Wheat Powdery Mildew in Central China:

Pathogen Population Structure and Host Resistance

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Propositions

1. Sexual reproduction increases the genetic diversity of pathogen populations by disrupting associations between alleles and phenotypes. *This thesis.*

2. When avirulence genes are combined in the sexual stage, the frequency of virulent phenotypes in the pathogen population will fall. *This thesis.*

3. The term "relatively low level of disease" is somewhat ambiguous. The vertical distance from a variety data point to the fitted regression line of severity against virulence frequencies makes a "relative level" an "absolute level". *This thesis.*

4. Intensive cultivation of successive crops to reduce the population of wheat volunteers and therefore to reduce severity of mildew is possible. *This thesis.*

5. When a resistance gene is withdrawn from commercial varieties, the frequency of the corresponding virulence may either rise or fall. *James Brown et. al., In The gene-for-gene relationship in plant –parasite interaction. 119-138.*

6. Co-evolution occurs as much between control strategies and the pathogens as between plant host and pathogens. *Martin Wolfe, Ann. Rev. Phytopathol. 32: 89-113.*

7. Regional resistance gene deployment strategies that focus on virulence present in distant populations as well as in local populations are more likely to be successful if there is extensive gene flow between populations separated by long distances. *J. M. Boeger et al, Phytopathology 83: 1148-1154.*

8. Durable resistance is a descriptive term and does not provide any explanation of its underlying causes, therefore, no experimental method can be as effective in detecting durable resistance as growing varieties in commercial agriculture. *By Roy. Johnson, Ann. Rev. Phytopathol.22: 309-329.*

9. Mostly, the highlands have a lesser economy and the nether-lands a greater economy.

10. The sum of study and inquiry is knowledge. Confucius, an ancient Chinese philosopher.

11. The way that a lost person wants to find is under his nose. A Chinese proverb.

12. Modesty is an attitude indicating that one wants to learn more; pride is an expression of confidence that one can learn more.

Propositions attached to the thesis: **Wheat Powdery Mildew in Central China: Pathogen Population Structure and Host Resistance** by Yu Dazhao, defended on 10 of May 2000.

Preface

The work presented in this thesis would not have been accomplished without the support, encouragement and help of many people.

First of all, I am so grateful to my supervisor, formerly professor of Wageningen University and Research Centre, the Netherlands, and now at Wye College of University of London, the United Kingdom, Dr Michael J. Jeger. Special thanks go to my co-promoter, phytopathologist at John Innes Centre, Norwich, the United Kingdom, Dr James K. M. Brown. Mike and James, the winter and the winter wind coming from the Little Bælt to Roskilde, Denmark in 1995 could be never forgotten. You went there from Wageningen and from Norwich, England and I from Zurich, Switzerland. I submitted my proposal to you with little confidence. After you read my proposal and had a brief discussion with me about it, I felt the strong wind was not a winter wind, but a spring breeze that makes the wheat seedlings green on a vast piece of Chinese farming land. Mike, I would not have had full confidence without your visit from Europe to China in the autumn of 1997. I gained great encouragement from your visit although it was a very short one. My stay in Wye College was so wonderful when you spent a lot of time revising my papers although you had just settled into your new chair and had so many things around your new desk at Wye. James, I would even now have a very superficial understanding of population biology concepts of wheat mildew without your frequently excellent guidance through e-mail. You told me that my plan was too ambitious but you always encouraged my ambition. When I was in your office you always put a note saying "do not disturb" on the door, which moved me so much. I would extend my many thanks to Diane. Diane, it was you who had all your housework done allowing James to spend evenings and weekends revising my papers, which made it possible for me to complete this thesis in such a limited time. This thesis would never have been completed without my two-month stay in Norwich.

