	2.82	0.099
Residual	12	3383.6		
Total	35	15943.1		

Table 30. Summary of ANOVA table at GS 31 for heat-dried methods which analysed with Taq-man to detected *Z. tritic*i, (all data transform to Log10) from three field plot which treated with alternative fertilizers as a factorial combination. All sample from winter wheat cv. M. Huntsman. d.f. degree of freedom, s.s. sum of square, v.r. variance ratio, F.pr. *F*-test probability associated with variance ratio.

Source of variation	d.f.	s.s.	v.r.	F pr.
block stratum	2	0.21246	0.81	
Block / main plot				
Fungicide	1	0.06449	0.49	0.5
P. nodorum	2	0.69169	2.63	0.121
Fungicide × *P. nodorum*	2	0.05026	0.19	0.829
Residual	10	1.31497	2.28	
Block / main plot / subplot				
fertilizer	1	0.27089	4.71	0.050
Fertilizer × Fungicide	1	0.0746	1.3	0.277
Fertilizer × *P. nodorum*	2	0.11847	1.03	0.387
Fertilizer × Fungicide × *P. nodorum*	2	0.56291	4.89	0.028
Residual	12	0.69064		
Total	35	4.05137		

1-3-4- Field design of large scale DNA extraction:

The 2014-2015 field experiment was used to test the liquidised method under field conditions. All of this work was done with SYBR-green reagent. At GS 31, sulphur fertilizer reduced the concentration of *Z. tritici* DNA by 10% (P = 0.014; Figure 76; Table 31). At GS 80 the concentration of *Z. tritici* was reduced by around 6%, but non-significantly, by sulphur fertilizer (P = 0.8; Figure 77; Table 32).
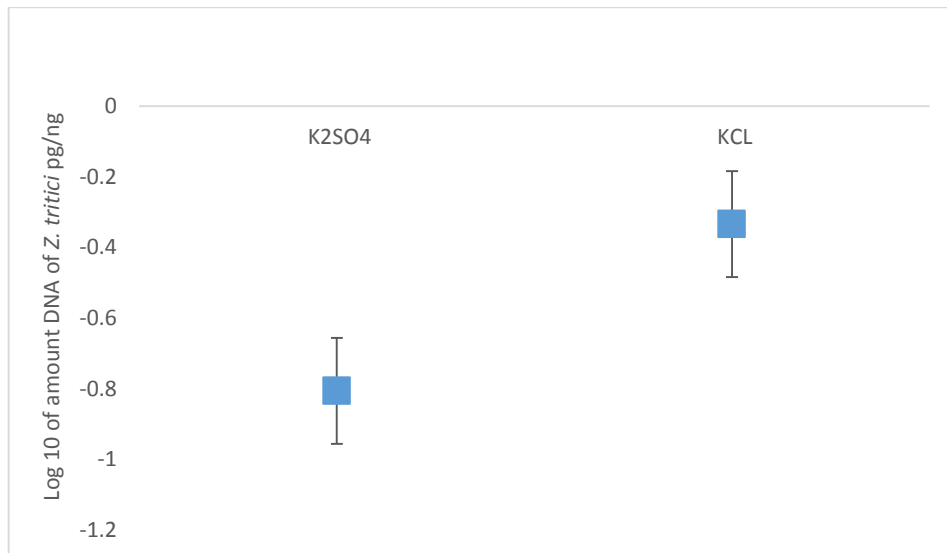
Figure 79. Fertilizer effect estimated using the liquidized method on *Z. tritici* DNA at growth stage 31from field samples of cv. M. Huntsman averaged over other factors. (Error bar = ± S.E.D. in two different replicates, P. = 0.014).
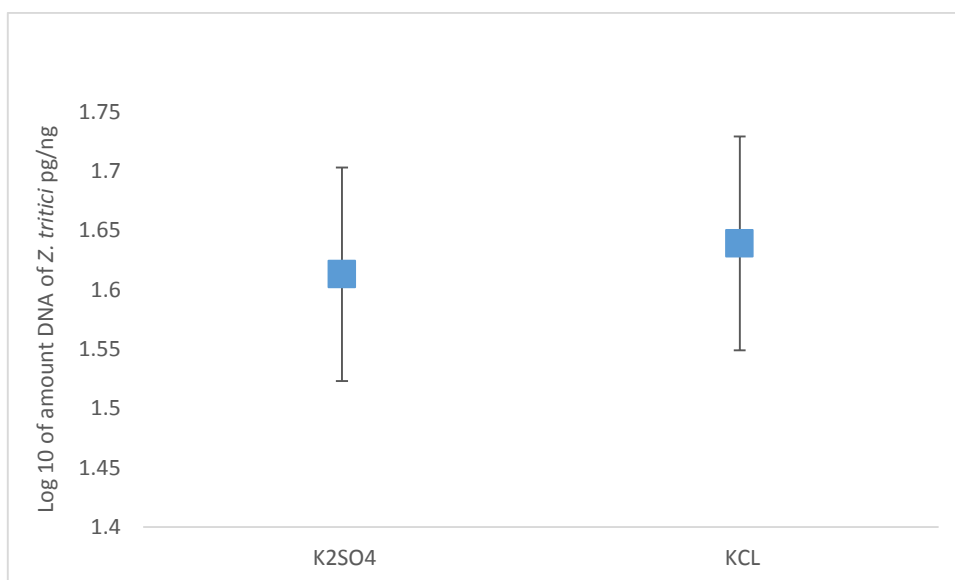
Figure 80. Fertilizer effect, estimated using the liquidized method, on *Z. tritici* DNA at growth stage 80 from field samples of cv. M. Huntsman averaged over other factors. (Error bar = ± S.E.D. in two different replicate, P. = 0.8).

Table 31. ANOVA table showing analysis of DNA concentration in samples taken at GS 31 from field experiment in 2014-2015 (ch 4-5) and extracted following liquidisation. DNA estimated using SYBR-green to detect *Z. tritici*. d.f. degree of freedom, s.s. sum of square, v.r. variance ratio, F.pr. *F*-test probability associated with variance ratio.

Source of variation	d.f.	(m.v.)	s.s.	v.r.	F pr.
block stratum	2		1.7714	1.67	
Block / main plot stratum					
Fungicide	1		0.0059	0.01	0.92
P. nodorum inoculation	2		1.4023	1.32	0.32
Fungicide × *P. nodorum* inoculation	2		0.1328	0.13	0.88
Residual	8	-2	4.243	2.77	
Block / main plot /subplot stratum					
fertilizer	1		2.0014	10.45	0.014
Fertilizer × Fungicide	1		0.0595	0.31	0.60
Fertilizer × *P. nodorum* inoculation	2		0.7841	2.05	0.20
Fertilizer × Fungicide × *P. nodorum* inoculation	2		0.1858	0.49	0.64
Residual	7	-5	1.3402		
Total	28	-7	11.0971		

Table 32, ANOVA table showing analysis of DNA concentration in samples taken at GS 80 from field experiment in 2014-2015 (ch 4-6) and extracted by liquidisation. DNA concentration estimated by qPCR using SYBR-green to detect *Z. tritici*. d.f. degree of freedom, s.s. sum of square, v.r. variance ratio, F.pr. *F*-test probability associated with variance ratio.

Source of variation	d.f.	(m.v.)	s.s.	v.r.	F pr.
block stratum	2		0.88436	1.65	
Block/ main plot stratum					
Fungicide	1		0.01703	0.06	0.80
P. nodorum inoculation	2		0.15767	0.29	0.75
Fungicide × *P. nodorum* inoculation	2		1.50923	2.82	0.11
Residual	10		2.67917	3.6	
Block /main plot /subplot stratum					
Fertilizer	1		0.00594	0.08	0.78
Fertilizer × Fungicide	1		0.00068	0.01	0.93
Fertilizer × *P. nodorum* inoculation	2		0.18534	1.25	0.33
Fertilizer × Fungicide × *P. nodorum* inoculation	2		0.30743	2.07	0.18
Residual	9	-3	0.66946		
Total	32	-3	5.53441		

1-3-5- Correlation between DNA assessment and other assessment methods:

There was a weak positive correlation between proportion of infected leaves or spore concentration with DNA estimated by the liquidisation method at growth stage 31 (r = 0.4 and 0.3 respectively; Figure 78 A and B), using SYBR-green to detect *Z. tritici*.

There were very slight and negative correlations between proportions of leaves infected or spore concentration of *Z. tritici* at growth stage 31 and *Z. tritici* DNA concentration measured with oven dried samples at GS 31, using either SYBR-green (r = -0.02, and -0.13; Figure 79 A and B) or Taq-man and to detect *Z. tritici* (r = -0.1, -0.14; Figure 80 A and B) respectively.

A   B
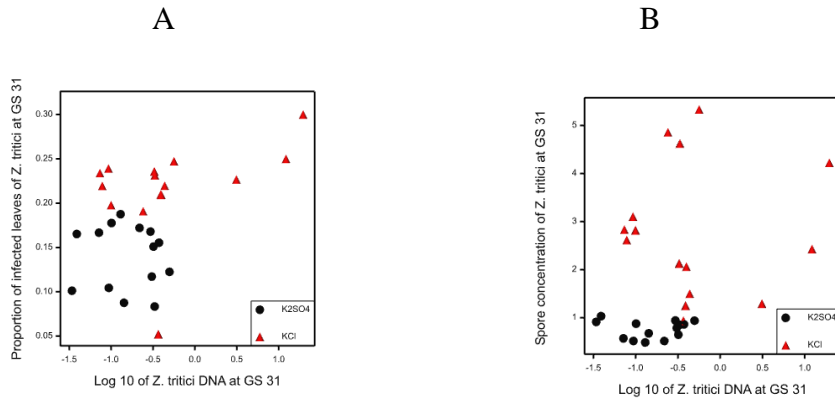
Figure 81, liquidized method analysed with SYBR-green at growth stage 31 correlation with (A) proportion of infected leaves r= 0.42 (B) spore concentration 10^3/ml, (r = 0.3), both at growth stage 31.
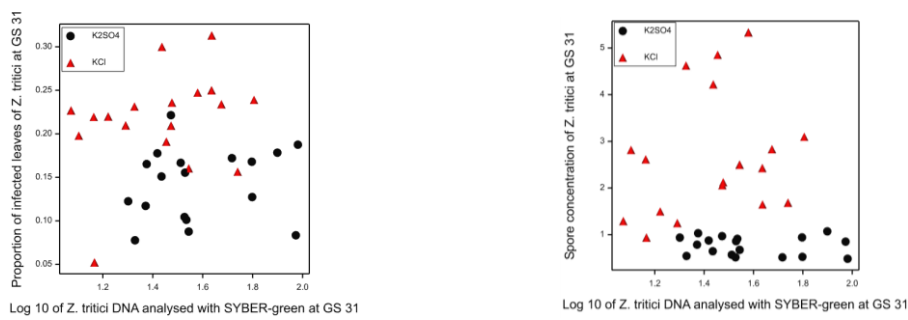
Figure 82, DNA concentration in dried samples of wheat taken at GS31 and oven-dried then analysed with SYBR-green plotted against (A) proportion of infected leaves r= -0.02 (B) spore concentration 10^3/ml (r = -0.13), both at growth stage 31.
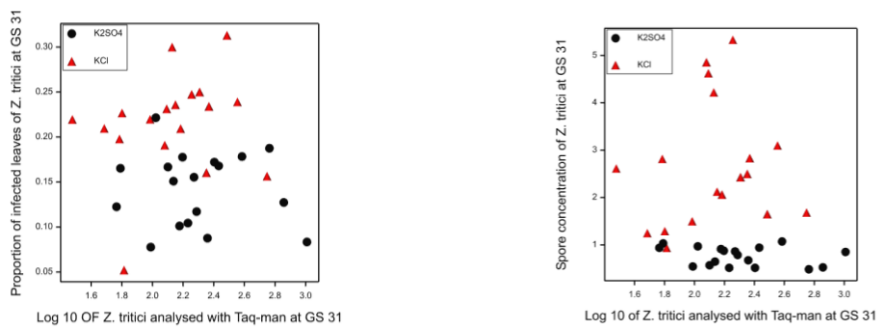
Figure 83. DNA concentration in dried samples of wheat taken at GS31 and oven-dried for 48 hours then analysed with Taq-man, plotted against (A) proportion of infected leaves r= -0.1 (B) spore concentration 10^3/ml (r = -0.14), both at growth stage 31.

1-4- Discussion:

Despite phosphate buffer having some disadvantages such as precipitating DNA in ethanol, the results have shown that phosphate buffer gave only slightly lower DNA concentration than CTAB buffer when DNA was extracted from fresh samples. The amount and quantity of DNA was variable between samples and sampling occasions. With seedling (GS13) samples the amount of DNA obtained was a bit higher than the samples in late season GS 31 and GS 80. This may be due to the early growth stage of sampling, or to intrinsic variation between samples. The two buffers showed significant differences. This could be due to the fact that phosphate buffer can precipitate in ethanol which could lead to precipitation of some DNA from the sample. Phosphate and CTAB both gave more stable DNA amounts with 6ml of buffer with each gram of plant sample.

There are various parts of the procedure which might lead to poor DNA yields and excessive variation between samples and need further optimisation. The washing stages by AW1 and AW2 buffers (steps 6-9 in the Quick-start protocol, in DNeasy plant mini kit) might need to be more thorough, to get rid of salts that might be precipitated during elution. Another reason for variation in DNA recovery and purity when using the DNeasy mini kit is that the buffers work specifically with 0.1g of wet sample or 0.02g of lyophilized samples. In my case 700µl of sample will have decreased the buffer concentration. Thus, more investigation is needed to optimise buffer concentrations.

The amount of *Z. tritici* DNA in the first three samples varied. This is what is expected from this experiment, because the actual amount of pathogen in each sample will have varied. These samples were collected from same plot, but there will have been differences in the amount of pathogen in replicate samples. Fresh samples gave more stable DNA concentration in both buffers than samples that were stored for various times and under different conditions. The samples that were kept at room temperature for 48 hours or in a fridge for 48 hours gave larger concentrations of *Z. tritici* DNA. This situation could arise in two different ways. Firstly, these conditions might encourage multiplication of pathogen. Secondly, these samples might by chance have initially had more pathogen than other samples which then gave more DNA of *Z. tritici*. To distinguish these cases another experiment was done specifically to test storage conditions. Within this experiment there were no significant differences, although the sample kept at 4^0C overnight again had a high mean value. This result was therefore inconclusive. There was little evidence of interaction between buffer types and storage methods, and the fresh sample and the sample which was kept at 4^0C for 48 hours gave nearly the same mean value in both buffers. The sample that was kept at -20ºC for

48 hours gave a good amount of good quality DNA, presumably because enzyme activity is reduced. Thus, these results suggested that DNA can be extracted either directly from fresh sample, or from samples kept in a freezer (-20°C) for 48 hours.

It is well known that the amount and quality of DNA extracted can be affected by many factors. The field samples that were used in this chapter had DNA extracted by using DNeasy mini kit, which is specialised for wet and lyophilized tissue but in small amounts. The first step of the protocol involves incubating the sample with AP1 buffer and RNase for 10 minutes; this could lead to fermentation and the production of ethanol, interfering with subsequent steps. Moreover, the use of 700μl of liquidised sample with same amount of buffers recommended for ≤ 100 mg of wet sample might be sub-optimal and lead to more dilutions of the buffers used. In consequence, the amount and quality of DNA will be reduced, and more inhibitor materials such as salts might be in the extracted sample of DNA.

Samples were collected from two different plots, one treated with and one without sulphur. At growth stage 15 there was no significant effect of fertilizers on *Z. tritici* DNA concentration.

Sulphate fertilization significantly decreased the concentration of *Z. tritici* at growth stage 31. This result agrees with visual assessment. At growth stage 80 *Z. tritici* was reduced by sulphur fertilizer but not significantly. This could be due to the long latent period of *Z. tritici* (Verreet *et al.*, 2000), which telemorph stage has longer period than anamorph (Kema *et al.*, 1996a). The level of contamination in upper leaves may have been increased by external ascospore contamination, as the position of these leaves (Flag leaf 1, Flag leaf 2) allows them to capture more airborne spores than bottom leaves (Selim *et al.*, 2014). Ascospores can be spread for long distance (Fraaije *et al.*, 2005), in comparison with pycnidiospores which spread only a few metres (Boeger *et al.*, 1993).

Samples extracted by the dried method at growth stage 31 were analysed with two different chemical reagents to detect *Z. tritici* DNA under two alternative fertilizers. Each dried sample from growth stage 31 was analysed in two different ways, with SYBR-green and with Taq-man. Both reagents gave similar results. This justifies the use of the cheaper, but potentially non-specific SYBR-green method. Using dried samples gave opposite results from the liquidized samples, the dried samples showed more *Z. tritici* in the sulphur treated plants (Figure 72 A and B). The liquidised samples showed the opposite, in agreement with the visual assessments (Figure 76).

Many studies have used PCR and qPCR techniques to estimate or detect diseases. Most of these studies used few leaves with expensive chemical reagents. These studies, using a few leaves or part

of a leaf, are not useful to quantify the pathogen in a field-scale, or even pot-scale. Kuzdraliński *et al.* (2015) used 10-15 leaves were collected at tillering stage in spring and at 3-4 leaves stage in autumn, and only 30-40 mg of leaves was used to detect *Z. tritici* at early stage by PCR. This study used liquid nitrogen to grind the sample by pestle to make a fine powder before DNA extraction. So this way may not be convenient to extract large number of sample due to the hazard and the cost. Csontos & Tamas (2011), found that 800-1000 leaves were needed to estimate the fungal species present on the phylloplane of plants in a site with high diversity coupled with low evenness values. This suggests that large numbers of leaf samples are required to quantify pathogen under field conditions and to detect the variation that may occur in the field.

The results from this chapter are encouraging for a new way to measure the amount of pathogen in a field crop, because it promises to provide a low-cost way to measure amount of pathogen in a large number of samples objectively. This chapter suggest that by using phosphate buffer in large amounts, and liquidizing many field samples directly, qPCR may provide a quantitative way to assess pathogens in field samples.

2- Tables of ANOVA and means of glasshouse and field experiments:

2-G 1. A summary table for the general ANOVA on Log10 of the proportion of infected leaves in glasshouse experiment for different isolates of alternative pathogen-inoculated different wheat cultivars and that treated with two alternative fertilizers as factorial combinations. d.f. the degree of freedom, s.s. sum of the square, v.r. variance ratio, F.pr. *F*-test probability associated with variance ratio.

Source of variation	d.f.	s.s.	v.r.	F pr.
block stratum	3	1.98	11.6	
Within blocks				
Cultivar	2	2.33	20.4	<0.001
Fertilizer	1	0.67	11.8	<0.001
isolate	3	23.00	134.4	<0.001
Cultivar × Fertilizer	2	0.04	0.3	0.70
Cultivar × isolate	6	0.81	2.3	0.04
Fertilize × isolate	3	0.38	2.2	0.10
Cultivar × Fertilizer × isolate	6	0.33	0.9	0.40
Residual	69	3.94		
Total	95	33.49		

2-G 2. A summary of ANOVA table for percentage of dead leaves in glaahouse experiment. Different wheat cultivars were inoculated with or without inoculation of three isolates of Z. tritici and one isolate of *P. nodorum* and treated with two alternative fertilizers as a factorial combination under glasshouse conditions. d.f. the degree of freedom, s.s. sum of the square, v.r. variance ratio, F.pr. *F*-test probability associated with variance ratio.

Source of variation	d.f.	s.s.	v.r.	F pr.
block stratum	3	589.72	5.31	
Within blocks				
Cultivar	2	5349.8	74.6	<0.001
Fertilizer	1	1.2	0.03	0.86
isolate	4	556.9	3.9	0.006
Cultivar × Fertilizer	2	30.4	0.4	0.66
Cultivar × isolate	8	608.2	2.1	0.04
Fertilize × isolate	4	188.1	1.3	0.27
Cultivar × Fertilizer × isolate	8	201.3	0.7	0.69
Residual	87	3120.1		
Total	119	10645.6		

2-F 1. A summary table for the general ANOVA on the proportion of infected leaves with *P. nododrum* at GS 31 (2013), from spring wheat cultivar, Paragon. Treated with four alternative fertilizers as a factorial combination. d.f. degree of freedom, s.s. sum of square, v.r. variance ratio, F.pr. *F*-test probability associated with variance ratio.

Source of variation	d.f.	s.s.	v.r.	F pr.
Block stratum	2	0.003	0.64	
Within Block				
Inoculation with *P. nodorum*	1	0.403	1.76	<0.001
Inoculation with *Z. tritici*	1	0.003	1.45	0.27
P. nodorum × *Z. tritici*	1	0.003	1.45	0.27
Residual	6	0.013	0.16	
Block × Main plot × subplot				
Fertiliser	3	0.031	0.72	0.55
Fertiliser × *P. nodorum* inoculation	3	0.031	0.72	0.55
Fertiliser × *Z. tritic*i inoculation	3	0.018	0.42	0.74
Fertiliser × *P. nodorum* × *Z. tritici*	3	0.018	0.42	0.74
Residual	24	0.35		
Total	47	0.876		

2-F 2. A summary table for the general ANOVA on disease severity of *P. nodorum* at GS 31 (2013), from spring wheat cultivar Paragon, treated with four alternative fertilizers as a factorial combination. d.f. degree of freedom, s.s. sum of square, v.r. variance ratio, F.pr. *F*-test probability associated with variance ratio.

Source of variation	d.f.	s.s.	v.r.	F pr.
Block stratum	2	3.6	0.1	
Within Block				
Inoculation with *P. nodorum*	1	462.5	26.3	0.002
Inoculation with *Z. tritici*	1	1.2	0.07	0.79
P. nodorum × *Z. tritici*	1	1.2	0.07	0.79
Residual	6	105.5	1.2	
Block × Main plot × subplot				
Fertiliser	3	9.1	0.2	0.89
Fertiliser × *P. nodorum* inoculation	3	9.1	0.2	0.89
Fertiliser × *Z. tritic*i inoculation	3	26.8	0.6	0.62
Fertiliser × *P. nodorum* × *Z. tritici*	3	26.8	0.6	0.62
Residual	24	362.2		
Total	47	1008.1		

2-F 3. A summary table for the general ANOVA of proportion of leaves infected with *Blumiria garaminis* (Powdery mildew) at GS 31 (2013), from spring wheat cultivar Paragon, treated with four alternative fertilizers in a factorial combination with a split-plot design. d.f. degree of freedom, s.s. sum of square, v.r. variance ratio, F.pr. *F*-test probability associated with variance ratio.

Source of variation	d.f.	s.s.	v.r.	F pr.
Block stratum	2	0.670	4.14	
Within Block				
Inoculation with *P. nodorum*	1	0.083	1.03	0.4
Inoculation with *Z. tritici*	1	0.213	2.63	0.15
P. nodorum × *Z. tritici*	1	0.213	2.63	0.15
Residual	6	0.486	0.63	
Block × Main plot × subplot				
Fertiliser	3	0.291	0.76	0.5
Fertiliser × *P. nodorum* inoculation	3	0.221	0.57	0.64
Fertiliser × *Z. tritici* inoculation	3	0.378	0.98	0.42
Fertiliser × *P. nodorum* × *Z. tritici*	3	0.028	0.07	0.98
Residual	24	3.09		
Total	47	5.676		

2-F 4. A summary table for the general ANOVA on proportion of leaves infected with *P. nodorum* at GS 59 (2013), from spring wheat cultivar Paragon, treated with four alternative fertilizers as a factorial combination. d.f. degree of freedom, s.s. sum of square, v.r. variance ratio, F.pr. *F*-test probability associated with variance ratio.

Source of variation	d.f.	s.s.	v.r.	F pr.
Block stratum	1	0.003	0.8	
Within Block				
Inoculation with *P. nodorum*	1	1.333	316.6	<.001
Inoculation with *Z. tritici*	1	0.044	10.6	0.05
P. nodorum × *Z. tritici*	1	0.044	10.6	0.05
Residual	3	0.012	0.2	
Block × Main plot × subplot				
Fertiliser	3	0.440	6.7	0.006
Fertiliser × *P. nodorum* inoculation	3	0.440	6.7	0.006
Fertiliser × *Z. tritici* inoculation	3	0.091	1.4	0.3
Fertiliser × *P. nodorum* × *Z. tritici*	3	0.091	1.4	0.3
Residual	12	0.261		
Total	31	2.762		

2-F 5. A summary table for the general ANOVA on proportion of plants infected with *P. nodorum* at GS 59 (2013), from spring wheat cultivar Paragon, treated with four alternative fertilizers as a factorial combination. d.f. degree of freedom, s.s. sum of square, v.r. variance ratio, F.pr. *F*-test probability associated with variance ratio.

Source of variation	d.f.	s.s.	v.r.	F pr.
Block stratum	1	0.070	2.45	
Within Block				
Inoculation with *P. nodorum*	1	0.812	28.37	0.013
Inoculation with *Z. tritici*	1	0.003	0.1	0.77
P. nodorum × *Z. tritici*	1	0.003	0.1	0.77
Residual	3	0.086	1.92	
Block × Main plot × subplot				
Fertiliser	3	0.208	4.66	0.022
Fertiliser × *P. nodorum* inoculation	3	0.208	4.66	0.022
Fertiliser × *Z. tritici* inoculation	3	0.043	0.97	0.44
Fertiliser × *P. nodorum* × *Z. tritici*	3	0.043	0.97	0.44
Residual	12	0.179		
Total	31	1.6571		

2-F 6. A summary table for the general ANOVA on disease severity of *P. nodorum* at GS 59 (2013), from spring wheat cultivar Paragon, treated with four alternative fertilizers as a factorial combination. d.f. degree of freedom, s.s. sum of square, v.r. variance ratio, F.pr. *F*-test probability associated with variance ratio.

Source of variation	d.f.	(m.v.)	s.s.	v.r.	F pr.
Block stratum	1		131.8	2.02	
Within Block					
Inoculation with *P. nodorum*	1		1195.2	18.3	0.02
Inoculation with *Z. tritici*	1		39.9	0.6	0.49
P. nodorum × *Z. tritici*	1		39.9	0.6	0.49
Residual	3		195.5	2.1	
Block × Main plot × subplot					
Fertiliser	3		356.3	3.8	0.04
Fertiliser × *P. nodorum* inoculation	3		356.3	3.8	0.04
Fertiliser × *Z. tritici* inoculation	3		112.5	1.2	0.36
Fertiliser × *P. nodorum* × *Z. tritici*	3		112.5	1.2	0.36
Residual	11	-1	343.9		
Total	30	-1	2873.5		

2-F 7. A summary table for the general ANOVA on proportion of plants infected with *Z. tritici* at GS 59 (2013), from spring wheat cultivar Paragon, treated with four alternative fertilizers as a factorial combination. d.f. degree of freedom, s.s. sum of square, v.r. variance ratio, F.pr. *F*-test probability associated with variance ratio.

Source of variation	d.f.	s.s.	v.r.	F pr.
Block stratum	1	0.061	1.8	
Within Block				
Inoculation with *P. nodorum*	1	0.011	0.3	0.60
Inoculation with *Z. tritici*	1	0.281	8.3	0.06
P. nodorum × *Z. tritici*	1	0.02	0.6	0.45
Residual	3	0.101	1.9	
Block × Main plot × subplot				
Fertiliser	3	0.182	3.5	0.05
Fertiliser × *P. nodorum* inoculation	3	0.021	0.4	0.75
Fertiliser × *Z. tritici* inoculation	3	0.101	1.9	0.18
Fertiliser × *P. nodorum* × *Z. tritici*	3	0.032	0.6	0.61
Residual	12	0.207		
Total	31	1.02		

2-F 8. A summary table for the general ANOVA on proportion of leaves infected with *Z. tritici* at GS 59 (2013), from spring wheat cultivar Paragon, treated with four alternative fertilizers as a factorial combination. d.f. degree of freedom, s.s. sum of square, v.r. variance ratio, F.pr. *F*-test probability associated with variance ratio.

Source of variation	d.f.	s.s.	v.r.	F pr.
Block stratum	1	0.099	33.8	
Within Block				
Inoculation with *P. nodorum*	1	0.023	7.9	0.07
Inoculation with *Z. tritici*	1	0.210	71.4	0.003
P. nodorum × *Z. tritici*	1	0.053	18.3	0.024
Residual	3	0.008	0.1	
Block × Main plot × subplot				
Fertiliser	3	0.317	4.3	0.028
Fertiliser × *P. nodorum* inoculation	3	0.102	1.4	0.29
Fertiliser × *Z. tritic*i inoculation	3	0.182	2.5	0.11
Fertiliser × *P. nodorum* × *Z. tritici*	3	0.211	2.9	0.08
Residual	12	0.296		
Total	31	1.506		

2-F 9. A summary table for the general ANOVA on disease severity of *Z. tritici* at GS 59 (2013), from spring wheat cultivar Paragon, treated with four alternative fertilizers as a factorial combination. d.f. degree of freedom, s.s. sum of square, v.r. variance ratio, F.pr. *F*-test probability associated with variance ratio.

Source of variation	d.f.	(m.v.)	s.s.	v.r.	F pr.
Block stratum	1		20.1	0.25	
Within Block					
Inoculation with *P. nodorum*	1		5.7	0.07	0.80
Inoculation with *Z. tritici*	1		145.8	1.8	0.27
P. nodorum × *Z. tritici*	1		126.6	1.6	0.30
Residual	3		236.7	0.7	
Block × Main plot × subplot					
Fertiliser	3		82.3	0.3	0.86
Fertiliser × *P. nodorum* inoculation	3		187	0.6	0.65
Fertiliser × *Z. tritic*i inoculation	3		49.2	0.2	0.92
Fertiliser × *P. nodorum* × *Z. tritici*	3		210.3	0.6	0.60
Residual	11	-1	1197.1		
Total	30	-1	2260.3		

2-F 10. A summary table for the general ANOVA on proportion of leaves infected with *B. graminis* (Powdery mildew) at GS 59 (2013), from spring wheat cultivar Paragon, treated with four alternative fertilizers as a factorial combination. d.f. degree of freedom, s.s. sum of square, v.r. variance ratio, F.pr. *F*-test probability associated with variance ratio.

Source of variation	d.f.	s.s.	v.r.	F pr.
Block stratum	1	0.045	14.83	
Within Block				
Inoculation with *P. nodorum*	1	0.022	7.54	0.07
Inoculation with *Z. tritici*	1	0.005	1.76	0.27
P. nodorum × *Z. tritici*	1	0.004	1.5	0.30
Residual	3	0.009	1.27	
Block × Main plot × subplot				
Fertiliser	3	0.020	2.89	0.08
Fertiliser × *P. nodorum* inoculation	3	0.004	0.69	0.57
Fertiliser × *Z. tritici* inoculation	3	0.005	0.8	0.51
Fertiliser × *P. nodorum* × *Z. tritici*	3	0.001	0.22	0.88
Residual	12	0.028		
Total	31	0.148		

2-F 11. A summary table for the general ANOVA on proportion of leaves infected with *Z. tritici* at GS 72 (2013), from spring wheat cultivar Paragon, treated with four alternative fertilizers as a factorial combination. d.f. degree of freedom, s.s. sum of square, v.r. variance ratio, F.pr. *F*-test probability associated with variance ratio.

Source of variation	d.f.	s.s.	v.r.	F pr.
Block stratum	1	0.0008	0.02	
Within Block				
Inoculation with *P. nodorum*	1	0.003	0.09	0.8
Inoculation with *Z. tritici*	1	0.009	0.25	0.65
P. nodorum × *Z. tritici*	1	0.078	2.12	0.24
Residual	3	0.110	1.64	
Block × Main plot × subplot				
Fertiliser	3	0.258	3.82	0.04
Fertiliser × *P. nodorum* inoculation	3	0.027	0.4	0.76
Fertiliser × *Z. tritici* inoculation	3	0.068	1.01	0.42
Fertiliser × *P. nodorum* × *Z. tritici*	3	0.039	0.59	0.64
Residual	12	0.269		
Total	31	0.864		

2-F 12. A summary table for the general ANOVA on proportion of leaves infected with *P. nodorum* at GS 72 (2013), from spring wheat cultivar Paragon, treated with four alternative fertilizers as a factorial combination. d.f. degree of freedom, s.s. sum of square, v.r. variance ratio, F.pr. *F*-test probability associated with variance ratio.

Source of variation	d.f.	s.s.	v.r.	F pr.
Block stratum	1	0.074	0.8	
Within Block				
Inoculation with *P. nodorum*	1	0.715	7.7	0.07
Inoculation with *Z. tritici*	1	0.373	4.0	0.14
P. nodorum × *Z. tritici*	1	0.373	4.0	0.14
Residual	3	0.279	5.4	
Block × Main plot × subplot				
Fertiliser	3	0.028	0.5	0.67
Fertiliser × *P. nodorum* inoculation	3	0.028	0.5	0.67
Fertiliser × *Z. tritic*i inoculation	3	0.066	1.3	0.33
Fertiliser × *P. nodorum* × *Z. tritici*	3	0.066	1.3	0.33
Residual	12	0.207		
Total	31	2.210		

2-F 13. A summary table for the general ANOVA on proportion of leaves infected with *Z. tritici* at GS 31 (2013-2014), from two winter wheat cultivars Gallant and M. Huntsman, treated with three alternative fertilizers as a factorial combination. d.f. degree of freedom, s.s. sum of square, v.r. variance ratio, F.pr. *F*-test probability associated with variance ratio

Source of variation	d.f.	(m.v.)	s.s.	v.r.	F pr.
Within Block	2		0.242	3.24	
Block × main plot					
Cultivar	1		0.263	7.04	0.02
Inoculation with *Z. tritici*	1		0.003	0.08	0.8
Inoculation with *P. nodorum*	1		0.008	0.23	0.64
Cultivar × *Z. tritici* inoculation	1		0.000001	0	1.0
Cultivar × *P. nodorum* inoculation	1		0.012	0.32	0.6
Z. tritici × *P. nodorum*	1		0.0012	0.03	0.9
Cultivar × *Z. tritici* × *P. nodorum*	1		0.005	0.13	0.7
Residual	14		0.523	10.68	
Block × main plot × subplot					
Fertilizer	2		0.043	6.12	0.006
Cultivar × Fertilizer	2		0.052	7.39	0.002
Fertilizer × *Z. tritici* Inoculation	2		0.015	2.1	0.14
Fertilizer × *P. nodorum* inoculation	2		0.043	6.05	0.006
Cultivar × Fertilizer × *Z. tritici*	2		0.008	1.2	0.31
Cultivar × fertilizer × *P. nodorum*	2		0.006	0.85	0.43
Fertilizer × *Z. tritici* × *P. nodorum*	2		0.023	3.24	0.05
Cultivar × Fertilizer × *Z. tritici* × *P. nodorum*	2		0.006	0.98	0.386
Residual	31	-1	0.108		
Total	70	-1	1.360		

2-F 14. A summary table for the general ANOVA on spore concentration of Z. *tritici* at GS 31 (2013-2014), from two winter wheat cultivars Gallant and M. Huntsman, treated with three alternative fertilizers as a factorial combination. d.f. degree of freedom, s.s. sum of square, v.r. variance ratio, F.pr. *F*-test probability associated with variance ratio.

Source of variation	d.f.	(m.v.)	s.s.	v.r.	F pr.
Within Block	2		18.5	0.41	
Block × main plot					
Cultivar	1		1096.9	48.63	<0.001
Inoculation with *Z. tritici*	1		4.8	0.21	0.65
Inoculation with *P. nodorum*	1		2.3	0.1	0.76
Cultivar × *Z. tritici* inoculation	1		31.0	1.37	0.26
Cultivar × *P. nodorum* inoculation	1		0.06	0	0.96
Z. tritici × *P. nodorum*	1		21.2	0.94	0.35
Cultivar × *Z. tritici* × *P. nodorum*	1		46.7	2.07	0.17
Residual	13	-1	293.3	1.68	
Block × main plot × subplot					
Fertilizer	2		25.3	0.94	0.40
Cultivar × Fertilizer	2		9.06	0.34	0.72
Fertilizer × *Z. tritici* Inoculation	2		71.1	2.64	0.09
Fertilizer × *P. nodorum* inoculation	2		20.6	0.76	0.48
Cultivar × Fertilizer × *Z. tritici*	2		63.9	2.37	0.11
Cultivar × fertilizer × *P. nodorum*	2		44.2	1.64	0.21
Fertilizer × *Z. tritici* × *P. nodorum*	2		51.8	1.92	0.17
Cultivar × Fertilizer × *Z. tritici* × *P. nodorum*	2		46.2	1.72	0.20
Residual	24	-8	322.9		
Total	62	-9	1803.9		

2-F 15. A summary table for the general ANOVA on proportion of leaves infected with *Z. tritici* at GS 59 (2013-2014), from two winter wheat cultivars Gallant and M. Huntsman, treated with three alternative fertilizers as a factorial combination. d.f. degree of freedom, s.s. sum of square, v.r. variance ratio, F.pr. *F*-test probability associated with variance ratio.

Source of variation	d.f.	(m.v.)	s.s.	v.r.	F pr.
Within Block	2		0.018	0.94	
Block × main plot					
Cultivar	1		0.001	0.11	0.74
Inoculation with *Z. tritici*	1		0.005	0.55	0.47
Inoculation with *P. nodorum*	1		0.0002	0.03	0.87
Cultivar × *Z. tritici* inoculation	1		0.00008	0.01	0.93
Cultivar × *P. nodorum* inoculation	1		0.002	0.17	0.69
Z. tritici × *P. nodorum*	1		0.003	0.31	0.59
Cultivar × *Z. tritici* × *P. nodorum*	1		0.005	0.52	0.48
Residual	14		0.132	3.36	
Block × main plot × subplot					
Fertilizer	2		0.052	9.18	<0.001
Cultivar × Fertilizer	2		0.008	1.53	0.23
Fertilizer × *Z. tritici* Inoculation	2		0.0006	0.11	0.90
Fertilizer × *P. nodorum* inoculation	2		0.012	2.05	0.15
Cultivar × Fertilizer × *Z. tritici*	2		0.011	2.06	0.15
Cultivar × fertilizer × *P. nodorum*	2		0.011	2.01	0.15
Fertilizer × *Z. tritici* × *P. nodorum*	2		0.003	0.5	0.61
Cultivar × Fertilizer × *Z. tritici* × *P. nodorum*	2		0.003	0.56	0.58
Residual	30	-2	0.084		
Total	69	-2	0.341		

2-F 16. A summary table for the general ANOVA on spore concentration of *Z. tritici* at GS 59 (2013-2014), from two winter wheat cultivars Gallant and M. Huntsman, treated with three alternative fertilizers as a factorial combination. d.f. degree of freedom, s.s. sum of square, v.r. variance ratio, F.pr. *F*-test probability associated with variance ratio.

Source of variation	d.f.	(m.v.)	s.s.	v.r.	F pr.
Within Block	2		824.71	3.27	
Block × main plot					
Cultivar	1		37.25	0.3	0.60
Inoculation with *Z. tritici*	1		0.51	0	0.95
Inoculation with *P. nodorum*	1		259.91	2.06	0.17
Cultivar × *Z. tritici* inoculation	1		69.29	0.55	0.47
Cultivar × *P. nodorum* inoculation	1		0.20	0	0.97
Z. tritici × *P. nodorum*	1		61.55	0.49	0.50
Cultivar × *Z. tritici* × *P. nodorum*	1		23.79	0.19	0.67
Residual	14		1765.78	2.32	
Block × main plot × subplot					
Fertilizer	2		1126.57	10.38	<0.001
Cultivar × Fertilizer	2		21.95	0.2	0.82
Fertilizer × *Z. tritici* Inoculation	2		72.75	0.67	0.52
Fertilizer × *P. nodorum* inoculation	2		120.68	1.11	0.34
Cultivar × Fertilizer × *Z. tritici*	2		162.22	1.49	0.24
Cultivar × fertilizer × *P. nodorum*	2		41.09	0.38	0.69
Fertilizer × *Z. tritici* × *P. nodorum*	2		100.04	0.92	0.41
Cultivar × Fertilizer × *Z. tritici* × *P. nodorum*	2		37.81	0.35	0.71
Residual	27	-5	1464.94		
Total	66	-5	6073.85		

2-F 17. A summary table for the general ANOVA on DNA amount of Z. tritici at GS 59 (2013-2014), from two winter wheat cultivars Gallant and M. Huntsman, treated with three alternative fertilizers as a factorial combination. d.f. degree of freedom, s.s. sum of square, v.r. variance ratio, F.pr. *F*-test probability associated with variance ratio.