I am very much indebted to Dr Gert H. J. Kema, phytopathologist at the Research Institute for Plant Protection (IPO-DLO), the Netherlands. Gert, as a friend and colleague, it was all your initiative to invite me as a visiting fellow to your group in 1992. This invitation paved the way for me. It could never be forgotten how we exulted over seeing some difference of *Septoria tritici* mycelium in the tissue of wheat leaves from stage to stage. Although Septoria leaf blotch is another disaster of wheat production which is different from powdery mildew, I gained a lot from the experience and the general knowledge in plant pathology, which helped me to approach some aspects of powdery mildew. It could be never forgotten how we chatted over so many things that happened in the past and nowadays on weekend evenings. Many thanks go to Hanna and children for their hospitality.

Many thanks go to Els for her wonderful help she offered to me when I worked

in IPO and when I did edition of this thesis. I am very grateful to Ali and Corrie for their help.

It was such a wonderful opportunity that I could meet Professor Martin Wolfe, an international authority in the field of cereal powdery mildew, and Dr Eckhard Limpert at the foot of the Alps. Eckhard, thanks for inviting me to ETH, Zurich, Switzerland. My stay in Switzerland was a memorable one. The landscape of every city backed by a mountain and half surrounded by a lake is the most harmonious combination of human beings and nature. The peaks of the mountains in Interlaken district are covered with snow all the year round, but hundreds of thousands of fish leap with joy in the lakes. The landscape attracted me so much, but the most attractive aspect was the research work done in your group. I learnt a lot from you and other experts in ETH. Thanks, Eckhard and Christina, thank you for your hospitalities you gave to me when I stayed in your home, a home away from home.

I would like to express my heartfelt thanks to Professor Martin Wolfe. Martin, it was all your arrangement that I could fulfil my study under Mike and James' supervision. Your arrangement of my travelling to Denmark had paved a smooth way for me to go from Wageningen to Wageningen. Thanks for the examination you gave to me for the re-registration as a PhD student at Wageningen. I cannot forget your humour when I asked about the result of my examination: " I am not a Chinese dragon". It is an honour to have met you and studied under your supervision at the first stage of my Ph.D. programme.

This thesis would not have been possible without the solid support I got from my friends. Particular thanks go to Professor Dr Zhihua Ye, the Chief of the Administrative Bureau of Science and Technology, Chinese Academy of Agricultural Sciences (CAAS), Kaizhong Zhou, the Chief of the Department of Science and Technology, State Ministry of Agriculture of China and Professor Yazhong Cao, Director of the Plant Protection Institute, CAAS, for the arrangement of the key research project in the Ninth Five Year Plan in which I participate. Zhihua and Kaizhong, I understand what your support means to me. I am very grateful to the colleagues in the State Ministry of Agriculture of China, Zhengyun Shao and Wancai Liu for their excellent collaboration for many years which leads me to have more understanding of wheat mildew, especially in its epidemics.

I owe a great deal to my colleagues in my home institute. Professor Wang Shaonan took over all of my administrative responsibilities when I was away from my home institute twice, even when I was in the institute but busy with my research work, he also made nothing of the hardship of bearing the burden I should bear. Professor Gu Shouli and Mrs Zhao Yongyu shared many of my duties. I am so grateful to my former professor Gan Qifang who always gives me encouragement and help. The help offered to my family, when I am away from home, by all colleagues in the administrative office of my home institute headed by Mr Li Lequan,

moved me so much.

I cannot find words to mention the deeds my students, colleagues and assistants, Yang Xiaojun and Yang Lijun, did for the research. Xiaojun, although the mountains in central China are not so imposing and marvelous as the Alps, and the rivers there are not so romantic as the Rhine, they belong to our homeland. Your work made people outside know the mountains and rivers in our homeland. I believe that you will make nothing of the hardships again to go there on foot for four to five days in the mountain chain to track the pathway of wheat powdery mildew with your isolate collecting boxes. You will again stay in a farmer's home at midnight to plan in the moonlight where you will go tomorrow. You always understand that you are doing good for the poor farmers in our homeland. Lijun, scientific research mixes depression and excitement, which are moulding my temperament as well as yours. Thanks for growing so many wheat seedlings, cutting so many leaf segments and recording so many sets of data. Two Yangs, thanks for your valuable time you spent with me in the laboratory so many weekends.