Source of variation	d.f.	s.s.	v.r.	F pr.
Within Block	1	1147.6	1.12	
Block × main plot				
Cultivars	1	8524.5	8.3	0.02
Inoculation with *Z. tritici*	1	1251.5	1.22	0.3
Inoculation with *P. nodorum*	1	60.8	0.06	0.8
Cultivar × *Z. tritici* inoculation	1	410.7	0.4	0.5
Cultivar × *P. nodorum* inoculation	1	165.5	0.16	0.7
Z. tritici × *P. nodorum*	1	1363.8	1.33	0.3
Cultivar × *Z. tritici* × *P. nodorum*	1	1883.8	1.83	0.2
Residual	7	7191.6	2.72	
Block × main plot × subplot				
Fertilizer	2	3953.9	5.23	0.02
Cultivar × Fertilizer	2	174.4	0.23	0.8
Fertilizer × *Z. tritici*	2	1777.5	2.35	0.1
Fertilizer × *P. nodorum*	2	2960.4	3.92	0.04
Cultivar × fertilizer × *Z. tritici*	2	809.1	1.07	0.4
Cultivar × Fertilizer × *P. nodorum*	2	2473.6	3.27	0.06
Fertilizer × *Z. tritici* × *P. nodorum*	2	2626.1	3.48	0.06
Cultivar × Fertilizer × *Z. tritici* × *P. nodorum*	2	4776.4	6.32	0.009
Residual	16	6044.8		
Total	47	47595.8		

2-F 18. A summary table for the general ANOVA on proportion of leaves infected with yellow rust at GS 59 (2013-2014), from two winter wheat cultivars Gallant and M. Huntsman, treated with three alternative fertilizers as a factorial combination. d.f. degree of freedom, s.s. sum of square, v.r. variance ratio, F.pr. *F*-test probability associated with variance ratio

Source of variation	d.f.	(m.v.)	s.s.	v.r.	F pr.
Within Block	2		0.301	4.01	
Block × main plot					
Cultivar	1		0.740	19.7	<0.001
Inoculation with *Z. tritici*	1		0.030	0.8	0.4
Inoculation with *P. nodorum*	1		0.0004	0.01	0.9
Cultivar × *Z. tritici* inoculation	1		0.030	0.8	0.4
Cultivar × *P. nodorum* inoculation	1		0.000005	0	1
Z. tritici × *P. nodorum*	1		0.004	0.11	0.7
Cultivar × *Z. tritici* × *P. nodorum*	1		0.005	0.14	0.7
Residual	14		0.526	8.27	
Block × main plot × subplot					
Fertilizer	2		0.036	3.97	0.03
Cultivar × Fertilizer	2		0.037	4.05	0.03
Fertilizer × *Z. tritici* Inoculation	2		0.005	0.51	0.6
Fertilizer × *P. nodorum* inoculation	2		0.004	0.45	0.6
Cultivar × Fertilizer × *Z. tritici*	2		0.003	0.35	0.7
Cultivar × fertilizer × *P. nodorum*	2		0.006	0.67	0.5
Fertilizer × *Z. tritici* × *P. nodorum*	2		0.0002	0.02	0.9
Cultivar × Fertilizer × *Z. tritici* × *P. nodorum*	2		0.00007	0.01	0.99
Residual	30	-2	0.136		
Total	69	-2	1.375		

2-F 19. A summary table for the general ANOVA on proportion of leaves infected with *Z. tritici* at GS 31 (2014-2015), from winter wheat cultivar M. Huntsman, treated either with or without sulphur fertilizer as a factorial combination. d.f. degree of freedom, s.s. sum of square, v.r. variance ratio, F.pr. *F*-test probability associated with variance ratio.

Source of variation	d.f.	s.s.	v.r.	F pr.
block stratum	2	0.002755	0.39	
Within block				
Fungicide	1	0.0016	0.45	0.52
Inoculation with *P. nodorum*	2	0.002598	0.37	0.70
Fungicide × *P. nodorum* Inoculation	2	0.006013	0.84	0.46
Residual	10	0.035587	1.4	
Block × main plot × subplot				
fertilizer	1	0.049162	19.38	<0.001
Fertilizer × Fungicide	1	0	0	0.99
Fertilizer × *P. nodorum* Inoculation	2	0.002594	0.51	0.61
Fertilizer × Fungicide × *P. nodorum*	2	0.001985	0.39	0.69
Residual	12	0.030436		
Total	35	0.13273		

2-F 20. A summary table for the general ANOVA on spore concentration of *Z. tritici* at GS 31 (2014-2015), from winter wheat cultivar M. Huntsman, treated either with or without sulphur fertilizer as a factorial combination. d.f. degree of freedom, s.s. sum of square, v.r. variance ratio, F.pr. *F*-test probability associated with variance ratio.

Source of variation	d.f.	s.s.	v.r.	F pr.
block stratum	2	2.8667	1.47	
Within block				
Fungicide	1	0.6087	0.63	0.447
Inoculation with *P. nodorum*	2	1.5379	0.79	0.48
Fungicide × *P. nodorum* Inoculation	2	1.3669	0.7	0.518
Residual	10	9.7185	1.12	
Block × main plot × subplot				
fertilizer	1	32.3552	37.34	<0.001
Fertilizer × Fungicide	1	0.4526	0.52	0.484
Fertilizer × *P. nodorum* Inoculation	2	1.1488	0.66	0.533
Fertilizer × Fungicide × *P. nodorum*	2	1.9847	1.15	0.351
Residual	12	10.3981		
Total	35	62.4381		

2-F 21. A summary table for the general ANOVA on proportion of leaves infected with *Z. tritici* at GS 59 (2014-2015), from winter wheat cultivar M. Huntsman, treated either with or without sulphur fertilizer as a factorial combination. d.f. degree of freedom, s.s. sum of square, v.r. variance ratio, F.pr. *F*-test probability associated with variance ratio.

Source of variation	d.f.	s.s.	v.r.	F pr.
block stratum	2	0.004167	0.93	
Within block				
Fungicide	1	0.002783	1.25	0.29
Inoculation with *P. nodorum*	2	0.00303	0.68	0.53
Fungicide × *P. nodorum* Inoculation	2	0.021101	4.72	0.04
Residual	10	0.022333	0.3	
Block × main plot × subplot				
fertilizer	1	0.062102	8.23	0.014
Fertilizer × Fungicide	1	0.000141	0.02	0.89
Fertilizer × *P. nodorum* Inoculation	2	0.004458	0.3	0.75
Fertilizer × Fungicide × *P. nodorum*	2	0.004024	0.27	0.77
Residual	12	0.090563		
Total	35	0.214702		

2-F 22. A summary table for the general ANOVA on proportion of leaves infected with *Z. tritici* at GS 77 (2014-2015), from winter wheat cultivar M. Huntsman, treated either with or without sulphur fertilizer as a factorial combination. d.f. degree of freedom, s.s. sum of square, v.r. variance ratio, F.pr. *F*-test probability associated with variance ratio.

Source of variation	d.f.	s.s.	v.r.	F pr.
block stratum	2	0.064271	2.06	
Within block				
Fungicide	1	0.001563	0.1	0.758
Inoculation with *P. nodorum*	2	0.020642	0.66	0.537
Fungicide × *P. nodorum* Inoculation	2	0.018491	0.59	0.571
Residual	10	0.155966	3.85	
Block × main plot × subplot				
fertilizer	1	0.068282	16.85	0.001
Fertilizer × Fungicide	1	0.005336	1.32	0.273
Fertilizer × *P. nodorum* Inoculation	2	0.019162	2.36	0.136
Fertilizer × Fungicide × *P. nodorum*	2	0.001822	0.22	0.802
Residual	12	0.048615		
Total	35	0.40415		

2-F 23. A summary table for the general ANOVA on disease severity of *Z. tritici* at GS 77 (2014-2015), from winter wheat cultivar M. Huntsman, treated either with or without sulphur fertilizer as a factorial combination. d.f. degree of freedom, s.s. sum of square, v.r. variance ratio, F.pr. *F*-test probability associated with variance ratio.

Source of variation	d.f.	s.s.	v.r.	F pr.
block stratum	2	166.11	30.67	
Within block				
Fungicide	1	1.10	0.41	0.54
Inoculation with *P. nodorum*	2	7.15	1.32	0.31
Fungicide × *P. nodorum* Inoculation	2	5.7	1.05	0.39
Residual	10	27.08	1.33	
Block × main plot × subplot				
fertilizer	1	18.35	9	0.01
Fertilizer × Fungicide	1	3.97	1.95	0.19
Fertilizer × *P. nodorum* Inoculation	2	1.17	0.29	0.76
Fertilizer × Fungicide × *P. nodorum*	2	9.02	2.21	0.15
Residual	12	24.48		
Total	35	264.17		

2-F 24. A summary table for the general ANOVA on spore concentration of *Z. tritici* at GS 77 (2014-2015), from winter wheat cultivar M. Huntsman, treated either with or without sulphur fertilizer as a factorial combination. d.f. degree of freedom, s.s. sum of square, v.r. variance ratio, F.pr. *F*-test probability associated with variance ratio.

Source of variation	d.f.	s.s.	v.r.	F pr.
block stratum	2	1799.6	4.5	
Within block				
Fungicide	1	916.7	4.59	0.06
Inoculation with *P. nodorum*	2	486.7	1.22	0.34
Fungicide × *P. nodorum* Inoculation	2	281.4	0.7	0.52
Residual	10	1998.5	1.86	
Block × main plot × subplot				
fertilizer	1	11776.9	109.62	<0.001
Fertilizer × Fungicide	1	169.7	1.58	0.23
Fertilizer × *P. nodorum* Inoculation	2	130.6	0.61	0.56
Fertilizer × Fungicide × *P. nodorum*	2	103.9	0.48	0.63
Residual	12	1289.2		
Total	35	18953.4		

2-F 25. A summary table for the general ANOVA on proportion of leaves infected with *P. nodorum* at GS 77 (2014-2015), from winter wheat cultivar M. Huntsman, treated either with or without sulphur fertilizer as a factorial combination. d.f. degree of freedom, s.s. sum of square, v.r. variance ratio, F.pr. *F*-test probability associated with variance ratio.

Source of variation	d.f.	s.s.	v.r.	F pr.
block stratum	2	0.00395	2.72	
Block × main plot				
Fungicide	1	0.025143	34.57	0.028
Residual	2	0.001454	0.12	
Block ×main plot × subplot				
fertilizer	1	0.03767	6.1	0.07
Fertilizer ×Fungicide	1	0.002882	0.47	0.532
Residual	4	0.024692		
Total	11	0.095792		

2-F 26. A summary table for the general ANOVA on disease severity of *P. nodorum* at GS 77 (2014-2015), from winter wheat cultivar M. Huntsman, treated either with or without sulphur fertilizer as a factorial combination. d.f. degree of freedom, s.s. sum of square, v.r. variance ratio, F.pr. *F*-test probability associated with variance ratio.

Source of variation	d.f.	s.s.	v.r.	F pr.
block stratum	2	0.5724	0.26	
Block × main plot				
Fungicide	1	0.5986	0.54	0.539
Residual	2	2.2222	2.61	
Block ×main plot × subplot				
fertilizer	1	1.5389	3.62	0.13
Fertilizer ×Fungicide	1	0.2933	0.69	0.453
Residual	4	1.7019		
Total	11	6.9273		

2-F 27. A summary table for the general ANOVA on spore concentration of *P. nodorum* at GS 77 (2014-2015), from winter wheat cultivar M. Huntsman, treated either with or without sulphur fertilizer as a factorial combination. d.f. degree of freedom, s.s. sum of square, v.r. variance ratio, F.pr. *F*-test probability associated with variance ratio.

Source of variation	d.f.	s.s.	v.r.	F pr.
block stratum	2	165.597	2.25	
Block × main plot				
Fungicide	1	31.256	0.85	0.454
Residual	2	73.67	4.37	
Block ×main plot × subplot				
fertilizer	1	40.394	4.79	0.094
Fertilizer ×Fungicide	1	10.407	1.23	0.329
Residual	4	33.745		
Total	11	355.069		

2-F 28. A summary of ANOVA table of S.D.S from spring wheat cv. Paragon (2013) inoculated either with or without *Z. tritici* and *P. nodorum* and treated with four alternative fertilizers. d.f. degree of freedom; s.s. sum of square; v.r. variance ratio, F.pr. *F*-test probability associated with variance ratio.

Source of variation	d.f.	s.s.	v.r.	F pr.
Block	2	15.29	1	
Within Block				
Inoculation with *Z. tritici*	1	0.02	0	0.96
Inoculation with *P. nodorum*	1	0.52	0.07	0.80
Z. tritici × *P. nodorum*	1	1.0	0.13	0.70
Residual	6	45.87	1.49	
Block × Main-plot ×subplot				
Fertilizer	3	52.89	3.43	0.03
Fertilizer × *Z. tritici* inoculation	3	13.22	0.86	0.50
Fertilizer × *P. nodorum* inoculation	3	8.062	0.52	0.70
Fertilizer × *Z. tritici* × *P. nodorum*	3	7.56	0.49	0.70
Residual	24	123.5		
Total	47	267.97		

2-F 29. A summary of ANOVA table of Specific weight from spring wheat cv. Paragon (2013) inoculated either with or without *Z. tritici* and *P. nodorum* and treated with four alternative fertilizers. d.f. degree of freedom; s.s. sum of square; v.r. variance ratio, F.pr. *F*-test probability associated with variance ratio.

Source of variation	d.f.	s.s.	v.r.	F pr.
Block	2	19.93	1.1	
Within Block				
Inoculation with *Z. tritici*	1	18.19	2	0.21
Inoculation with *P. nodorum*	1	1.56	0.17	0.69
Z. tritici × *P. nodorum*	1	0.02	0	0.96
Residual	6	54.56	1.46	
Block × Main-plot ×subplot				
Fertilizer	3	122.86	6.57	0.002
Fertilizer × *Z. tritici* inoculation	3	24.57	1.31	0.29
Fertilizer × *P. nodorum* inoculation	3	1.27	0.07	0.98
Fertilizer × *Z. tritici* × *P. nodorum*	3	8.17	0.44	0.73
Residual	24	149.59		
Total	47	400.72		

2-F 30. A summary of ANOVA table of Harvest index from spring wheat cv. Paragon (2013) inoculated either with or without *Z. tritici* and *P. nodorum* and treated with four alternative fertilizers. d.f. degree of freedom; s.s. sum of square; v.r. variance ratio, F.pr. *F*-test probability associated with variance ratio.

Source of variation	d.f.	s.s.	v.r.	F pr.
Block	2	0.000693	0.39	
Within Block				
Inoculation with *Z. tritici*	1	0.001259	1.41	0.28
Inoculation with *P. nodorum*	1	0.0000142	0.02	0.90
Z. tritici × *P. nodorum*	1	0.001005	1.13	0.33
Residual	6	0.005359	2.12	
Block × Main-plot ×subplot				
Fertilizer	3	0.006943	5.49	0.005
Fertilizer × *Z. tritici* inoculation	3	0.000909	0.72	0.55
Fertilizer × *P. nodorum* inoculation	3	0.001154	0.91	0.45
Fertilizer × *Z. tritici* × *P. nodorum*	3	0.000339	0.27	0.85
Residual	24	0.010111		
Total	47	0.027787		

2-F 31. A summary of ANOVA table of Sulphur concentration in flour from spring wheat cv. Paragon (2013) inoculated either with or without *Z. tritici* and *P. nodorum* and treated with four alternative fertilizers. d.f. degree of freedom; s.s. sum of square; v.r. variance ratio, F.pr. *F*-test probability associated with variance ratio.

Source of variation	d.f.	s.s.	v.r.	F pr.
Block	2	0.00023	2.37	
Within Block				
Inoculation with *Z. tritici*	1	0.00017	3.41	0.11
Inoculation with *P. nodorum*	1	0.000010	0.22	0.66
Z. tritici × *P. nodorum*	1	0.000036	0.74	0.42
Residual	6	0.00029	1.22	
Block × Main-plot ×subplot				
Fertilizer	3	0.01979	164.46	<0.001
Fertilizer × *Z. tritici* inoculation	3	0.00015	1.24	0.32
Fertilizer × *P. nodorum* inoculation	3	0.000023	0.19	0.90
Fertilizer × *Z. tritici* × *P. nodorum*	3	0.000068	0.56	0.64
Residual	24	0.00096		
Total	47	0.02173		

2-F 32. A summary of ANOVA table of N/S ratio from spring wheat cv. Paragon (2013) inoculated either with or without *Z. tritici* and *P. nodorum* and treated with four alternative fertilizers. d.f. degree of freedom; s.s. sum of square; v.r. variance ratio, F.pr. *F*-test probability associated with variance ratio.

Source of variation	d.f.	s.s.	v.r.	F pr.
Block	2	3.705	2.04	
Within Block				
Inoculation with *Z. tritici*	1	0.800	0.88	0.38
Inoculation with *P. nodorum*	1	0.163	0.18	0.69
Z. tritici × *P. nodorum*	1	1.268	1.39	0.28
Residual	6	5.455	2.76	
Block × Main-plot ×subplot				
Fertilizer	3	96.028	97.08	<0.001
Fertilizer × *Z. tritici* inoculation	3	0.157	0.16	0.92
Fertilizer × *P. nodorum* inoculation	3	0.198	0.2	0.89
Fertilizer × *Z. tritici* × *P. nodorum*	3	0.158	0.16	0.92
Residual	24	7.913		
Total	47	115.847		

2-F 33. A summary of ANOVA table of Hagberg falling number from spring wheat cv. Paragon (2013) inoculated either with or without *Z. tritici* and *P. nodorum* and treated with four alternative fertilizers. d.f. degree of freedom; s.s. sum of square; v.r. variance ratio, F.pr. *F*-test probability associated with variance ratio.

Source of variation	d.f.	s.s.	v.r.	F pr.
Block	2	1849	0.94	
Within Block				
Inoculation with *Z. tritici*	1	0.3	0	0.99
Inoculation with *P. nodorum*	1	14.1	0.01	0.91
Z. tritici × *P. nodorum*	1	5.3	0.01	0.94
Residual	6	5887.1	1.98	
Block × Main-plot ×subplot				
Fertilizer	3	5288.1	3.56	0.03
Fertilizer × *Z. tritici* inoculation	3	878.5	0.59	0.62
Fertilizer × *P. nodorum* inoculation	3	1744.4	1.17	0.34
Fertilizer × *Z. tritici* × *P. nodorum*	3	2095.2	1.41	0.26
Residual	24	11895.8		
Total	47	29657.9		

2-F 34. A summary of ANOVA table of Total yield from spring wheat cv. Paragon (2013) inoculated either with or without *Z. tritici* and *P. nodorum* and treated with four alternative fertilizers. d.f. degree of freedom; s.s. sum of square; v.r. variance ratio, F.pr. *F*-test probability associated with variance ratio.

Source of variation	d.f.	s.s.	v.r.	F pr.
Block	2	76381	0.26	
Within Block				
Inoculation with *Z. tritici*	1	746109	5.09	0.065
Inoculation with *P. nodorum*	1	5727	0.04	0.85
Z. tritici × *P. nodorum*	1	22059	0.15	0.71
Residual	6	879169	1.75	
Block × Main-plot ×subplot				
Fertilizer	3	234616	0.94	0.44
Fertilizer × *Z. tritici* inoculation	3	211734	0.84	0.48
Fertilizer × *P. nodorum* inoculation	3	12496	0.05	0.99
Fertilizer × *Z. tritici* × *P. nodorum*	3	38796	0.15	0.93
Residual	24	2005049		
Total	47	4232137		

2-F 35. A summary of ANOVA table of TGW from spring wheat cv. Paragon (2013) inoculated either with *Z. tritici* or not, averaged over other factors. d.f. degree of freedom; s.s. sum of square; v.r. variance ratio, F.pr. *F*-test probability associated with variance ratio.

Source of variation	d.f.	s.s.	v.r.	F pr.
Block	2	12.01	1.04	
Within Block				
Inoculation with *Z. tritici*	1	24.51	4.23	0.08
Inoculation with *P. nodorum*	1	2.29	0.4	0.55
Z. tritici × *P. nodorum*	1	1.57	0.27	0.62
Residual	6	34.75	1.7	
Block × Main-plot ×subplot				
Fertilizer	3	56.69	5.53	0.005
Fertilizer × *Z. tritici* inoculation	3	17.91	1.75	0.18
Fertilizer × *P. nodorum* inoculation	3	1.18	0.12	0.95
Fertilizer × *Z. tritici* × *P. nodorum*	3	3.04	0.3	0.82
Residual	24	81.94		
Total	47	235.92		

2-F 36. A summary of ANOVA table of Nitrogen concentration in flour from spring wheat cv. Paragon (2013) inoculated either with or without *Z. tritici* and *P. nodorum* and treated with four alternative fertilizers. d.f. degree of freedom; s.s. sum of square; v.r. variance ratio, F.pr. *F*-test probability associated with variance ratio.

Source of variation	d.f.	s.s.	v.r.	F pr.
Block	2	0.028	0.41	
Within Block				
Inoculation with *Z. tritici*	1	0.154	4.38	0.08
Inoculation with *P. nodorum*	1	0.00001	0	0.99
Z. tritici × *P. nodorum*	1	0.002	0.06	0.81
Residual	6	0.211	1.85	
Block × Main-plot ×subplot				
Fertilizer	3	0.908	15.91	<0.001
Fertilizer × *Z. tritici* inoculation	3	0.065	1.14	0.35
Fertilizer × *P. nodorum* inoculation	3	0.007	0.12	0.95
Fertilizer × *Z. tritici* × *P. nodorum*	3	0.014	0.25	0.86
Residual	24	0.457		
Total	47	1.84769		

2-F 37. A summary of ANOVA table of TGW from two winter wheat cultivars Gallant and M. Huntsman (2013-2014) inoculated either with or with *Z. tritici* and *P. nodorum* and treated with three different fertilizers. d.f. degree of freedom; s.s. sum of square; v.r. variance ratio, F.pr. *F*-test probability associated with variance ratio.

Source of variation	d.f.	(m.v.)	s.s.	v.r.	F pr.
block stratum	2		9.092	0.06	
Within Block					
Cultivar	1		248.75	3.07	0.10
Inoculation with *Z. tritici*	1		25.24	0.31	0.59
Inoculation with *P. nodorum*	1		51.06	0.63	0.44
Cultivar × *Z. tritici* inoculation	1		19.46	0.24	0.63
Cultivar × *P. nodorum* inoculation	1		163.71	2.02	0.18
Z. tritici × *P. nodorum*	1		75.70	0.94	0.35
Cultivar × *Z. tritici* × *P. nodorum*	1		40.16	0.5	0.49
Residual	14		1132.81	20.11	
Block × main-plot × subplot					
Fertilizer	2		45.08	5.6	0.009
Cultivar × fertilizer	2		8.88	1.1	0.35
Fertilizer × *Z. tritici* inoculation	2		11.46	1.42	0.26
Fertilizer × *P. nodorum* inoculation	2		3.78	0.47	0.63
Cultivar × fertilizer × *Z. tritici* inoculation	2		8.84	1.1	0.35
Cultivar × fertilizer × *P. nodorum* inoculation	2		18.18	2.26	0.12
Fertilizer × *Z. tritici* × *P. nodorum*	2		6.99	0.87	0.43
Cultivar × fertilizer × *Z. tritici* × *P. nodorum*	2		6.84	0.85	0.44
Residual	29	-3	116.68		
Total	68	-3	1768.07		

2-F 38. A summary of ANOVA table of Specific weight from two winter wheat cultivars Gallant and M. Huntsman (2013-2014) inoculated either with or with *Z. tritici* and *P. nodorum* and treated with three different fertilizers. d.f. degree of freedom; s.s. sum of square; v.r. variance ratio, F.pr. *F*-test probability associated with variance ratio.

Source of variation	d.f.	(m.v.)	s.s.	v.r.	F pr.
block stratum	2		11.452	0.06	
Within Block					
Cultivar	1		122.09	1.31	0.27
Inoculation with *Z. tritici*	1		12.97	0.14	0.72
Inoculation with *P. nodorum*	1		37.49	0.4	0.54
Cultivar × *Z. tritici* inoculation	1		24.22	0.26	0.62
Cultivar × *P. nodorum* inoculation	1		76.14	0.82	0.38
Z. tritici × *P. nodorum*	1		27.33	0.29	0.59
Cultivar × *Z. tritici* × *P. nodorum*	1		59.11	0.63	0.44
Residual	14		1305.68	13.15	
Block × main-plot × subplot					
Fertilizer	2		44.92	3.17	0.056
Cultivar × fertilizer	2		3.48	0.25	0.78
Fertilizer × *Z. tritici* inoculation	2		2.55	0.18	0.84
Fertilizer × *P. nodorum* inoculation	2		11.92	0.84	0.44
Cultivar × fertilizer × *Z. tritici* inoculation	2		1.96	0.14	0.87
Cultivar × fertilizer × *P. nodorum* inoculation	2		17.31	1.22	0.31
Fertilizer × *Z. tritici* × *P. nodorum*	2		18.79	1.33	0.28
Cultivar × fertilizer × *Z. tritici* × *P. nodorum*	2		1.09	0.08	0.93
Residual	30	-2	212.71		
Total	69	-2	1912.63		

2-F 39. A summary of ANOVA table of Harvest index from two winter wheat cultivars Gallant and M. Huntsman (2013-2014) inoculated either with or with *Z. tritici* and *P. nodorum* and treated with three different fertilizers. d.f. degree of freedom; s.s. sum of square; v.r. variance ratio, F.pr. *F*-test probability associated with variance ratio.

Source of variation	d.f.	(m.v.)	s.s.	v.r.	F pr.
block stratum	2		0.00154	0.15	
Within Block					
Cultivar	1		0.0069	1.37	0.26
Inoculation with *Z. tritici*	1		0.0009	0.17	0.69
Inoculation with *P. nodorum*	1		0.0027	0.55	0.47
Cultivar × *Z. tritici* inoculation	1		0.0061	1.22	0.29
Cultivar × *P. nodorum* inoculation	1		0.0113	2.25	0.16
Z. tritici × *P. nodorum*	1		0.0009	0.18	0.68
Cultivar × *Z. tritici* × *P. nodorum*	1		0.0001	0.02	0.90
Residual	14		0.0703	4.63	
Block × main-plot × subplot					
Fertilizer	2		0.0029	1.32	0.28
Cultivar × fertilizer	2		0.0019	0.9	0.42
Fertilizer × *Z. tritici* inoculation	2		0.005	2.39	0.11
Fertilizer × *P. nodorum* inoculation	2		0.0050	2.33	0.12
Cultivar × fertilizer × *Z. tritici* inoculation	2		0.0001	0.07	0.93
Cultivar × fertilizer × *P. nodorum* inoculation	2		0.0047	2.18	0.13
Fertilizer × *Z. tritici* × *P. nodorum*	2		0.0009	0.43	0.66
Cultivar × fertilizer × *Z. tritici* × *P. nodorum*	2		0.0039	1.79	0.19
Residual	30	-2	0.0325		
Total	69	-2	0.145		

2-F 40. A summary of ANOVA table of Sulphur DW% in flour from two winter wheat cultivars Gallant and M. Huntsman (2013-2014) inoculated either with or with *Z. tritici* and *P. nodorum* and treated with three different fertilizers. d.f. degree of freedom; s.s. sum of square; v.r. variance ratio, F.pr. *F*-test probability associated with variance ratio.

Source of variation	d.f.	(m.v.)	s.s.	v.r.	F pr.
block stratum	2		0.0009867	0.54	
Within Block					
Cultivar	1		0.0034	3.77	0.07
Inoculation with *Z. tritici*	1		0.0004	0.42	0.53
Inoculation with *P. nodorum*	1		0.0013	1.38	0.26
Cultivar × *Z. tritici* inoculation	1		0.0001	0	0.97
Cultivar × *P. nodorum* inoculation	1		0.0006	0.76	0.39
Z. tritici × *P. nodorum*	1		0.0015	1.73	0.21
Cultivar × *Z. tritici* × *P. nodorum*	1		0.0001	0.13	0.72
Residual	14		0.0128	6.57	
Block × main-plot × subplot					
Fertilizer	2		0.0056	20.32	<0.001
Cultivar × fertilizer	2		0.0003	1.04	0.37
Fertilizer × *Z. tritici* inoculation	2		0.0002	0.86	0.43
Fertilizer × *P. nodorum* inoculation	2		0.0002	0.6	0.55
Cultivar × fertilizer × *Z. tritici* inoculation	2		0.0001	0.17	0.84
Cultivar × fertilizer × *P. nodorum* inoculation	2		0.0007	2.31	0.12
Fertilizer × *Z. tritici* × *P. nodorum*	2		0.0007	2.56	0.09
Cultivar × fertilizer × *Z. tritici* × *P. nodorum*	2		0.0001	0.05	0.95
Residual	30	-2	0.0042		
Total	69	-2	0.0296		

2-F 41. A summary of ANOVA table of Hagberg falling number from two winter wheat cultivars Gallant and M. Huntsman (2013-2014) inoculated either with or with *Z. tritici* and *P. nodorum* and treated with three different fertilizers. d.f. degree of freedom; s.s. sum of square; v.r. variance ratio, F.pr. *F*-test probability associated with variance ratio.

Source of variation	d.f.	(m.v.)	s.s.	v.r.	F pr.
block stratum	2		2010	0.75	
Within Block					
Cultivar	1		173884	130.19	<0.001
Inoculation with *Z. tritici*	1		3780	2.83	0.12
Inoculation with *P. nodorum*	1		215	0.16	0.69
Cultivar × *Z. tritici* inoculation	1		2263	1.69	0.21
Cultivar × *P. nodorum* inoculation	1		5862	4.39	0.055
Z. tritici × *P. nodorum*	1		422	0.32	0.58
Cultivar × *Z. tritici* × *P. nodorum*	1		6	0	0.95
Residual	14		18698	1.11	
Block × main-plot × subplot					
Fertilizer	2		310	0.13	0.88
Cultivar × fertilizer	2		1698	0.71	0.50
Fertilizer × *Z. tritici* inoculation	2		2458	1.02	0.37
Fertilizer × *P. nodorum* inoculation	2		1027	0.43	0.66
Cultivar × fertilizer × *Z. tritici* inoculation	2		4267	1.77	0.19
Cultivar × fertilizer × *P. nodorum* inoculation	2		5163	2.14	0.14
Fertilizer × *Z. tritici* × *P. nodorum*	2		1683	0.7	0.50
Cultivar × fertilizer × *Z. tritici* × *P. nodorum*	2		363	0.15	0.86
Residual	30	-2	36116		
Total	69	-2	255656		

2-F 42. A summary of ANOVA table of green leaf area from two winter wheat cultivars Gallant and M. Huntsman (2013-2014) inoculated either with or with *Z. tritici* and *P. nodorum* and treated with three different fertilizers. d.f. degree of freedom; s.s. sum of square; v.r. variance ratio, F.pr. *F*-test probability associated with variance ratio.

Source of variation	d.f.	(m.v.)	s.s.	v.r.	F pr.
block stratum	2		297.65	0.65	
Within Block					
Cultivar	1		16790.86	73.2	<0.001
Inoculation with *Z. tritici*	1		98.95	0.43	0.52
Inoculation with *P. nodorum*	1		11.69	0.05	0.83
Cultivar × *Z. tritici* inoculation	1		10.24	0.04	0.84
Cultivar × *P. nodorum* inoculation	1		81.78	0.36	0.56
Z. tritici × *P. nodorum*	1		50.02	0.22	0.65
Cultivar × *Z. tritici* × *P. nodorum*	1		13.6	0.06	0.81
Residual	14		3211.41	3.78	
Block × main-plot × subplot					
Fertilizer	2		262.5	2.16	0.13
Cultivar × fertilizer	2		418.19	3.45	0.04
Fertilizer × *Z. tritici* inoculation	2		195.56	1.61	0.22
Fertilizer × *P. nodorum* inoculation	2		131.65	1.09	0.35
Cultivar × fertilizer × *Z. tritici* inoculation	2		28.28	0.23	0.80
Cultivar × fertilizer × *P. nodorum* inoculation	2		343.02	2.83	0.08
Fertilizer × *Z. tritici* × *P. nodorum*	2		19.21	0.16	0.85
Cultivar × fertilizer × *Z. tritici* × *P. nodorum*	2		404.19	3.33	0.05
Residual	30	-2	1819.88		
Total	69	-2	24016.52		

2-F 43. A summary of ANOVA table of total yield from two winter wheat cultivars Gallant and M. Huntsman (2013-2014) inoculated either with or with *Z. tritici* and *P. nodorum* and treated with three different fertilizers. d.f. degree of freedom; s.s. sum of square; v.r. variance ratio, F.pr. *F*-test probability associated with variance ratio.

Source of variation	d.f.	(m.v.)	s.s.	v.r.	F pr.
block stratum	2		2.29	0.48	
Within Block					
Cultivar	1		58.79	24.4	<0.001
Inoculation with *Z. tritici*	1		0.99	0.41	0.53
Inoculation with *P. nodorum*	1		1.01	0.42	0.53
Cultivar × *Z. tritici* inoculation	1		1.99	0.83	0.38
Cultivar × *P. nodorum* inoculation	1		4.35	1.81	0.2
Z. tritici × *P. nodorum*	1		0.03	0.01	0.92
Cultivar × *Z. tritici* × *P. nodorum*	1		3.90	1.62	0.22
Residual	14		33.73	6.81	
Block × main-plot × subplot					
Fertilizer	2		5.35	7.55	0.002
Cultivar × fertilizer	2		0.39	0.55	0.59
Fertilizer × *Z. tritici* inoculation	2		0.17	0.24	0.79
Fertilizer × *P. nodorum* inoculation	2		1.71	2.42	0.11
Cultivar × fertilizer × *Z. tritici* inoculation	2		0.09	0.14	0.87
Cultivar × fertilizer × *P. nodorum* inoculation	2		0.17	0.24	0.79
Fertilizer × *Z. tritici* × *P. nodorum*	2		1.87	2.64	0.08
Cultivar × fertilizer × *Z. tritici* × *P. nodorum*		2	1.68	2.38	0.11
Residual	30	-2	10.62		
Total	69	-2	120.44		

2-F 44. A summary of ANOVA table of Nitrogen DW% in flour from two winter wheat cultivars Gallant and M. Huntsman (2013-2014) inoculated either with or with *Z. tritici* and *P. nodorum* and treated with three different fertilizers. d.f. degree of freedom; s.s. sum of square; v.r. variance ratio, F.pr. *F*-test probability associated with variance ratio.

Source of variation	d.f.	(m.v.)	s.s.	v.r.	F pr.
block stratum	2		0.0124	0.25	
Within Block					
Cultivar	1		2.0431	80.9	<0.001
Inoculation with *Z. tritici*	1		0.0361	1.43	0.25
Inoculation with *P. nodorum*	1		0.0295	1.17	0.29
Cultivar × *Z. tritici* inoculation	1		0.0111	0.44	0.52
Cultivar × *P. nodorum* inoculation	1		0.0079	0.32	0.58
Z. tritici × *P. nodorum*	1		0.0001	0	0.96
Cultivar × *Z. tritici* × *P. nodorum*	1		0.0013	0.05	0.83
Residual	14		0.3536	8.13	
Block × main-plot × subplot					
Fertilizer	2		0.1934	31.12	<.001
Cultivar × fertilizer	2		0.0166	2.67	0.08
Fertilizer × *Z. tritici* inoculation	2		0.0049	0.78	0.47
Fertilizer × *P. nodorum* inoculation	2		0.0064	1.03	0.37
Cultivar × fertilizer × *Z. tritici* inoculation	2		0.0039	0.63	0.54
Cultivar × fertilizer × *P. nodorum* inoculation	2		0.0213	3.42	0.046
Fertilizer × *Z. tritici* × *P. nodorum*	2		0.0305	4.9	0.014
Cultivar × fertilizer × *Z. tritici* × *P. nodorum*	2		0.002	0.32	0.73
Residual	30	-2	0.0932		
Total	69	-2	2.7735		

2-F 45. A summary of ANOVA table of N/S ratio in flour from two winter wheat cultivars Gallant and M. Huntsman (2013-2014) inoculated either with or with *Z. tritici* and *P. nodorum* and treated with three different fertilizers. d.f. degree of freedom; s.s. sum of square; v.r. variance ratio, F.pr. *F*-test probability associated with variance ratio.

Source of variation	d.f.	(m.v.)	s.s.	v.r.	F pr.
block stratum	2		34.02	1.09	
Within Block					
Cultivar	1		13.26	0.85	0.37
Inoculation with *Z. tritici*	1		1.64	0.11	0.75
Inoculation with *P. nodorum*	1		15.49	1	0.34
Cultivar × *Z. tritici* inoculation	1		2.81	0.18	0.68
Cultivar × *P. nodorum* inoculation	1		10.42	0.67	0.43
Z. tritici × *P. nodorum*	1		49.81	3.21	0.095
Cultivar × *Z. tritici* × *P. nodorum*	1		8.68	0.56	0.47
Residual	14		217.5	3	
Block × main-plot × subplot					
Fertilizer	2		224.14	21.64	<0.001
Cultivar × fertilizer	2		2.70	0.26	0.77
Fertilizer × *Z. tritici* inoculation	2		4.64	0.45	0.64
Fertilizer × *P. nodorum* inoculation	2		6.77	0.65	0.53
Cultivar × fertilizer × *Z. tritici* inoculation	2		5.19	0.5	0.61
Cultivar × fertilizer × *P. nodorum* inoculation	2		23.18	2.24	0.12
Fertilizer × *Z. tritici* × *P. nodorum*	2		37.77	3.65	0.038
Cultivar × fertilizer × *Z. tritici* × *P. nodorum*		2	1.45	0.14	0.87
Residual	30	-2	155.35		
Total	69	-2	778.77		

2-F 46. A summary of ANOVA table of S.D.S from two winter wheat cultivars Gallant and M. Huntsman (2013-2014) inoculated either with or with *Z. tritici* and *P. nodorum* and treated with three different fertilizers. d.f. degree of freedom; s.s. sum of square; v.r. variance ratio, F.pr. *F*-test probability associated with variance ratio.

Source of variation	d.f.	(m.v.)	s.s.	v.r.	F pr.
block stratum	2		368.07	1.5	
Within Block					
Cultivar	1		16178.7	131.86	<0.001
Inoculation with *Z. tritici*	1		11.5	0.09	0.77
Inoculation with *P. nodorum*	1		334.9	2.73	0.12
Cultivar × *Z. tritici* inoculation	1		104.4	0.85	0.37
Cultivar × *P. nodorum* inoculation	1		25.3	0.21	0.66
Z. tritici × *P. nodorum*	1		655.8	5.34	0.037
Cultivar × *Z. tritici* × *P. nodorum*	1		317.9	2.59	0.13
Residual	14		1717.8	3.11	
Block × main-plot × subplot					
Fertilizer	2		278.6	3.53	0.04
Cultivar × fertilizer	2		212.0	2.69	0.08
Fertilizer × *Z. tritici* inoculation	2		22.7	0.29	0.75
Fertilizer × *P. nodorum* inoculation	2		48.4	0.61	0.55
Cultivar × fertilizer × *Z. tritici* inoculation	2		4.6	0.06	0.94
Cultivar × fertilizer × *P. nodorum* inoculation	2		108.1	1.37	0.27
Fertilizer × *Z. tritici* × *P. nodorum*	2		135.4	1.72	0.19
Cultivar × fertilizer × *Z. tritici* × *P. nodorum*		2	58.1	0.74	0.49
Residual	30	-2	1182.8		
Total	69	-2	20538.9		

2-F 47. A summary of ANOVA table of S.D.S. from winter wheat cultivar M. Huntsman (2014-2015) inoculated either with or without *P. nodorum* and treated with fungicide and high and low sulphur fertilization. d.f. degree of freedom; s.s. sum of square; v.r. variance ratio, F.pr. *F*-test probability associated with variance ratio.