I would like to thank my contemporary in my former university, the president and professor of Hubei Academy of Agricultural Sciences, Dr Liu Dinfu for his invaluable support, help and understanding. Dinfu, first you set an example for me, and then gave strong backing to me. What you have done for me is an impetus making me go forward.

It is a great pleasure to have the opportunity to extend my special thanks to Mr Hu Weihua for his help in many ways.

I would like to express my gratitude with deep feelings to my parents for teaching me the values of life, how to lead and enjoy a wonderful life and encouraging me to pursue higher education. Particular thanks go to my brothers and sisters, nieces and nephews for their help to my family during I am away from home.

Last but not least, I am so very grateful to my wife Jiuzhen and my daughter Li. Jiuzhen, thank you for making tea for me when I was sleepy at a busy midnight, thanks for making soup for me when I felt thirsty at a hot midday. Thanks, Li, thank you for understanding of me when I could not take care of you due to my obsession for work, thank you for doing your utmost in your study so that I felt relieved.

To the people I love,

Dazhao

Wageningen, the Netherlands, April 2000

Abstract

Wheat powdery mildew, causal agent *Erysiphe graminis* f. sp. *tritici*, has been a serious disease in central China since the late 1970s.

The wheat growing area in central China can be divided into three zones defined by altitude. Over 800m altitude, *E. graminis* f. sp. *tritici* can oversummer as the sexual stage. Cleistothecia are produced which release ascospores infecting wheat volunteers and early emerging seedlings in autumn. At 500-800m altitudes, ascospores transmit the disease across seasons only by infecting volunteers. In the lowlands below 500m, neither cleistothecia nor wheat volunteers survive the summer, and the disease is re-established each year through immigration of the pathogen from the highlands and external sources. A set of differential cultivars was selected from local varieties important in commercial production and monitoring with this differential set revealed regional differences in the pathogen population. Although there were differences between the mid and high altitude zones in oversummering of the pathogen, the population structures were very similar and can therefore be treated as a single epidemiological unit.

Five known *Pm* genes, *Pm*1 *Pm*3b, *Pm*3c, *Pm*5 and *Pm*8, were postulated to be present in the wheat varieties grown along the Yangtze River valley, but in each case, they were associated with unknown resistance factors which differed across varieties. Most varieties tested carried resistance which cannot be identified by the reference differential set used in Europe and North America. *Pm*3d, *Pm*4b and *Pm* 1+2+9 had less than a 1% frequency of matching virulences in the pathogen population in central China. This indicates that these genes have value for wheat resistance breeding in central China. Several local varieties also had a very low frequency of matching virulence in the pathogen population and would also be valuable in resistance breeding.

Wheat varieties grown in central China and some other regions were assessed for partial resistance in field trials and laboratory studies. Five varieties, Hx8541, E28547, Chun1066, Ze88pin6 and Lin5064, showed compatible interactions with a large proportion of the pathogen population, but had low disease severities. Component analysis showed that low germination rate of conidia of the pathogen, low formation of appressorium and haustorium, and small haustorium size contributed to this resistance. The most common and important components were longer latent period, low colony formation and low sporulation. Calculation of the vertical distance from a cultivar data point to a fitted regression line of disease severity against matching virulence frequency was used as a novel method for

assessing partial resistance. The method provided a clear evaluation of partial resistance.

Variation of responses to triadimefon in the pathogen population was high, and covered a 370-fold range of estimated ED50s. This indicates a fall in the performance of triadimefon in controlling wheat powdery mildew in central China.

Key words: Wheat powdery mildew, *Erysiphe graminis* f. sp. *tritici*, oversummering, population structure, population migration, differential set, Pm genes, partial resistance, triadimefon, variation of responses to fungicide

Contents

General Introduction

Wheat production in China

Archaeological evidence shows that wheat cultivation in China can be traced back to 5000 BC (Anon. 1958). By 600 BC, wheat had already been widely grown along the middle and lower reaches of the Yellow River. With the immigration of new groups of ancient people, its cultivation area further expanded. About 120 BC, wheat was discriminated into spring-sown wheat and autumn-sown wheat, which accelerated the spread of wheat cultivation in ancient China. Spring-sown wheat grew north of the Yellow River and autumn-sown wheat south of the Yellow River. In northern China, wheat was grown as a staple crop, but in the south, it was cultivated for two purposes. One was to provide food for people from late spring to early summer when the last rice had run out and new rice was not available, and the other was to make up for occasional losses of other food crops caused by flooding or drought (Jin, 1996).