Source of variation	d.f.	s.s.	v.r.	F pr.
block stratum	2	85.17	4.65	
Within Block				
Fungicide	1	5.44	0.6	0.46
Inoculation with *P. nodorum*	2	15.5	0.85	0.46
Fungicide × *P. nodorum* inoculation	2	24.39	1.33	0.31
Residual	10	91.5	0.67	
Block × main plot ×subplot				
Fertilizer	1	1045.44	76.5	<.001
Fertilizer × Fungicide	1	13.44	0.98	0.34
Fertilizer × *P. nodorum* inoculation	2	13.72	0.5	0.62
Fertilizer × Fungicide × *P. nodorum* inoculation	2	14.39	0.53	0.60
Residual	12	164		
Total	35	1473		

2-F 48. A summary of ANOVA table of Sulphur DW% in flour from winter wheat cultivar M. Huntsman (2014-2015) inoculated either with or without *P. nodorum* and treated with fungicide and high and low sulphur fertilization. d.f. degree of freedom; s.s. sum of square; v.r. variance ratio, F.pr. *F*-test probability associated with variance ratio.

Source of variation	d.f.	s.s.	v.r.	F pr.
block stratum	2	0.00001606	0.2	
Within Block				
Fungicide	1	0.00006348	1.59	0.24
Inoculation with *P. nodorum*	2	0.00009527	1.19	0.34
Fungicide × *P. nodorum* inoculation	2	0.00004321	0.54	0.59
Residual	10	0.00039937	0.54	
Block × main plot ×subplot				
Fertilizer	1	0.00941877	128.47	<0.001
Fertilizer × Fungicide	1	0.00004044	0.55	0.47
Fertilizer × *P. nodorum* inoculation	2	0.00000338	0.02	0.97
Fertilizer × Fungicide × *P. nodorum* inoculation	2	0.00002666	0.18	0.84
Residual	12	0.00087978		
Total	35	0.01098643		

2-F 49. A summary of ANOVA table of N/S in flour from winter wheat cultivar M. Huntsman (2014-2015) inoculated either with or without *P. nodorum* and treated with fungicide and high and low sulphur fertilization. d.f. degree of freedom; s.s. sum of square; v.r. variance ratio, F.pr. *F*-test probability associated with variance ratio.

Source of variation	d.f.	s.s.	v.r.	F pr.
block stratum	2	4.864	1.18	
Within Block				
Fungicide	1	0.015	0.01	0.93
Inoculation with *P. nodorum*	2	4.186	1.02	0.39
Fungicide × *P. nodorum* inoculation	2	6.52	1.58	0.25
Residual	10	20.57	0.75	
Block × main plot ×subplot				
Fertilizer	1	449.45	163.53	<0.001
Fertilizer × Fungicide	1	0.79	0.29	0.60
Fertilizer × *P. nodorum* inoculation	2	0.23	0.04	0.96
Fertilizer × Fungicide × *P. nodorum* inoculation	2	1.11	0.2	0.82
Residual	12	32.98		
Total	35	520.72		

2-F 50. A summary of ANOVA table of Sulphur DW% in whole-plant at GS 31 from winter wheat cultivar M. Huntsman (2014-2015) inoculated either with or without *P. nodorum* and treated with fungicide and high and low sulphur fertilization. d.f. degree of freedom; s.s. sum of square; v.r. variance ratio, F.pr. *F*-test probability associated with variance ratio.

Source of variation	d.f.	s.s.	v.r.	F pr.
block stratum	2	0.004537	0.5	
Within Block				
Fungicide	1	0.00052	0.11	0.74
Inoculation with *P. nodorum*	2	0.002326	0.26	0.78
Fungicide × *P. nodorum* inoculation	2	0.001133	0.12	0.88
Residual	10	0.045404	1.34	
Block × main plot ×subplot				
Fertilizer	1	0.316931	93.76	<.001
Fertilizer × Fungicide	1	0.000029	0.01	0.93
Fertilizer × *P. nodorum* inoculation	2	0.009451	1.4	0.29
Fertilizer × Fungicide × *P. nodorum* inoculation	2	0.002466	0.36	0.70
Residual	12	0.040563		
Total	35	0.423359		

2-F 51. A summary of ANOVA table of Sulphur DW% in whole-plant at GS 77 from winter wheat cultivar M. Huntsman (2014-2015) inoculated either with or without *P. nodorum* and treated with fungicide and high and low sulphur fertilization. d.f. degree of freedom; s.s. sum of square; v.r. variance ratio, F.pr. *F*-test probability associated with variance ratio.

Source of variation	d.f.	s.s.	v.r.	F pr.
block stratum	2	0.005596	0.52	
Within Block				
Fungicide	1	0.000028	0.01	0.94
Inoculation with *P. nodorum*	2	0.004676	0.44	0.66
Fungicide × *P. nodorum* inoculation	2	0.00832	0.78	0.48
Residual	10	0.053333	1.14	
Block × main plot ×subplot				
Fertilizer	1	0.049855	10.63	0.007
Fertilizer × Fungicide	1	0.000081	0.02	0.89
Fertilizer × *P. nodorum* inoculation	2	0.001508	0.16	0.85
Fertilizer × Fungicide × *P. nodorum* inoculation	2	0.009749	1.04	0.38
Residual	12	0.056268		
Total	35	0.189415		

2-F 52. A summary of ANOVA table of H.F.N. from winter wheat cultivar M. Huntsman (2014-2015) inoculated either with or without *P. nodorum* and treated with fungicide and high and low sulphur fertilization. d.f. degree of freedom; s.s. sum of square; v.r. variance ratio, F.pr. *F*-test probability associated with variance ratio.

Source of variation	d.f.	s.s.	v.r.	F pr.
block stratum	2	8177	9.9	
Within Block				
Fungicide	1	3	0.01	0.94
Inoculation with *P. nodorum*	2	3234	3.92	0.055
Fungicide × *P. nodorum* inoculation	2	2080	2.52	0.13
Residual	10	4131	0.21	
Block × main plot ×subplot				
Fertilizer	1	2178	1.11	0.31
Fertilizer × Fungicide	1	81	0.04	0.84
Fertilizer × *P. nodorum* inoculation	2	99	0.03	0.98
Fertilizer × Fungicide × *P. nodorum* inoculation	2	4323	1.1	0.36
Residual	12	23486		
Total	35	47792		

2-F 53. A summary of ANOVA table of Nitrogen DW% in flour from winter wheat cultivar M. Huntsman (2014-2015) inoculated either with or without *P. nodorum* and treated with fungicide and high and low sulphur fertilization. d.f. degree of freedom; s.s. sum of square; v.r. variance ratio, F.pr. *F*-test probability associated with variance ratio.

Source of variation	d.f.	s.s.	v.r.	F pr.
block stratum	2	0.0535	4.37	
Within Block				
Fungicide	1	0.0119	1.94	0.19
Inoculation with *P. nodorum*	2	0.0654	5.35	0.026
Fungicide × *P. nodorum* inoculation	2	0.0378	3.09	0.09
Residual	10	0.0611	1.51	
Block × main plot ×subplot				
Fertilizer	1	0.3058	75.46	<0.001
Fertilizer × Fungicide	1	0.000087	0.02	0.89
Fertilizer × *P. nodorum* inoculation	2	0.0045	0.56	0.58
Fertilizer × Fungicide × *P. nodorum* inoculation	2	0.00036	0.04	0.95
Residual	12	0.04863		
Total	35	0.58929		

2-F 54. A summary of ANOVA table of TGW from winter wheat cultivar M. Huntsman (2014-2015) inoculated either with or without *P. nodorum* and treated with fungicide and high and low sulphur fertilization. d.f. degree of freedom; s.s. sum of square; v.r. variance ratio, F.pr. *F*-test probability associated with variance ratio.

Source of variation	d.f.	s.s.	v.r.	F pr.
block stratum	2	0.3772	0.16	
Within Block				
Fungicide	1	3.87	3.18	0.11
Inoculation with *P. nodorum*	2	40.87	16.82	<0.001
Fungicide × *P. nodorum* inoculation	2	9.32	3.83	0.058
Residual	10	12.15	2.68	
Block × main plot ×subplot				
Fertilizer	1	121	266.91	<0.001
Fertilizer × Fungicide	1	0.59	1.3	0.28
Fertilizer × *P. nodorum* inoculation	2	1.55	1.71	0.22
Fertilizer × Fungicide × *P. nodorum* inoculation	2	7.60	8.38	0.005
Residual	12	5.44		
Total	35	202.76		

2-F 55. A summary of ANOVA table of Specific weight from winter wheat cultivar M. Huntsman (2014-2015) inoculated either with or without *P. nodorum* and treated with fungicide and high and low sulphur fertilization. d.f. degree of freedom; s.s. sum of square; v.r. variance ratio, F.pr. *F*-test probability associated with variance ratio.

Source of variation	d.f.	s.s.	v.r.	F pr.
block stratum	2	1.6689	5.22	
Within Block				
Fungicide	1	0.09	0.56	0.47
Inoculation with *P. nodorum*	2	1.8022	5.64	0.023
Fungicide × *P. nodorum* inoculation	2	0.6867	2.15	0.17
Residual	10	1.5978	0.32	
Block × main plot ×subplot				
Fertilizer	1	5.9211	11.82	0.005
Fertilizer × Fungicide	1	0.01	0.02	0.89
Fertilizer × *P. nodorum* inoculation	2	2.1489	2.14	0.16
Fertilizer × Fungicide × *P. nodorum* inoculation	2	0.6067	0.61	0.56
Residual	12	6.0133		
Total	35	20.5456		

2-F 56. A summary of ANOVA table of total yield from winter wheat cultivar M. Huntsman (2014-2015) inoculated either with or without *P. nodorum* and treated with fungicide and high and low sulphur fertilization. d.f. degree of freedom; s.s. sum of square; v.r. variance ratio, F.pr. *F*-test probability associated with variance ratio.

Source of variation	d.f.	s.s.	v.r.	F pr.
block stratum	2	2.9888	5.07	
Within Block				
Fungicide	1	1.2405	4.21	0.07
Inoculation with *P. nodorum*	2	2.5053	4.25	0.05
Fungicide × *P. nodorum* inoculation	2	1.9616	3.33	0.078
Residual	10	2.9456	0.69	
Block × main plot ×subplot				
Fertilizer	1	32.4254	75.48	<0.001
Fertilizer × Fungicide	1	0.0007	0	0.97
Fertilizer × *P. nodorum* inoculation	2	0.0029	0	0.99
Fertilizer × Fungicide × *P. nodorum* inoculation	2	0.0958	0.11	0.89
Residual	12	5.1548		
Total	35	49.3215		

A 1. Table of all means and L.S.D. forLog10 of proportion of infected leaves with septoria isolates. Glasshouse experiment.

Variation	Variable	Mean	L.S.D value		Variation	Variable	Mean	L.S.D value
Cultivars	Gallant	-1.31			cultivars x Fertilizers	Gallant x K2SO4	-1.40	
	M. Huntsman	-1.47	0.11			Gallant x KCl	-1.22	
	Paragon	-1.09				M. Huntsman x K2SO4	-1.57	0.17
						M. Huntsman x KCl	-1.36	
Isolates	*P. nodorum*	-0.45				Paragon x K2SO4	-1.14	
	Z. tritici 1	-1.50	0.13			Paragon x KCl	-1.03	
	Z. tritici 2	-1.64						
	Z. tritici 3	-1.58			CV x Isolates x Fertilizers	Gallant x *P.n* x S+	-0.52	
						Gallant x *P.n* x S-	-0.42	
Fertilizers	K2SO4	-1.37	0.09			Gallant x Z. t 1 x S+	-1.78	
	KCl	-1.21				Gallant x Z. t 1 x S-	-1.41	
						Gallant x Z. t 2 x S+	-1.71	
cultivars × isolates	Gallant x *P. n*	-0.48				Gallant x Z. t 2 x S-	-1.39	
	Gallant x *Z. t* 1	-1.60				Gallant x Z. t 3 x S+	-1.51	
	Gallant x *Z. t* 2	-1.56				Gallant x Z. t 3 x S-	-1.58	
	Gallant x *Z. t* 3	-1.63				M. Huntsman x *P.n* x S+	-0.63	0.33
	M. Huntsman x *P. n*	-0.48				M. Huntsman x *P.n* x S-	-0.32	
	M. Huntsman x *Z. t* 1	-1.77	0.23			M. Huntsman x Z. t 1 x S+	-1.86	
	M. Huntsman x *Z. t* 2	-1.87				M. Huntsman x Z. t 1 x S-	-1.67	
	M. Huntsman x *Z. t* 3	-1.75				M. Huntsman x Z. t 2 x S+	-1.94	
	Paragon x *P. n*	-0.39				M. Huntsman x Z. t 2 x S-	-1.8	
	Paragon x *Z. t* 1	-1.13				M. Huntsman x Z. t 3 x S+	-1.69	
	Paragon x *Z. t* 2	-1.48				M. Huntsman x Z. t 3 x S-	-1.58	
	Paragon x *Z. t* 3	-1.36				Paragon x *P.n* x S+	-0.49	

						Paragon x *P.n* x S-	-0.27	
Isolates x Fertilizers	*P.n* x S+	-0.55				Paragon x Z. t 1 x S+	-1.35	
	P.n x S-	-0.34				Paragon x Z. t 1 x S-	-0.90	
	Z.t 1 x S+	-1.67				Paragon x Z. t 2 x S+	-1.45	
	Z.t 1 x S-	-1.33	0.2			Paragon x Z. *t* 2 x S-	-1.51	
	Z.t 2 x S+	-1.70				Paragon x Z. *t* 3 x S+	-1.21	
	Z.t 2 x S-	-1.57				Paragon x Z. *t* 3 x S-	-1.27	
	Z.t 3 x S+	-1.58						
	Z.t 3 x S-	-1.58						

A 2. Table of all mean and L.S.D of Proportion of infected leaves with *Z. tritici* at GS 31, from two winter wheat cultivars in season 2013-2014 treated with three alternative fertilizers and inoculated either with or without two pathogens.

variation	Cultivars			Isolates					Fertilizers			cv. × isolates							
variable	Gallant	M. Huntsman		with Z. *t*	without Z. *t*	with P. n	without P. n		K2SO4	KCl	CaSiO3	Gallant × with Z. *t*	Gallant × without Z. *t*	Gallant × with P. n	Gallant × without P. n	M. Huntsman × with Z. *t*	M. Huntsman × without Z. *t*	M. Huntsman × with P. n	M. Huntsman × without P. n
mean	0.47	0.35		0.47	0.42	0.4	0.42		0.38	0.43	0.43	0.47	0.48	0.47	0.47	0.34	0.36	0.33	0.38
L.S.D	0.09			0.09					0.03			0.13							

variation	Fertilizers × isolates													cv. × Fertilizers						
variable	K2SO4 × with Z. *t*	K2SO4 × without Z. *t*	KCl × with Z. *t*	KCl × without Z. *t*	CaSiO3 × with Z. *t*	CaSiO3 × without Z. *t*	K2SO4 × with P. n	K2SO4 × without P. n	KCl × with P. n	KCl × without P. n	CaSiO3 × with P. n	CaSiO3 × without P. n		Gallant × K2SO4	Gallant × KCl	Gallant × CaSiO3	M. Huntsman × K2SO4	M. Huntsman × KCl	M. Huntsman n × CaSiO3	M. Huntsman × CaSiO3
mean	0.35	0.4	0.44	0.43	0.43	0.42	0.33	0.42	0.44	0.43	0.44	0.42		0.47	0.5	0.45	0.29	0.37	0.4	
L.S.D	0.1						0.1							0.1						

variation	Between isolates					cv. × Between isolates									Fertilizers × Between isolates					
variable	with Z. *t* × without P. n	with Z. *t* × with P. n	without Z. *t* × without P. n	without Z. *t* × with P. n		Gallant × with Z. *t* × without P. n	Gallant × with Z. *t* × with P. n	Gallant × without Z. *t* × without P. n	Gallant × without Z. *t* × with P. n	M. Huntsman × with Z. *t* × without P. n	M. Huntsman × with Z. *t* × with P. n	M. Huntsman × without Z. *t* × without P. n	M. Huntsman n × without Z. *t* × with P. n		K2SO4 × without Z. *t* × without P. n	K2SO4 × with Z. *t* × with P. n	K2SO4 × with Z. *t* × without P. n	K2SO4 × without Z. *t* × with P. n	KCl × with Z. *t* × with P. n	KCl × with Z. *t* × without P. n
mean	0.42	0.39	0.43	0.41		0.48	0.46	0.47	0.49	0.37	0.33	0.39	0.33		0.43	0.28	0.42	0.39	0.43	0.44
L.S.D	0.13					0.19									0.14					

Block 1

variation	Fertilizers × Between isolates							c.v × Fertilizers. × isolates											
variable	KCl × without Z.t × without P.n	KCl × without Z.t × with P.n	CaSiO3 × with Z.t × without P.n	CaSiO3 × with Z.t × with P.n	CaSiO3 × without Z.t × without P.n	CaSiO3 × without Z.t × with P.n		Gallant × with Z.t × K2SO4	Gallant × with Z.t × KCl	Gallant × with Z.t × CaSiO3	Gallant × without Z.t × K2SO4	Gallant × without Z.t × KCl	Gallant × without Z.t × CaSiO3	M. Huntsman × with Z.t × K2SO4	M. Huntsman × with Z.t × KCl	M.Huntsman × with Z.t × CaSiO3	M.Huntsman × without Z.t × K2SO4	M.Huntsman × without Z.t × KCl	M.Huntsman × without Z.t × CaSiO3
mean	0.43	0.43	0.41	0.45	0.42	0.42		0.46	0.5	0.45	0.46	0.5	0.46	0.25	0.38	0.42	0.33	0.36	0.38
L.S.D	0.14							0.14											

Block 2

variation	c.v × Fertilizers. × isolate													cv. × Fertilizers × Between isolates						
variable	Gallant × with P.n × K2SO4	Gallant × with P.n × KCl	Gallant × with P.n × CaSiO3	Gallant × without P.n × K2SO4	Gallant × without P.n × KCl	Gallant × without P.n × CaSiO3	M.Huntsman × with P.n × K2SO4	M.Huntsman × with P.n × KCl	M.Huntsman × with P.n × CaSiO3	M.Huntsman × without P.n × K2SO4	M.Huntsman × without P.n × KCl	M.Huntsman × without P.n × CaSiO3		Gallant × K2SO4 × with Z.t × without P.n	Gallant × K2SO4 × with Z.t × with P.n	Gallant × K2SO4 × without Z.t × without P.	Gallant × K2SO4 × without Z.t × with P.n	Gallant × KCl × with Z.t × without P.n	Gallant × KCl × with Z.t × with P.n	Gallant × KCl × without Z.t × without P.n
mean	0.45	0.5	0.47	0.49	0.49	0.43	0.22	0.37	0.4	0.36	0.37	0.4		0.52	0.39	0.45	0.51	0.5	0.5	0.49
L.S.D	0.014													0.2						