Nowadays, wheat is grown throughout the country. It is normally grown from 20° to 53° N latitude and from 75° to 135° E longitude, and from 154m below sea level in Turfan Basin, Xingjiang, to 4040m above sea level in Jiangzi prefecture, Tibet. It occupies a cultivated area of about 30.8 million hectares, accounting for 27% of the total food production area and 22% of total food production in the whole country (Anon., 1990). The species grown is mainly common wheat (*Triticum aestivum*), accounting for more than 99% of wheat growing area. Other species, such as *T. durum*, *T. compactum*, *T. turgidum* *T. turanicum* and *T. polonicum* account for less than 1% (Jin and Wu, 1959).

Wheat production in China has passed through four stages of development in the last half century (Fig 1). It took 15 years (1949-64) to increase total output from less than 20 million metric tons to 20 million metric tons, and 7 years (1964-71) for it to increase to 30 million metric tons. Then there was a very sharp increase from 30 million metric tons to 80 million metric tons (from 1971-1983). It took 6 more years, 1983-1989, to increase the output from 80 million metric tons to 90 million metric tons. Now the total yield has reached a plateau of between 90 and 100 million metric tons per year (Jin, 1996).

The low yield of wheat half a century ago can be attributed to several reasons. 1. The land for wheat growing was of low fertility and farmers had no fertilizer to apply to the soil. 2. The farmers grew wheat in an inappropriate way because of a lack of information on cultivation techniques. 3. Wheat in the northern part of China

frequently suffered from drought and in the south from waterlogging in spring. 4. The tall stature of varieties often caused lodging during ripening. 5. There were no effective methods of controlling disease and insect pests.
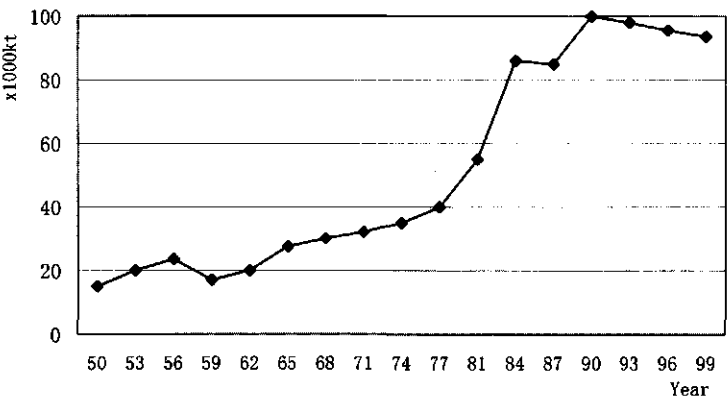
Figure 1. Development of the total output of wheat.

Both the expansion of the wheat growing acreage and the increase of the yield per unit area played very important roles in increasing the total annual yield of wheat in China. But the proportions contributed by the two factors to the increase in yield differed from decade to decade. Increasing yield per unit area contributed more to the total yield than increasing the wheat growing acreage, with advancement of wheat cultivation techniques (Fig. 2; Jin, 1996.).
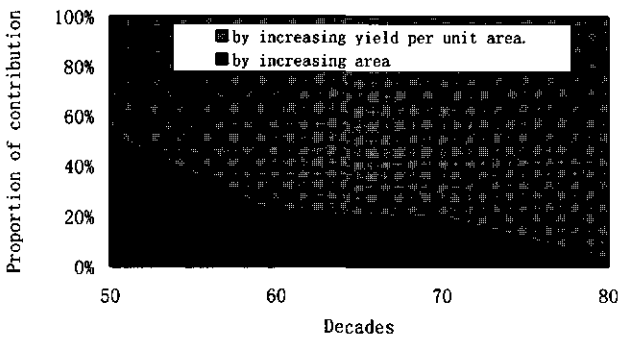
Figure 2. Contributions made by increasing wheat acreage and yield per unit area to the yearly total yield in China from the 1950s to the 1980s.

Although China now holds the first position in wheat production worldwide in terms of annual total output, it is also the biggest importer of wheat in the world. China imports 10 to 15 million tonnes of wheat annually accounting for 10-15% of the total international trade in wheat, and is much dependent on the global wheat market. So wheat production was yesterday, is today, and will continue to be an important feature of Chinese agriculture as it has such a large population.

Wheat production in Hubei province

Hubei province is located in central China between longitudes 108.2° to 116.2° E and latitudes 29° to 33.3° N. Of about 180,000 square kilometers of land area, 70% is hilly and mountainous; the lower, plain area is less than 30% of the area. The province slopes down from west to east with a highest altitude of 3105 m to a lowest altitude of 50 m above sea level. The Yangtze River, with 1000 distributaries, flows through the whole province. Nearly 60 million people in the province share some 7 million hectares of arable land.

The climate in Hubei is characterized by the subtropical humid monsoon. There are four distinct seasons during the year. Cold air moves south in the winter bringing heavy snow. The subtropical high moving north in the summer brings the so-called "plum" rains, the name given to the early monsoon rains that fall when plums are ripening, which lasts for about one month in most years. In spring, the wind moving to the East China Sea from the Bay of Bengal is obstructed by the Himalayan ranges, so the weather alternates between sunny and rainy days. In autumn, the sky is usually clear. Average annual rainfall in the northwest is about 800-1000mm, and about 1500mm per year elsewhere. With a maximum temperature of 42°C and a minimum temperature of −17.3°C, the annual mean temperature is 13-18°C, ranging from 1-6°C in January to 24-30°C in July. The frostless season is 220 to 300 days per year. The southeast part of the province has 1100 to 1600 hours' sunshine and the rest has 1700 to 2000 hours per year.

Wheat has been grown for more than 2000 years in Hubei. As in any other region, farming in Hubei has been largely moulded by geography and climate. Because of adequate rainfall, fertile land and other natural conditions, enabling diverse crops to be grown in the province, Hubei has historically been called the "food barn" of China. Wheat now holds the position of the second main food crop after rice in Hubei. The province grows 1.6 million hectares of wheat annually, which is rotated with cotton, rice, maize, potato, peanut and soybean.

The wheat growing area can be divided into three categories. 1. The areas below

500m, with sowing times from late September to late October and harvest times from late May to mid June. 2. The area with an altitude of 500-800m, with sowing times from mid-September to mid-October, and harvesting times from mid-June to early July. 3. The area between 800 and 1400m, with sowing times from late August to early September and harvest times from early to late July. Owing to the different environmental conditions of these regions, several wheat cultivars are used in Hubei. The main cultivars may cover 20% to 50% of the total wheat cropping area while others only cover 1 to 5%. The yield of wheat in Hubei province fluctuates greatly from year to year because of weather conditions and the occurrence of disease epidemics. Average yield is some 2.1 t/ha, with a highest yield of about 4.5 t/ha in the northern part of the province, because of more hours of sunshine and a longer growing season.

Wheat powdery mildew in China with emphasis on Hubei province

General situation

Wheat powdery mildew, caused by the obligate biotrophic fungus *Erysiphe graminis* f. sp. *tritici*, was a minor disease in China until relatively recently. It was only regarded as an economically important disease in the Southwest Plateau and the coastal zone of Shandong Peninsula where occasional yield losses were recorded in wheat a quarter of a century ago (Liu, 1989; Tao *et al.*, 1982).