Block 3

variation	cv. × Fertilizers × Between isolates																
variable	Gallant × KCl × without Z.t × with P.n	Gallant × CaSiO3 × with Z.t × without P.n	Gallant × CaSiO3 × with Z.t × with P.n	Gallant × CaSiO3 × without Z.t × with P.n	Gallant × CaSiO3 × without Z.t × without P.	M.Huntsman × K2SO4 × with Z.t × without P.n	M.Huntsman × K2SO4 × with Z.t × with P.n	M.Huntsman × K2SO4 × without Z.t × without P.	M.Huntsman × K2SO4 × without Z.t × with P.n	M.Huntsman × KCl × with Z.t × without P.n	M.Huntsman × KCl × with Z.t × with P.n	M.Huntsman × KCl × without Z.t × without P.	M.Huntsman × KCl × without Z.t × with P.n	M.Huntsman × CaSiO3 × with Z.t × without P.n	M.Huntsman × CaSiO3 × with Z.t × with P.n	M.Huntsman × CaSiO3 × without Z.t × without P.	M.Huntsman × CaSiO3 × without Z.t × with P.n
mean	0.51	0.41	0.48	0.45	0.46	0.33	0.16	0.39	0.27	0.36	0.39	0.38	0.34	0.41	0.42	0.39	0.38
L.S.D	0.2																

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A 3. Table of all mean and L.S.D of Proportion of leaves infected with *Z. tritici* at GS 59, from two winter wheat cultivars in season 2013-2014 treated with three alternative fertilizers and inoculated either with or without two pathogens.

variation	Cultivars			Isolates					Fertilizers				cv. × isolates							
variable	Gallant	M.Huntsman		with Z. *t*	without Z. *t*	with P. n	without P. n		K2SO4	KCl	CaSiO3		Gallant × with Z. *t*	Gallant × without Z. *t*	Gallant × with P. n	Gallant × without P. n	M.Huntsman × with Z. *t*	M.Huntsman × without Z. *t*	M.Huntsman× with P. n	M.Huntsman × without P. n
mean	0.66	0.65		0.64	0.66	0.65	0.65		0.62	0.68	0.66		0.65	0.66	0.66	0.65	0.64	0.66	0.64	0.65
L.S.D	0.05			0.05					0.03				0.07							

variation	Fertilizers × isolates													cv. × Fertilizers					
variable	K2SO4 × with Z. *t*	K2SO4 × without Z. *t*	KCl × with Z. *t*	KCl × without Z. *t*	CaSiO3 × with Z. *t*	CaSiO3 × without Z. *t*	K2SO4 × with P. n	K2SO4 × without P. n	KCl × with P. n	KCl × without P. n	CaSiO3 × with P. n	CaSiO3 × without P. n		Gallant × K2SO4	Gallant × KCl	Gallant × CaSiO3	M.Huntsman × K2SO4	M.Huntsman × KCl	M.Huntsman × CaSiO3
mean	0.61	0.62	0.67	0.69	0.65	0.67	0.6	0.63	0.68	0.68	0.67	0.64		0.64	0.67	0.66	0.6	0.69	0.66
L.S.D	0.06						0.06							0.06					

variation	Between isolates					cv. × Between isolates									Fertilizers. × Between isolates					
variable	with Z. *t* × without P. n	with Z. *t* × with P. n	without Z. *t* × without P. n	without Z. *t* × with P. n		Gallant × with Z. *t* × without P. n	Gallant × with Z. *t* × with P. n	Gallant × without Z. *t* × without P. n	Gallant × without Z. *t* × with P. n	M.Huntsman × with Z. *t* × without P. n	M.Huntsman × with Z. *t* × with P. n	M.Huntsman × without Z. *t* × without P. n	M.Huntsman × without Z. *t* × with P. n		K2SO4 × with Z. *t* × without P. n	K2SO4 × with Z. *t* × with P. n	K2SO4 × without Z. *t* × without P. n	K2SO4 × without Z. *t* × with P. n	KCl × with Z. *t* × without P. n	KCl × with Z. *t* × with P. n
mean	0.65	0.63	0.66	0.66		0.64	0.65	0.66	0.66	0.66	0.62	0.65	0.67		0.63	0.6	0.64	0.6	0.68	0.66
L.S.D	0.07					0.1									0.08					

Block 1

variation	variable	mean
Fertilizers. × Between isolates	KCl × without Z. t × without P. n	0.69
	KCl × without Z. t × with P. n	0.69
	CaSiO3 × with Z. t × without P. n	0.65
	CaSiO3 × with Z. t × with P. n	0.65
	CaSiO3 × without Z. t × without P. n	0.64
	CaSiO3 × without Z. t × with P. n	0.7
c.v × Fertilizers × isolates	Gallant × with Z. t × K2SO4	0.64
	Gallant × with Z. t × KCl	0.64
	Gallant × with Z. t × CaSiO3	0.66
	Gallant × without Z. t × K2SO4	0.62
	Gallant × without Z. t × KCl	0.7
	Gallant × without Z. t × CaSiO3	0.67
	M.Huntsman × with Z. t × K2SO4	0.58
	M.Huntsman × with Z. t × KCl	0.7
	M.Huntsman × with Z. t × CaSiO3	0.64
	M.Huntsman × without Z. t × K2SO4	0.61
	M.Huntsman × without Z. t × KCl	0.68
	M.Huntsman × without Z. t × CaSiO3	0.67

L.S.D = 0.08 (left) ; 0.08 (right)

Block 2

variation	variable	mean
c.v × Fertilizers × isolates	Gallant × with P.n × K2SO4	0.61
	Gallant × with P.n × KCl	0.66
	Gallant × with P. n × CaSiO3	0.7
	Gallant × without P.n × K2SO4	0.65
	Gallant × without P.n × KCl	0.68
	Gallant × without P. n × CaSiO3	0.63
	M.Huntsman × with P.n × K2SO4	0.59
	M.Huntsman × with P.n × KCl	0.69
	M.Huntsman × with P. n × CaSiO3	0.65
	M.Huntsman × without P.n × K2SO4	0.61
	M.Huntsman × without P.n × KCl	0.69
	M.Huntsman × without P. n × CaSiO3	0.66
cv. × Fertilizers × Between isolates	Gallant × K2SO4 × with Z. t × without P. n	0.65
	Gallant × K2SO4 × with Z. t × with P. n	0.63
	Gallant × K2SO4 × without Z. t × without	0.65
	Gallant × K2SO4 × without Z. t × with P. n	0.59
	Gallant × KCl × with Z. t × without P. n	0.66
	Gallant × KCl × with Z. t × with P. n	0.63
	Gallant × KCl × without Z. t × without	0.7

L.S.D = 0.08 (left) ; 0.11 (right)

Block 3

variation	variable	mean
cv. × Fertilizers × Between isolates	Gallant × KCl × without Z. t × with P. n	0.69
	Gallant × CaSiO3 × with Z. t × without P. n	0.62
	Gallant × CaSiO3 × with Z. t × with P. n	0.7
	Gallant × CaSiO3 × without Z. t × without P. n	0.63
	Gallant × CaSiO3 × without Z. t × with P. n	0.7
	M.Huntsman × K2SO4 × with Z. t × without P. n	0.6
	M.Huntsman × K2SO4 × with Z. t × with P. n	0.56
	M.Huntsman × K2SO4 × without Z. t × without P. n	0.62
	M.Huntsman × K2SO4 × without Z. t × with P. n	0.61
	M.Huntsman × KCl × with Z. t × without P. n	0.7
	M.Huntsman × KCl × with Z. t × with P. n	0.69
	M.Huntsman × KCl × without Z. t × without P. n	0.68
	M.Huntsman × KCl × without Z. t × with P. n	0.69
	M.Huntsman × CaSiO3 × with Z. t × with P. n	0.68
	M.Huntsman × CaSiO3 × with Z. t × without P. n	0.6
	M.Huntsman × CaSiO3 × without Z. t × with P. n	0.65
	M.Huntsman × CaSiO3 × without Z. t × with P. n	0.7

L.S.D = 0.11

A 4. Table of all mean and L.S.D of Proportion of leaves infected with *Puccinia graminis* yellow rust at GS 59, from two winter wheat cultivars in season 2013-2014 treated with three alternative fertilizers and inoculated either with or without two pathogens.

Cultivars

variable	Gallant	M.Huntsman
mean	1.7	1.5
L.S.D	0.1	

Isolate

variable	with Z. *t*	without Z. *t*	with P. n	without P. n
mean	0.13	0.09	0.11	0.1
L.S.D	0.1			

Fertilizers

variable	K2SO4	KCl	CaSiO3
mean	1.14	1.1	1.09
L.S.D	0.04		

cv. × isolates

variable	Gallant × with Z. *t*	Gallant × without Z. *t*	Gallant × with P. n	Gallant × without P. n	M.Huntsman × with Z. *t*	M.Huntsman × without Z. *t*	M.Huntsman × with P. n	M.Huntsman × without P. n
mean	0.25	0.17	0.21	0.2	0.004	0.004	0.007	0.0012
L.S.D	0.14							

Fertilizers × isolates

variable	K2SO4 × with Z. *t*	K2SO4 × without Z. *t*	KCl × with Z. *t*	KCl × without Z. *t*	CaSiO3 × with Z. *t*	CaSiO3 × without Z. *t*	K2SO4 × with P. n	K2SO4 × without P. n	KCl × with P. n	KCl × without P. n	CaSiO3 × with P. n	CaSiO3 × without P. n
mean	0.17	0.11	0.11	0.066	0.1	0.08	0.15	0.12	0.08	0.09	0.09	0.09
L.S.D	0.11											

cv. × Fertilizers

variable	Gallant × K2SO4	Gallant × KCl	Gallant × CaSiO3	M.Huntsman × K2SO4	M.Huntsman × KCl	M.Huntsman × CaSiO3
mean	1.3	1.18	1.17	1.002	1.004	1.006
L.S.D	0.11					

Between isolates

variable	with Z. *t* × without P. n	with Z. *t* × with P. n	without Z. *t* × without P. n	without Z. *t* × with P. n
mean	0.12	0.14	0.09	0.08
L.S.D	0.14			

cv. × Between isolate

variable	Gallant × with Z. *t* × without P. n	Gallant × with Z. *t* × with P. n	Gallant × without Z. *t* × without P. n	Gallant × without Z. *t* × with P. n	M.Huntsman × with Z. *t* × without P. n	M.Huntsman × with Z. *t* × with P. n	M.Huntsman × without Z. *t* × without P. n	M.Huntsman × without Z. *t* × with P. n
mean	0.23	0.27	0.18	0.15	0.002	0.006	0	0.008
L.S.D	0.2							

Fertilizers × Between isolates

variable	K2SO4 × with Z. *t* × without P. n	K2SO4 × with Z. *t* × with P. n	K2SO4 × without Z. *t* × without P. n	K2SO4 × without Z. *t* × with P. n	KCl × with Z. *t* × without P. n	KCl × with Z. *t* × with P. n
mean	0.14	0.19	0.11	0.11	0.11	0.11
L.S.D	0.15					

variation: Fertilizers × Between isolates | c.v × Fertilizers × isolates

variable	KCl × without Z. t × without P.n	KCl × without Z. t × with P.n	CaSiO3 × with Z. t × without P.n	CaSiO3 × with Z. t × with P.n	CaSiO3 × without Z. t × without P.n	CaSiO3 × without Z. t × with P.n		Gallant × with Z. t × K2SO4	Gallant × with Z. t × KCl	Gallant × with Z. t × CaSiO3	Gallant × without Z. t × K2SO4	Gallant × without Z. t × KCl	Gallant × without Z. t × CaSiO3	M.Huntsman × with Z. t × K2SO4	M.Huntsman × with Z. t × KCl	M.Huntsman × with Z. t × CaSiO3	M.Huntsman × without Z. t × K2SO4	M.Huntsman × without Z. t × KCl	M.Huntsman × without Z. t × CaSiO3	
mean	0.08	0.06	0.09	0.1	0.09	0.07		0.32	0.23	0.19	0.22	0.13	0.15	0.004	0.004	0.013	0.004	0	0	
L.S.D	0.15							0.15												

variation: c.v × Fertilizers × isolates | cv. × Fertilizers × Between isolates

variable	Gallant × with P.n × K2SO4	Gallant × with P.n × KCl	Gallant × with P. n × CaSiO3	Gallant × without P.n × K2SO4	Gallant × without P.n × KCl	Gallant × without P. n × CaSiO3	M.Huntsman × with P.n × K2SO4	M.Huntsman × with P.n × KCl	M.Huntsman × with P. n × CaSiO3	M.Huntsman × without P.n × K2SO4	M.Huntsman × without P. n × KCl	M.Huntsman × without P. n × CaSiO3		Gallant × K2SO4 × with Z. t × without	Gallant × K2SO4× with Z. t × with P. n	Gallant × K2SO4 × with Z. t × without	Gallant×K2SO4×without Z. t × with P.	Gallant × K2SO4 × without Z. t × without	Gallant × KCl × with Z. t × with P. n	Gallant × KCl × with Z. t × with P. n	Gallant × KCl × without Z. t ×
mean	0.3	0.16	0.17	0.24	0.19	0.18	0.004	0.004	0.013	0.004	0	0		0.28	0.37	0.21	0.23	0.22	0.23	0.15	
L.S.D	0.15													0.21							

variation: cv. × Fertilizers × Between isolates

variable	Gallant × KCl × without Z. t × with P. n	Gallant × CaSiO3 × with Z. t × without P.	Gallant × CaSiO3 × with Z. t × with P.	Gallant × CaSiO3 × without Z. t × without P. n	Gallant × CaSiO3 × without Z. t × with P. n	M.Huntsman × K2SO4 × with Z. t × with P. n	M.Huntsman × K2SO4 × without Z. t × without P. n	M.Huntsman × K2SO4 × with Z. t × with P. n	M.Huntsman × K2SO4 × without Z. t × with P. n	M.Huntsman × KCl × without Z. t × with P. n	M.Huntsman × KCl × with Z. t × with P. n	M.Huntsman × KCl × without Z. t × without P. n	M.Huntsman × KCl × without Z. t × with P. n	M.Huntsman × CaSiO3 × with Z. t × with P. n	M.Huntsman × CaSiO3 × with Z. t × with P. n	M.Huntsman × CaSiO3 × without Z. t × without P. n	M.Huntsman × CaSiO3 × without Z. t × with P. n			
mean	0.1	0.18	0.2	0.18	0.13	0.007	0.008	0	0	0	0	0	0.008	0	0.009	0	0.016			
L.S.D	0.21																			

A 5. Table of all mean and L.S.D of *Z. tritici* spore concentration at GS 31, from two winter wheat cultivars in season 2013-2014 treated with three alternative fertilizers and inoculated either with or without two pathogens.

Cultivars

variable	Gallant	M.Huntsman
mean	10.25	2.5
L.S.D	2.4	

Isolates

variable	with Z. *t*	without Z. *t*	with P. n	without P. n
mean	6.1	6.6	6.2	6.6
L.S.D	2.4			

Fertilizers

variable	K_2SO_4	KCl	$CaSiO_3$
mean	5.8	6	7.2
L.S.D	2.2		

cv. × isolates

variable	Gallant × with Z. *t*	Gallant × without Z. *t*	Gallant × with P. n	Gallant × without P. n	M.Huntsman × with Z. *t*	M.Huntsman × without Z. *t*	M.Huntsman × with P. n	M.Huntsman × without P. n
mean	9.3	11.2	10.1	10.4	2.8	2.1	2.2	2.7
L.S.D	3.4							

Fertilizers × isolates

variable	K_2SO_4 × with Z. *t*	K_2SO_4 × without Z. *t*	KCl × with Z. *t*	KCl × without Z. *t*	$CaSiO_3$ × with Z. *t*	$CaSiO_3$ × without Z. *t*	K_2SO_4 × with P. n	K_2SO_4 × without P. n	KCl × with P. n	KCl × without P. n	$CaSiO_3$ × with P. n	$CaSiO_3$ × without P. n
mean	6.3	5.4	6.5	5.6	5.5	4.8	6.4	5.3	5.6	6.5	6.5	7.9
L.S.D	3.4											

cv. × Fertilizers

variable	Gallant × K_2SO_4	Gallant × KCl	Gallant × $CaSiO_3$	M.Huntsman × K_2SO_4	M.Huntsman × KCl	M.Huntsman × $CaSiO_3$
mean	9.6	9.6	11.6	2	2.5	2.8
L.S.D	3.4					

Between isolates

variable	with Z. *t* × without P. n	with Z. *t* × with P. n	without Z. *t* × without P. n	without Z. *t* × with P. n
mean	5.7	6.5	7.3	5.9
L.S.D	3.4			

cv. × Between isolates

variable	Gallant × with Z. *t* × without P. n	Gallant × with Z. *t* × with P. n	Gallant × without Z. *t* × without P. n	Gallant × without Z. *t* × with P. n	M.Huntsman × with Z. *t* × without P. n	M.Huntsman × with Z. *t* × with P. n	M.Huntsman × without Z. *t* × without P. n	M.Huntsman × without Z. *t* × with P. n
mean	8.1	10.5	12.7	9.7	3.3	2.4	2	2.1
L.S.D	4.8							

Fertilizers × Between isolates

variable	K_2SO_4 × with Z. *t* × without P. n	K_2SO_4 × with Z. *t* × with P. n	K_2SO_4 × without Z. *t* × without P. n	K_2SO_4 × without Z. *t* × with P. n	KCl × with Z. *t* × without P. n	KCl × with Z. *t* × with P. n
mean	5.4	7.1	5.2	5.7	7.3	5.7
L.S.D	4.8					

variation: Fertilizers × Between isolates | c.v × Fertilizers × isolates

variable	mean
KCl × without Z. t × without P. n	5.6
KCl × without Z. t × with P. n	5.5
CaSiO3 × with Z. t × without P. n	4.5
CaSiO3 × with Z. t × with P. n	6.5
CaSiO3 × without Z. t × without P. n	11.2
CaSiO3 × without Z. t × with P. n	6.5
Gallant × with Z. t × K2SO4	10
Gallant × with Z. t × KCl	10.2
Gallant × with Z. t × CaSiO3	7.9
Gallant × without Z. t × K2SO4	9.3
Gallant × without Z. t × KCl	9
Gallant × without Z. t × CaSiO3	15.2
M.Huntsman × with Z. t × K2SO4	2.6
M.Huntsman × with Z. t × KCl	2.9
M.Huntsman × with Z. t × CaSiO3	3.1
M.Huntsman × without Z. t × K2SO4	1.5
M.Huntsman × without Z. t × KCl	2.2
M.Huntsman × without Z. t × CaSiO3	2.5

L.S.D (Fertilizers × Between isolates): 4.8 L.S.D (c.v × Fertilizers × isolates): 4.8

variation: c.v × Fertilizers × isolates | cv. × Fertilizers × Between isolates

variable	mean
Gallant × with P.n × K2SO4	11.2
Gallant × with P.n × KCl	9.1
Gallant × with P. n × CaSiO3	10
Gallant × without P.n × K2SO4	8
Gallant × without P.n × KCl	10
Gallant × without P. n × CaSiO3	13
M.Huntsman × with P.n × K2SO4	1.5
M.Huntsman × with P.n × KCl	2.2
M.Huntsman × with P.n × CaSiO3	3
M.Huntsman × without P.n × K2SO4	2.5
M.Huntsman × without P. n × KCl	2.8
M.Huntsman × without P. n × CaSiO3	2.6
Gallant × K2SO4 × with Z. t × without P. n	7.07
Gallant × K2SO4 × with Z. t × with P. n	12.83
Gallant × K2SO4 × without Z. t × without P.	8.97
Gallant × K2SO4 × without Z. t × with P. n	9.64
Gallant × KCl × with Z. t × without P. n	11.27
Gallant × KCl × with Z. t × with P. n	9.03
Gallant × KCl × without Z. t × without P. n	8.9

L.S.D (c.v × Fertilizers × isolates): 7.8 L.S.D (cv. × Fertilizers × Between isolates): 6.7

variation: cv. × Fertilizers × Between isolates

variable	mean
Gallant × KCl × without Z. t × with P. n	9.08
Gallant × CaSiO3 × with Z. t × without P. n	6.07
Gallant × CaSiO3 × with Z. t × with P. n	9.74
Gallant × CaSiO3 × without Z. t × without P. n	20.12
Gallant × CaSiO3 × without Z. t × with P. n	10.28
M.Huntsman × K2SO4 × with Z. t × without P. n	3.68
M.Huntsman × K2SO4 × with Z. t × with P. n	1.42
M.Huntsman×K2SO4×without Z. t × without P. n	1.32
M.Huntsman × K2SO4 × without Z. t × with P. n	1.66
M.Huntsman × KCl × with Z. t × without P. n	3.29
M.Huntsman × KCl × with Z. t × with P. n	2.42
M.Huntsman × KCl × without Z. t × without P. n	2.38
M.Huntsman × KCl × without Z. t × with P. n	1.97
M.Huntsman × CaSiO3 × with Z. t × without P. n	2.95
M.Huntsman × CaSiO3 × with Z. t × with P. n	3.27
M.Huntsman × CaSiO3 × without Z. t × without P. n	2.28
M.Huntsman × CaSiO3 × without Z. t × with P. n	2.68