With changes in wheat cultivation methods, such as increasing densities of plant population, nitrogen fertilizer inputs, shifts in cultivars from local landraces to dwarf and semi-dwarf yield-improved cultivars and the expansion of the irrigated area in northern areas, wheat mildew has now become a major disease in terms of both severity and area of occurrence. This has been marked by two major phases of mildew expansion (Fig. 3; Liu and Shao, 1994). The first was during the late 1970s and early 1980s and occurred over the Yangtze River basin, including Hubei, Anhui, Jiangsu and Zhejiang provinces. The second was in the mid-1980s when the epidemic area expanded from south to north. In 1990 and 1991, the epidemic area covered 24 provinces with 12 million hectares attacked and 3 million metric tons of yield lost per year, which caused a crisis in Chinese wheat production.

Hubei was in the region affected by the first phase of wheat powdery mildew expansion and continues to be most heavily attacked. During the last two decades, the affected area was about 0.7 million hectares per year with an average annual yield loss of approximately 210 thousand tones.

I: Original epidemic zone

II: Expanded epidemic zone since the late 1970s
(Shaded area is Hubei Province)

III: Further expanded epidemic zone in 1980s
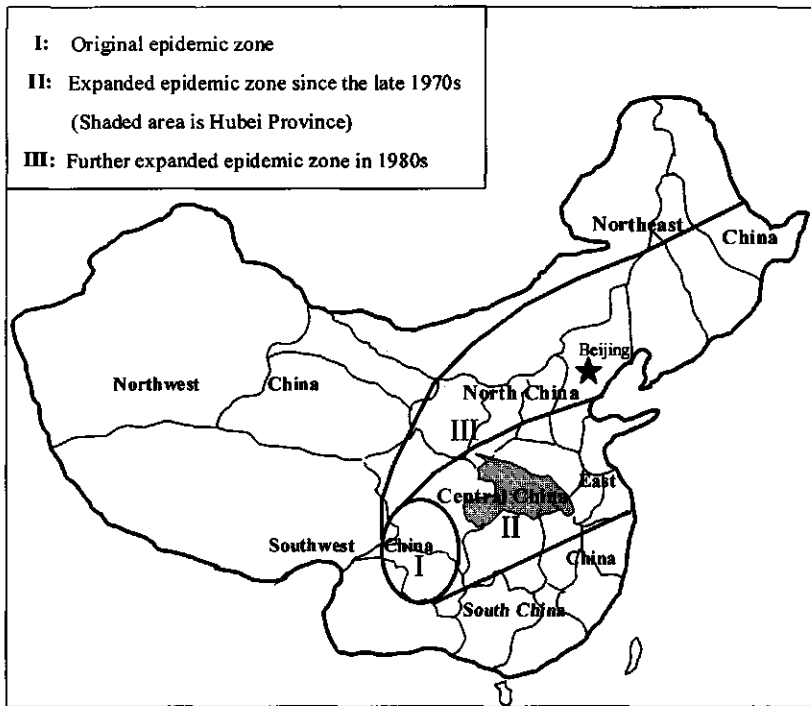
Figure 3. Wheat powdery mildew epidemic area in China, showing two major phases of expansion.

Epidemics of wheat powdery mildew

Wheat powdery mildew is a wind-dispersed disease. Knowledge of the source and amount of initial inoculum is fundamental to an understanding of epidemics. Various authors in China have discussed these aspects to some extent (Liu, 1989). Cleistothecia, the sexual fruiting bodies of the pathogen, are formed each year on senescent wheat leaves during ripening. Most authors report that cleistothecia cannot survive the whole summer until autumn-sown seedlings appear, and therefore play no role in epidemics of wheat mildew (Wu, 1984; Wang, 1984; Liu, 1984; Zhang, 1986). Other authors (Du, 1983; He, 1985), however, report that cleistothecia dispersed with wheat seeds form the main initial inoculum in Zhenjiang prefecture, Jiangsu province, and that cleistothecia can survive the whole summer in some regions. Conidia, the asexual spores of the pathogen, are widely thought to be the initial inoculum on autumn-sown seedlings. Wu (1984) reported that conidia in Guiyang region, at a latitude of 26.5° N, oversummered on volunteers in the