L.S.D: 6.7

A 6. Table of all mean and L.S.D of Z. *tritici* spore concentration at GS 59, from two winter wheat cultivars in season 2013-2014 treated with three alternative fertilizers and inoculated either with or without two pathogens.

variation	Cultivars			Isolates					Fertilizers				cv. × isolates							
variable	Gallant	M.Huntsman		with Z. *t*	without Z. *t*	with P. n	without P. n		K2SO4	KCl	CaSiO3		Gallant × with Z. *t*	Gallant × without Z. *t*	Gallant × with P. n	Gallant × without P. n	M.Huntsman × with Z. *t*	M.Huntsman × without P. n	M.Huntsman× with P. n	M.Huntsman × without P. n
mean	9.5	8		8.9	8.7	6.9	10.7		5.5	14.8	10		8.6	10.4	7.5	11.5	9.1	7	6.2	9.9
L.S.D	5.7			5.7					4.4				8.03							

variation	Fertilizers × isolates													cv. × Fertilizers						
variable	K2SO4 × with Z. *t*	K2SO4 × without Z. *t*	KCl × with Z. *t*	KCl × without Z. *t tritici*	CaSiO3 × with Z. *t*	CaSiO3 × without Z. *t*	K2SO4 × with P. n	K2SO4 × without P. n	KCl × with P. n	KCl × without P. n	CaSiO3 × with P. n	CaSiO3 × without P. n		Gallant × K2SO4	Gallant × KCl	Gallant × CaSiO3	M.Huntsman × K2SO4	M.Huntsman × KCl	M.Huntsman × CaSiO3	
mean	4.1	4	15.1	12.4	7.4	9.6	3.8	4.4	10.3	17.3	6.6	10.4		4.2	15.2	9.1	3.9	12.3	7.9	
L.S.D	7.3													7.3						

variation	Between isolates					cv. × Between isolates										Fertilizers × Between isolates						
variable	with Z. *t* × without P. n	with Z. *t* × with P. n	without Z. *t* × without P. n	without Z. *t* × with P. n		Gallant × with Z. *t* × without P. n	Gallant × with Z. *t* × with P. n	Gallant × without Z. *t* × without P. n	Gallant × without Z. *t* × with P. n	M.Huntsman × with Z. *t* × without P. n	M.Huntsman × with Z. *t* × with P. n	M.Huntsman × without Z. *t* × without P. n	M.Huntsman×without Z. *t* × with P. n		K2SO4 × with Z. *t* × without P. n	K2SO4 × with Z. *t* × with P. n	K2SO4 × without Z. *t* × without P. n	K2SO4 × without Z. *t* × with P. n	KCl × with Z. *t* × without P. n	KCl × with Z. *t* × with P. n
mean	11.7	6	9.7	7.7		12.1	5.2	10.9	9.9	11.3	6.9	8.5	5.5		4.3	3.9	4.5	3.6	21.2	9
L.S.D	8.03					11.4									10.4					

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Fertilizers × Between isolates and **c.v × Fertilizers × isolates**

variable	mean
KCl × without Z. t × without P. n	13.4
KCl × without Z. t × with P. n	11.5
CaSiO3 × without Z. t × without P. n	9.6
CaSiO3 × with Z. t × with P. n	5.2
CaSiO3 × with Z. t × without P. n	11.2
CaSiO3 × without Z. t × with P. n	8.1
Gallant × with Z. t × K2SO4	4.5
Gallant × with Z. t × KCl	13.4
Gallant × with Z. t × CaSiO3	7.9
Gallant × without Z. t × K2SO4	3.9
Gallant × without Z. t × KCl	17
Gallant × without Z. t × CaSiO3	10.3
M.Huntsman × with Z. t × K2SO4	3.8
M.Huntsman × with Z. t × KCl	16.7
M.Huntsman × with Z. t × CaSiO3	6.8
M.Huntsman×without Z. t × K2SO4	4.1
M.Huntsman×without Z. t × KCl	7.9
M.Huntsman×without Z. t × CaSiO3	9

L.S.D (left) = 10.4 ; L.S.D (right) = 10.4

c.v × Fertilizers × isolates and **cv. × Fertilizers × Between isolates**

variable	mean
Gallant × with P. n × K2SO4	4
Gallant × with P. n × KCl	10.7
Gallant × with P. n × CaSiO3	8
Gallant × without P.n × K2SO4	4.4
Gallant × without P.n × KCl	19.8
Gallant × without P. n × CaSiO3	10.2
M.Huntsman×with P.n× K2SO4	3.5
M.Huntsman × with P.n × KCl	9.9
M.Huntsman × with P. n × CaSiO3	5.3
M.Huntsman × without P.n × K2SO4	4.4
M.Huntsman × without P.n × KCl	14.7
M.Huntsman × without P. n × CaSiO3	10.6
Gallant × K2SO4 × with Z. t × without P. n	5.76
Gallant × K2SO4 × with Z. t × with P. n	3.16
Gallant × K2SO4 × without Z. t × without P.	3.03
Gallant × K2SO4 × without Z. t × with P. n	4.86
Gallant × KCl × with Z. t × without P. n	21.51
Gallant × KCl × with Z. t × with P. n	5.37
Gallant × KCl × without Z. t × with P. n	18.02

L.S.D (left) = 10.4 ; L.S.D (right) = 14.7

cv. × Fertilizers × Between isolates

variable	mean
Gallant × KCl × without Z. t × with P. n	15.94
Gallant × CaSiO3 × with Z. t × without P. n	8.9
Gallant × CaSiO3 × with Z. t × with P. n	6.91
Gallant × CaSiO3 × without Z. t × without P.	11.49
Gallant × CaSiO3 × without Z. t × with P. n	9.02
M.Huntsman × K2SO4 × with Z. t × without P. n	2.93
M.Huntsman × K2SO4 × with Z. t × with P. n	4.69
M.Huntsman×K2SO4×without Z. t×without P. n	5.89
M.Huntsman×K2SO4 × without Z. t × with P. n	2.3
M.Huntsman × KCl × with Z. t × with P. n	20.8
M.Huntsman × KCl × with Z. t × without P. n	12.66
M.Huntsman × KCl × without Z. t × without P. n	8.68
M.Huntsman × KCl × without Z. t × with P. n	7.07
M.Huntsman×CaSiO3× with Z. t × without P. n	10.23
M.Huntsman×CaSiO3× with Z. t × with P. n	3.44
M.Huntsman×CaSiO3×without Z. t × without P. n	10.9
M.Huntsman × CaSiO3 × without Z. t × with P. n	7.13

L.S.D = 14.7

A 7. Table of all mean and L.S.D of proportion of leaves infected with *Z. tritici* at GS 31, from winter wheat cultivars M.Huntsman in season 2014-2015 treated either with or without sulphate fertilizer and inoculated with *P. nodorum*.

variation	Fertilizer		Fungicide		isolate			Fertilizer × Fungicide				Fertilizer × isolate					
variable	Sulphate +	Sulphate -	with fungicide	without fungicide	seed *P. n*	spray *P. n*	without *P. n*	Sulphate+ × with fungicide	Sulphate- × with fungicide	Sulphate+ × without fungicide	Sulphate- × without fungicide	Sulphate + × seed *P. n*	Sulphate + × spray *P. n*	Sulphate + × none *P. n*	Sulphate - × seed *P. n*	Sulphate - × spray *P. n*	Sulphate - × none *P. n*
mean	0.142	0.216	0.186	0.172	0.179	0.169	0.189	0.149	0.222	0.135	0.209	0.134	0.128	0.164	0.224	0.209	0.215
L.S.D	0.036		0.044		0.054			0.054				0.066					

variation	Fungicide × isolate						Fertilizer × Fungicide × isolate											
variable	with fungicide × seed *P. n*	with fungicide × spray *P. n*	with fungicide × none *P. n*	without fungicide × seed *P. n*	without fungicide × spray *P. n*	without fungicide × none *P. n*	Sulphate + × with fungicide × seed *P. n*	Sulphate + × with fungicide × spray *P. n*	Sulphate + × with fungicide × none *P. n*	Sulphate + × without fungicide × seed *P. n*	Sulphate + × without fungicide × spray *P. n*	Sulphate + × without fungicide × none *P. n*	Sulphate - × with fungicide × seed *P. n*	Sulphate - × with fungicide × spray *P. n*	Sulphate - × with fungicide × none *P. n*	Sulphate - × without fungicide × seed *P. n*	Sulphate - × without fungicide × spray *P. n*	Sulphate - × without fungicide × none *P. n*
mean	0.198	0.181	0.178	0.16	0.156	0.201	0.144	0.14	0.162	0.124	0.116	0.166	0.252	0.222	0.194	0.196	0.196	0.235
L.S.D	0.076						0.093											

A 8. Table of all mean and L.S.D of *Z. tritici* spore concentration at GS 31, from winter wheat cultivars M.Huntsman in season 2014-2015 treated either with or without sulphate fertilizer and inoculated with *P. nodorum*.

variation	Fertilizer			Fungicide			isolate				Fertilizer × Fungicide					Fertilizer × isolate					
variable	Sulphate +	Sulphate -		with fungicide	without fungicide		seed *P. n*	spray *P. n*	without *P. n*		Sulphate+ × with fungicide	Sulphate- × with fungicide	Sulphate+ × without fungicide	Sulphate- × without fungicide		Sulphate + × seed *P. n*	Sulphate + × spray *P. n*	Sulphate + × none *P. n*	Sulphate - × seed *P. n*	Sulphate - × spray *P. n*	Sulphate - × none *P. n*
mean	0.76	2.66		1.58	1.85		1.48	1.67	1.98		0.74	2.42	0.78	2.9		0.76	0.7	0.83	2.2	2.64	3.13
L.S.D	0.67			0.73			0.89				0.93					1.14					

variation	Fungicide × isolate							Fertilizer × Fungicide × isolate													
variable	with fungicide × seed *P. n*	with fungicide × spray *P. n*	with fungicide × none *P. n*	without fungicide × seed *P. n*	without fungicide × spray *P. n*	without fungicide × none *P. n*		Sulphate + × with fungicide × seed *P. n*	Sulphate + × with fungicide × spray *P. n*	Sulphate + × with fungicide × none *P. n*	Sulphate + × without fungicide × seed *P.*	Sulphate + × without fungicide × spray *P.*	Sulphate + × without fungicide × none *P.*	Sulphate - × with fungicide × seed *P. n*	Sulphate - × with fungicide × spray *P. n*	Sulphate - × with fungicide × none *P. n*	Sulphate - × without fungicide × seed *P.*	Sulphate - × without fungicide × spray *P.*	Sulphate - × without fungicide × none *P.*		
mean	1.48	1.27	2	1.48	2.08	1.96		0.77	0.73	0.73	0.75	0.67	0.92	2.18	1.8	3.26	2.21	3.49	3		
L.S.D	1.26							1.62													

A 9. Table of all mean and L.S.D of proportion of leaves infected with *Z. tritici* at GS 59, from winter wheat cultivars M. Huntsman in season 2014-2015 treated either with or without sulphate fertilizer and inoculated with *P. nodorum*.

variation	Fertilizer			Fungicide			isolate				Fertilizer × Fungicide					Fertilizer × isolate					
variable	Sulphate +	Sulphate -		with fungicide	without fungicide		seed *P. n*	spray *P. n*	without *P. n*		Sulphate+ × with fungicide	Sulphate- × with fungicide	Sulphate+ × without fungicide	Sulphate- × without fungicide		Sulphate + × seed *P. n*	Sulphate + × spray *P. n*	Sulphate + × none *P. n*	Sulphate - × seed *P. n*	Sulphate - × spray *P. n*	Sulphate - × none *P. n*
mean	0.027	0.110		0.077	0.060		0.060	0.081	0.065		0.034	0.121	0.020	0.099		0.034	0.036	0.012	0.086	0.127	0.117
L.S.D	0.063			0.035			0.043				0.069					0.084					

variation	Fungicide × isolate							Fertilizer × Fungicide × isolate													
variable	with fungicide × seed *P. n*	with fungicide × spray *P. n*	with fungicide × none *P. n*	without fungicide × seed *P. n*	without fungicide × spray *P. n*	without fungicide × none *P. n*		Sulphate + × with fungicide × seed *P. n*	Sulphate + × with fungicide × spray *P. n*	Sulphate + × with fungicide × none *P. n*	Sulphate + × without fungicide × seed *P. n*	Sulphate + × without fungicide × spray *P. n*	Sulphate + × without fungicide × none *P. n*	Sulphate - × with fungicide × seed *P. n*	Sulphate - × with fungicide × spray *P. n*	Sulphate - × with fungicide × none *P. n*	Sulphate - × without fungicide × seed *P. n*	Sulphate - × without fungicide × spray *P. n*	Sulphate - × without fungicide × none *P. n*		
mean	0.027	0.118	0.042	0.048	0.045	0.087		0.030	0.710	0.000	0.037	0.000	0.024	0.114	0.165	0.084	0.059	0.089	0.150		
L.S.D	0.060							0.119													

A 10. Table of all mean and L.S.D of proportion of leaves infected with Z. *tritici* at GS 77, from winter wheat cultivars M. Huntsman in season 2014-2015 treated either with or without sulphate fertilizer and inoculated with *P. nodorum*.

variation	Fertilizer			Fungicide			isolate				Fertilizer × Fungicide					Fertilizer × isolate					
variable	Sulphate +	Sulphate -		with fungicide	without fungicide		seed *P. n*	spray *P. n*	without *P. n*		Sulphate+ × with fungicide	Sulphate- × with fungicide	Sulphate+ × without fungicide	Sulphate- × without fungicide		Sulphate + × seed *P. n*	Sulphate + × spray *P. n*	Sulphate + × none *P. n*	Sulphate - × seed *P. n*	Sulphate - × spray *P. n*	Sulphate - × none *P. n*
mean	0.607	0.694		0.644	0.657		0.664	0.617	0.671		0.612	0.675	0.601	0.713		0.626	0.542	0.652	0.701	0.691	0.69
L.S.D	0.046			0.092			0.113				0.099					0.122					

variation	Fungicide × isolate							Fertilizer × Fungicide × isolate											
variable	with fungicide × seed *P. n*	with fungicide × spray *P. n*	with fungicide × none *P. n*	without fungicide × seed *P. n*	without fungicide × spray *P. n*	without fungicide × none *P. n*		Sulphate + × with fungicide × seed *P. n*	Sulphate + × with fungicide × spray *P. n*	Sulphate + × with fungicide × none *P. n*	Sulphate + × without fungicide × seed *P. n*	Sulphate + × without fungicide × spray *P. n*	Sulphate + × without fungicide × none *P. n*	Sulphate - × with fungicide × seed *P. n*	Sulphate - × with fungicide × spray *P. n*	Sulphate - × with fungicide × none *P. n*	Sulphate - × without fungicide × seed *P. n*	Sulphate - × without fungicide × spray *P. n*	Sulphate - × without fungicide × none *P. n*
mean	0.652	0.64	0.639	0.675	0.594	0.703		0.621	0.575	0.642	0.632	0.51	0.662	0.684	0.705	0.636	0.717	0.678	0.744
L.S.D	0.16							0.172											

A 11. Table of all mean and L.S.D of disease severity of *Z. tritici* at GS 77, from winter wheat cultivars M. Huntsman in season 2014-2015 treated either with or without sulphate fertilizer and inoculated with *P. nodorum*.

variation	Fertilizer			Fungicide			isolate				Fertilizer × Fungicide					Fertilizer × isolate					
variable	Sulphate +	Sulphate -		with fungicide	without fungicide		seed *P. n*	spray *P. n*	without *P. n*		Sulphate+ × with fungicide	Sulphate- × with fungicide	Sulphate+ × without fungicide	Sulphate- × without fungicide		Sulphate + ×seed *P. n*	Sulphate + ×spray *P. n*	Sulphate + ×none *P. n*	Sulphate - ×seed *P. n*	Sulphate - ×spray *P. n*	Sulphate - ×none *P. n*
mean	3.87	5.3		4.41	4.76		5.01	3.97	4.78		3.36	5.46	4.38	5.14		4.05	3.3	4.26	5.96	4.64	5.3
L.S.D	1.03			1.22			1.49				1.51					1.85					

variation	Fungicide × isolate							Fertilizer × Fungicide × isolate												
variable	with fungicide × seed *P. n*	with fungicide × spray *P. n*	with fungicide × none *P. n*	without fungicide × spray *P. n*	without fungicide × seed *P. n*	without fungicide × none *P. n*		Sulphate + × fungicide ×seed *P. n*	Sulphate + × fungicide ×spray *P. n*	Sulphate + × fungicide ×none *P. n*	Sulphate + × without fungicide ×seed *P. n*	Sulphate + × without fungicide ×spray *P. n*	Sulphate + × without fungicide ×none *P. n*	Sulphate - × with fungicide ×seed *P. n*	Sulphate - × with fungicide ×spray *P. n*	Sulphate - × with fungicide ×none *P. n*	Sulphate - × without fungicide ×seed *P. n*	Sulphate - × without fungicide ×spray *P. n*	Sulphate - × without fungicide ×none *P. n*	
Mean	5.18	4	4.05	4.83	3.93	5.51		3.19	3.3	3.6	4.92	3.3	4.92	7.17	4.7	4.49	4.75	4.57	6.11	
L.S.D	2.11							2.61												

A 12. Table of all mean and L.S.D of spore concentration of *Z. tritici* at GS 77, from winter wheat cultivars M. Huntsman in season 2014-2015 treated either with or without sulphate fertilizer and inoculated with *P. nodorum*.

variation	Fertilizer			Fungicide			isolate				Fertilizer × Fungicide					Fertilizer × isolate					
variable	Sulphate +	Sulphate -		with fungicide	without fungicide		seed *P. n*	spray *P. n*	without *P. n*		Sulphate+ × with fungicide	Sulphate- × with fungicide	Sulphate+ × without fungicide	Sulphate- × without fungicide		Sulphate + × seed *P. n*	Sulphate + × spray *P. n*	Sulphate + × none *P. n*	Sulphate - × seed *P. n*	Sulphate - × spray *P. no*	Sulphate - × none *P. n*
mean	28.7	64.8		41.7	51.8		48.6	41.6	50		25.8	57.6	31.5	72		28	24	34	69.3	59.2	66
L.S.D	7.5			10.5			12.9				12.2					15					

variation	Fungicide × isolate							Fertilizer × Fungicide × isolate													
variable	with fungicide × seed *P. n*	with fungicide × spray *P. n*	with fungicide × none *P. n*	without fungicide × seed *P. n*	without fungicide × spray *P. n*	without fungicide × none *P. n*		Sulphate + × with fungicide × seed *P. n*	Sulphate + × with fungicide × spray *P. n*	Sulphate + × with fungicide × none *P. n*	Sulphate + × without fungicide × seed *P. n*	Sulphate + × without fungicide × spray *P. n*	Sulphate + × without fungicide × none *P. n*	Sulphate - × with fungicide × seed *P. n*	Sulphate - × with fungicide × spray *P. n*	Sulphate - × with fungicide × none *P. n*	Sulphate - × without fungicide × seed *P. n*	Sulphate - × without fungicide × spray *P. n*	Sulphate - × without fungicide × none *P. n*		
mean	39.7	39	46.5	57.6	44.2	53.3		18.8	24.9	33.6	37.2	23	34.4	60.5	53	59.3	78	65.5	72.7		
L.S.D	18.2							21.2													

A 13. Table of all mean and L.S.D of proportion of leaves infected with *P. nodorum* at GS 77, from winter wheat cultivars M. Huntsman in season 2014-2015 treated either with or without sulphate fertilizer and inoculated with *P. nodorum*.

variation	Fertilizer			Fungicide			isolate				Fertilizer × Fungicide					Fertilizer × isolate					
variable	Sulphate +	Sulphate -		with fungicide	without fungicide		seed *P. n*	spray *P. n*	without *P. n*		Sulphate+ × with fungicide	Sulphate- × with fungicide	Sulphate+ × without fungicide	Sulphate- × without fungicide		Sulphate +× seed *P. n*	Sulphate +× spray *P. n*	Sulphate +× none *P. n*	Sulphate -× seed *P. n*	Sulphate -× spray *P. n*	Sulphate -× none *P. n*
mean	0.174	0.235		0.225	0.185		0.05	0.6	0		0.196	0.253	0.152	0.218		0.006	0.516	0	0.078	0.628	0
L.S.D	0.047			0.041			0.05				0.059					0.072					

variation	Fungicide × isolate							Fertilizer × Fungicide × isolate													
variable	with fungicide × seed *P. n*	with fungicide × spray *P. n*	with fungicide × none *P. n*	without fungicide × seed *P. n*	without fungicide × spray *P. n*	without fungicide × none *P. n*		Sulphate + × with fungicide × seed *P. n*	Sulphate + × with fungicide × spray *P. n*	Sulphate + × with fungicide × none *P. n*	Sulphate + × without fungicide × seed *P. n*	Sulphate + × without fungicide × spray *P. n*	Sulphate + × without fungicide × none *P. n*	Sulphate - × with fungicide × seed *P. n*	Sulphate - × with fungicide × spray *P. n*	Sulphate - × with fungicide × none *P. n*	Sulphate - × without fungicide × seed *P. n*	Sulphate - × without fungicide × spray *P. n*	Sulphate - × without fungicide × none *P. n*		
mean	0.056	0.618	0	0.028	0.527	0		0.012	0.578	0	0	0	0.455	0.1	0.659	0	0.056	0.598	0		
L.S.D	0.071							0.102													

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A 14. Table of all mean and L.S.D of spore concentration of *P. nodorum* at GS 77, from winter wheat cultivars M. Huntsman in season 2014-2015 treated either with or without sulphate fertilizer and inoculated with *P. nodorum*.

variation	Fertilizer			Fungicide			isolate				Fertilizer × Fungicide					Fertilizer × isolate					
variable	Sulphate +	Sulphate -		with fungicide	without fungicide		seed *P. n*	spray *P. n*	without *P. n*		Sulphate+ × with fungicide	Sulphate- × with fungicide	Sulphate+ × without fungicide	Sulphate- × without fungicide		Sulphate + × seed *P. n*	Sulphate + × spray *P. n*	Sulphate + × none *P. n*	Sulphate - × seed *P. n*	Sulphate - × spray *P. n*	Sulphate - × none *P. n*
mean	2.49	3.72		2.57	3.64		0	9.31	0		2.27	2.87	2.72	4.56		0	7.48	0	0	11.15	0
L.S.D	1.21			3.18			3.9				3.31					4.06					

variation	Fungicide × isolate							Fertilizer × Fungicide × isolate												
variable	with fungicide × seed *P. n*	with fungicide × spray *P. n*	with fungicide × none *P. n*	without fungicide × seed *P. n*	without fungicide × spray *P. n*	without fungicide × none *P. n*		Sulphate + × with fungicide × seed *P. n*	Sulphate + × with fungicide × spray *P. n*	Sulphate + × with fungicide × none *P. n*	Sulphate + × without fungicide × seed *P. n*	Sulphate + × without fungicide × spray *P. n*	Sulphate + × without fungicide × none *P. n*	Sulphate - × with fungicide × seed *P. n*	Sulphate - × with fungicide × spray *P. n*	Sulphate - × with fungicide × none *P. n*	Sulphate - × without fungicide × seed *P. n*	Sulphate - × without fungicide × spray *P. n*	Sulphate - × without fungicide × none *P. n*	
mean	0	7.7	0	0	10.93	0		0	6.8	0	0	8.16	0	0	8.6	0	0	13.69	0	
L.S.D	5.51							5.74												

A 15. Table of all means and L.S.D. value for the test the homogeneity of samples that liquidized with phosphate buffer.

variation	variable	mean	L.S.D
Samples	A	184	
	B	88	179
	C	298	
Replicate	1	190	
	2	190	179
	3	190	
Samples × Replicate	A×1	184	
	A×2	184	
	A×3	184	
	B×1	88	
	B×2	88	309
	B×3	88	
	C×1	298	
	C×2	298	
	C×3	298	

A 16. Table of means and L.S.D. value for buffers, storage conditions and volume experiment, all samples from wheat cultivar M. Huntsman of two field replicates.

variation	Buffers		Storage						Volume		
variable	POS	CTAB	Fresh	fridge 2 days	fridge Overnight	min. 20 overnight	room 2 days	room Overnight	2ml/g	6ml/g	20ml/g
mean	137	240	172	270	118	126	312	213	172	242	126
L.S.D	77		114						95		

variation	Buffers x Storage											
variable	POS x Fresh	POS x fridge 2 days	POS x fridge overnight	POS x min. 20 overnight	POS x room 2 days	POS x room overnight	CTAB x Fresh	CTAB x fridge 2 days	CTAB x fridge overnight	CTAB x min. 20 overnight	CTAB x room 2 days	CTAB x room overnight
mean	147.5	259.6	49.6	83.3	70	62.6	188.3	280.5	159.6	161.7	457.5	283
L.S.D	191											

Variation	Buffer x volume						Storage x volume					
variable	POS x 2ml/g	POS x 6ml/g	POS x 20ml/g	CTAB x 2ml/g	CTAB x 6ml/g	CTAB x 20ml/g	Fresh x 2ml/g	fridge 2 days x 2ml/g	fridge Overnight x 2ml/g	min. 20 overnight x 2ml/g	room 2 days x 2ml/g	room Overnight x 2ml/g
mean	73	207.2	95.7	238.8	265.5	160.5	147.3	125.2	104.1	121.1	373	289.4
L.S.D	135						210					

variation	Storage x volume											
variable	Fresh x 6ml/g	fridge 2 days x 6ml/g	fridge Overnight x 6ml/g	min. 20 overnight x 6ml/g	room 2 days x 6ml/g	room Overnight x 6ml/g	Fresh x 20ml/g	fridge 2 days x 20ml/g	fridge Overnight x 20ml/g	min. 20 overnight x 20ml/g	room 2 days x 20ml/g	room Overnight x 20ml/g
mean	188.6	429.6	134.3	164.5	306.2	144.7	152.1	205.1	0	64.3	40.9	0
L.S.D	210											
variation	Buffer x Storage x volume											
variable	POS x Fresh x 2ml/g	POS x fridge 2 days x 2ml/g	POS x fridge overnight x 2ml/g	POS x min. 20 overnight x 2ml/g	POS x room 2 days x 2ml/g	POS x room overnight x 2ml/g	POS x Fresh x 6ml/g	POS x fridge 2 days x 6ml/g	POS x fridge overnight x 6ml/g	POS x min. 20 overnight x 6ml/g	POS x room 2 days x 6ml/g	POS x room overnight x 6ml/g
mean	0	105.4	41.6	69.3	93.3	20.8	179.7	443.8	65.4	122.4	65.6	100.4
L.S.D	264											

variation	Buffer x Storage x volume											
variable	POS x Fresh x 20ml/g	POS x fridge 2 days x 20ml/g	POS x fridge overnight x 20ml/g	POS x min. 20 overnight x 20ml/g	POS x room 2 days x 20ml/g	POS x room overnight x 20ml/g	CTAB x Fresh x 2ml/g	CTAB x fridge 2 days x 2ml/g	CTAB x fridge overnight x 2ml/g	CTAB x min. 20 overnight x 2ml/g	CTAB x room 2 days x 2ml/g	CTAB x room overnight x 2ml/g
mean	91.2	181.6	0	37.4	40.9	0	147.3	142.1	157.6	165.5	502.1	413.4
L.S.D	264											
variation	Buffer x Storage x volume											
variable	CTAB x Fresh x 6ml/g	CTAB x fridge 2 days x 6ml/g	CTAB x fridge overnight x 6ml/g	CTAB x min. 20 overnight x 6ml/g	CTAB x room 2 days x 6ml/g	CTAB x room overnight x 6ml/g	CTAB x Fresh x 20ml/g	CTAB x fridge 2 days x 20ml/g	CTAB x fridge overnight x 20ml/g	CTAB x min. 20 overnight x 20ml/g	CTAB x room 2 days x 20ml/g	CTAB x room overnight x 20ml/g
mean	196.2	417.4	161.5	200.7	417.2	165.1	204.2	242.4	0	87.4	0	0
L.S.D	264											

A 17. Table of mean and L.S.D. value for test storage conditions second experiment, all samples from wheat cultivar M. Huntsman, from five field replicates.

variation	variable	mean	L.S.D.
storage	fresh	238	279
	room over night	284	
	room 48 hours	318	
	fridge over night	471	
	fridge 48 hours	385	
	freezer	337	

A 18. Table of mean and L.S.D. value for test the methodology technique, all samples from wheat cultivar M. Huntsman, treated either with or without sulphur fertilization.

variation	variable	mean	L.S.D
Methods	Liquidised	198	
	Oven dry	268	152
	lyophilize	342	
Fertilizers	Sulphate +	294	
	Sulphate -	245	124
Methods x fertilizers	Liquidised x Sulphate +	169	
	Liquidised x Sulphate -	226	
	Oven dry x Sulphate +	287	
	Oven dry x Sulphate -	249	215
	Lyophilize x Sulphate +	425	
	Lyophilize x Sulphate -	260	

A 19. Table of mean for the samples at growth stage 31, liquidised with phosphate buffer and quantify by qPCR assay using SYBR-green chemical reagent.

variation	variable	mean	L.S.D
Fertilizer	Sulphate +	-0.806	0.34
	Sulphate -	-0.334	
Fungicide	with fungicide	-0.583	0.56
	without fungicide	-0.557	
P. nodorum inoculation	no inoculation	-0.307	0.69
	seed inoculation	-0.782	
	spray inoculation	-0.622	
Fertilizer x Fungicide	Sulphate + x with fungicide	-0.859	0.61
	Sulphate + x without Fungicide	-0.752	
	Sulphate - x with fungicide	-0.307	
	Sulphate - x without fungicide	-0.362	
Fertilizer x *P. nodorum* inoculation	Sulphate + x non inoculation *P. nodorum*	-0.748	0.75
	Sulphate + x seed inoculation *P. nodorum*	-0.947	
	Sulpate + x spray inoculation *P. nodorum*	-0.723	
	Sulphate - x non inoculation *P. nodorum*	0.134	
	Sulphate - x seed inoculation *P. nodorum*	-0.617	
	Sulphate - x spray inoculation *P. nodorum*	-0.521	
Fungicide x *P. nodorum* inoculation	with fungicide x non *P. nodorum*	-0.365	0.97
	with fungicide x seed inoculation *P. nodorum*	-0.836	
	with fungicide x spray inoculation *P. nodorum*	-0.549	
	without fungicide x non *P. nodorum*	-0.249	
	without fungicide x seed inoculation *P. nodorum*	-0.728	
	without fungicide x spray inoculation *P. nodorum*	-0.695	

Fertilizer x fungicide x *P. nodorum* inoculation	Sulphate + x with fungicide x non inoculation *P. nodorum*	-0.855	1.06
	Sulphate + x with fungicide x seed inoculation *P. nodorum*	-0.95	
	Sulphate + x with fungicide x spray inoculation *P. nodorum*	-0.773	
	Sulphate + x without fungicide x non inoculation *P. nodorum*	-0.641	
	Sulphate + x without fungicide x seed inoculation *P. nodorum*	-0.945	
	Sulphate + x without fungicide x spray inoculation *P. nodorum*	-0.672	
	Sulphate - x with fungicide x non inoculation *P. nodorum*	0.126	
	Sulphate - x with fungicide x seed inoculation *P. nodorum*	-0.722	
	Sulphate - x with fungicide x spray inoculation *P. nodorum*	-0.324	
	Sulphate - x without fungicide x non inoculation *P. nodorum*	0.142	
	Sulphate - x without fungicide x seed inoculation *P. nodorum*	-0.511	
	Sulphate - x without fungicide x spray inoculation *P. nodorum*	-0.718	

A 20. Table of mean for the samples at growth stage 80, liquidised with phosphate buffer and quantify by qPCR assay using SYBR-green chemical reagent.

variation	variable	mean	L.S.D
Fertilizer	Sulphate +	1.55	0.2
	Sulphate -	1.64	
Fungicide	with fungicide	1.61	0.36
	without fungicide	1.59	
P. nodorum inoculation	no inoculation	1.62	0.44
	seed inoculation	1.53	
	spray inoculation	1.63	
Fertilizer x Fungicide	Sulphate + x with fungicide	1.59	0.39
	Sulphate + x without Fungicide	1.52	
	Sulphate - x with fungicide	1.62	
	Sulphate - x without fungicide	1.66	
Fertilizer x *P. nodorum* inoculation	Sulphate + x non inoculation *P. nodorum*	1.65	0.48
	Sulphate + x seed inoculation *P. nodorum*	1.50	
	Sulpate + x spray inoculation *P. nodorum*	1.50	
	Sulphate - x non inoculation *P. nodorum*	1.60	
	Sulphate - x seed inoculation *P. nodorum*	1.56	
	Sulphate - x spray inoculation *P. nodorum*	1.76	
Fungicide x *P. nodorum* inoculation	with fungicide x non *P. nodorum*	1.63	0.62
	with fungicide x seed inoculation *P. nodorum*	1.28	
	with fungicide x spray inoculation *P. nodorum*	1.91	
	without fungicide x non *P. nodorum*	1.62	
	without fungicide x seed inoculation *P. nodorum*	1.79	
	without fungicide x spray inoculation *P. nodorum*	1.35	

Fertilizer x fungicide x *P. nodorum* inoculation	Sulphate + x with fungicide x non inoculation *P. nodorum*	1.64	
	Sulphate + x with fungicide x seed inoculation *P. nodorum*	1.18	
	Sulphate + x with fungicide x spray inoculation *P. nodorum*	1.94	
	Sulphate + x without fungicide x non inoculation *P. nodorum*	1.66	
	Sulphate + x without fungicide x seed inoculation *P. nodorum*	1.83	
	Sulphate + x without fungicide x spray inoculation *P. nodorum*	1.06	0.68
	Sulphate - x with fungicide x non inoculation *P. nodorum*	1.61	
	Sulphate - x with fungicide x seed inoculation *P. nodorum*	1.38	
	Sulphate - x with fungicide x spray inoculation *P. nodorum*	1.88	
	Sulphate - x without fungicide x non inoculation *P. nodorum*	1.59	
	Sulphate - x without fungicide x seed inoculation *P. nodorum*	1.74	
	Sulphate - x without fungicide x spray inoculation *P. nodorum*	1.64	

A 21. Table of log 10 mean for the samples at growth stage 31, dried oven sampled that quantify by qPCR using SYBR-green chemical reagent.

variation	variable	Mean	L.S.D
Fertilizer	Sulphate +	1.85	0.13
	Sulphate -	1.43	
Fungicide	with fungicide	1.46	0.16
	without fungicide	1.56	
P. nodorum inoculation	no inoculation	1.44	0.2
	seed inoculation	1.63	
	spray inoculation	1.46	
Fertilizer x Fungicide	Sulphate + x with fungicide	1.56	0.2
	Sulphate + x without Fungicide	1.6	
	Sulphate - x with fungicide	1.35	
	Sulphate - x without fungicide	1.51	
Fertilizer x *P. nodorum* inoculation	Sulphate + x non inoculation *P. nodorum*	1.47	0.24
	Sulphate + x seed inoculation *P. nodorum*	1.74	
	Sulpate + x spray inoculation *P. nodorum*	1.54	
	Sulphate - x non inoculation *P. nodorum*	1.41	
	Sulphate - x seed inoculation *P. nodorum*	1.52	
	Sulphate - x spray inoculation *P. nodorum*	1.38	
Fungicide x *P. nodorum* inoculation	with fungicide x non *P. nodorum*	1.41	0.29
	with fungicide x seed inoculation *P. nodorum*	1.62	
	with fungicide x spray inoculation *P. nodorum*	1.34	
	without fungicide x non *P. nodorum*	1.47	
	without fungicide x seed inoculation *P. nodorum*	1.64	
	without fungicide x spray inoculation *P. nodorum*	1.58	

Fertilizer x fungicide x *P. nodorum* inoculation	Sulphate + x with fungicide x non inoculation *P. nodorum*	1.51	
	Sulphate + x with fungicide x seed inoculation *P. nodorum*	1.65	
	Sulphate + x with fungicide x spray inoculation *P. nodorum*	1.53	
	Sulphate + x without fungicide x non inoculation *P. nodorum*	1.44	
	Sulphate + x without fungicide x seed inoculation *P. nodorum*	1.83	
	Sulphate + x without fungicide x spray inoculation *P. nodorum*	1.55	0.34
	Sulphate - x with fungicide x non inoculation *P. nodorum*	1.31	
	Sulphate - x with fungicide x seed inoculation *P. nodorum*	1.60	
	Sulphate - x with fungicide x spray inoculation *P. nodorum*	1.15	
	Sulphate - x without fungicide x non inoculation *P. nodorum*	1.50	
	Sulphate - x without fungicide x seed inoculation *P. nodorum*	1.44	
	Sulphate - x without fungicide x spray inoculation *P. nodorum*	1.60	

A 22. Table of log 10 mean for the samples at growth stage 31, dried oven sampled that quantify by qPCR using Taq-man chemical reagent.

variation	variable	Mean	L.S.D
Fertilizer	Sulphate +	2.30	0.17
	Sulphate -	2.13	
Fungicide	with fungicide	2.20	0.27
	without fungicide	2.25	
P. nodorum inoculation	no inoculation	2.06	0.33
	seed inoculation	2.39	
	spray inoculation	2.19	
Fertilizer x Fungicide	Sulphate + x with fungicide	2.30	0.3
	Sulphate + x without Fungicide	2.30	
	Sulphate - x with fungicide	2.04	
	Sulphate - x without fungicide	2.21	
Fertilizer x P. nodorum inoculation	Sulphate + x non inoculation P. nodorum	2.07	0.37
	Sulphate + x seed inoculation P. nodorum	2.50	
	Sulpate + x spray inoculation P. nodorum	2.33	
	Sulphate - x non inoculation P. nodorum	2.05	
	Sulphate - x seed inoculation P. nodorum	2.29	
	Sulphate - x spray inoculation P. nodorum	2.04	
Fungicide x P. nodorum inoculation	with fungicide x non P. nodorum	2.06	0.47
	with fungicide x seed inoculation P. nodorum	2.35	
	with fungicide x spray inoculation P. nodorum	2.10	
	without fungicide x non P. nodorum	2.05	
	without fungicide x seed inoculation P. nodorum	2.44	
	without fungicide x spray inoculation P. nodorum	2.27	

Fertilizer x fungicide x *P. nodorum* inoculation	Sulphate + x with fungicide x non inoculation *P. nodorum*	2.11	0.53
	Sulphate + x with fungicide x seed inoculation *P. nodorum*	2.35	
	Sulphate + x with fungicide x spray inoculation *P. nodorum*	2.45	
	Sulphate + x without fungicide x non inoculation *P. nodorum*	2.02	
	Sulphate + x without fungicide x seed inoculation *P. nodorum*	2.65	
	Sulphate + x without fungicide x spray inoculation *P. nodorum*	2.22	
	Sulphate - x with fungicide x non inoculation *P. nodorum*	2.01	
	Sulphate - x with fungicide x seed inoculation *P. nodorum*	2.35	
	Sulphate - x with fungicide x spray inoculation *P. nodorum*	1.75	
	Sulphate - x without fungicide x non inoculation *P. nodorum*	2.08	
	Sulphate - x without fungicide x seed inoculation *P. nodorum*	2.23	
	Sulphate - x without fungicide x spray inoculation *P. nodorum*	2.33	