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mountainous area at an altitude of 1100m. Between latitudes 35 to 36°, conidia survived summers at altitudes of 530m (Lei, 1982). In Zhibo prefecture (latitude 37°) of Shandong province, they survived at an altitude of 200m (Zhang, 1983). In the region of Beijing, the pathogen oversummered as mycelium on wheat seedlings (Liu, 1989). Because of the evidence that mildew grew on volunteers in the summer, they asserted that this region was an isolated epidemic zone. In Liaolin province in northeast China, above latitude 41° N, only spring-sown wheat is grown and no host plants are available for mildew to survive the winter. Yang *et al.* (1990) reported that mildew conidiospores were transported to the spring wheat regions by wind from the Shandong peninsula.

Previous studies showed that there were two kinds of epidemic patterns in Hubei province (Yu and Shi, 1991). In the northwest part of the province, mildew epidemics start in mid-November, but no disease is found between late December and the end of January; the epidemics start again in mid-February. In the lower hills and lower plains, epidemics start in late February or early March. In the high areas, sparse or dense wheat volunteers with mildew can be found in summer. These findings led to the suggestion that mildew survived summers on volunteers in the highlands and supplied wheat in lowlands with initial inoculum in the next spring. Cleistothecia had nothing to do with mildew epidemics (Shi, 1992). However, several questions remain. How do conidia bridge the period from adult wheat plants in the field to the volunteers that emerge subsequently? Do cleistothecia in both the highlands and lowlands survive the summer? Is the entire wheat growing area in Hubei an isolated epidemiological zone or are pathogen populations from outside the region also involved?

Answers to these questions are imperative to the understanding of population variation and to decisions about control strategies.

Structure and evolution of the pathogen population

Since the late 1970s, several research groups have conducted research on variation in wheat mildew pathogen in China (Table 1). Wu (1983b) detected 16 physiological races using a differential set of 8 cultivars in Guizhou province. At the same time and in the same province, Li (1983) detected 10 physiological races using a differential set of 7 cultivars. Zhang (1983) detected 23 races in Sichuan province with another differential set composed of 8 cultivars. Shi *et al.* (1987) identified 32 races in wheat mildew epidemic areas in China with nine cultivars as a differential set and Wang (1988) identified 25 races with the same differential set in Henan

province. Some of the cultivars in the differential sets which these authors used in their studies were the same and others were not. Their results were not strictly comparable so it was not easy to ascertain relationships between the pathogen populations. Most of the cultivars in the differential sets were highly resistant cultivars which are widely used as differentials in Europe and North America, and little is known about their relation to landraces in China or to Chinese commercial varieties (Wang *et al.*, 1992). For instance, Xiaobaidongmai, a landrace from Xinjiang Autonomous District, carries a resistance gene which is unidentified (Shi *et al.*, 1987; Xia *et al.*, 1995), but was included in the differential set used by Shi *et al* (1987) and Wang (1988). From the late 1970s until the present, the frequency of virulence to this cultivar in all populations monitored has never changed (Xiang, 1994). The resistance of many commercial cultivars, however, had broken down in the meantime. This indicated that Xiaobaidongmai had no discriminative ability because it had no relation to cultivars in commercial production or to germplasm in wheat breeding, and itself never entered wheat breeding programmes.

Table 1. Differential sets previously used to investigate mildew population in China

Author	Differential set	Sample size	Number of races	Sample Region
Wu (1983)	Funo, Kenaguil, Yuma, Khapli/8cc, Guinong17, Lovrin10, Baimian1, Baimian3	73	16	Guizhou
Li (1983)	Kenaguil, Funo, Khapli/8cc, Baimian3, Kavkaz, Maris Huntsman, Lovrin10	64	10	Guizhou
Zhang (1983)	Funo, Kavkaz, Lovrin13, Baimian3, Fan6, Timgalen/8cc, Maris Huntsman, Mianyang	88	23	Sichuan
Shi et al. (1987)	Funo, Ulka/8cc, Era, Kavkaz, CI12632, Maris Huntsman, Kenaguil, Baimian3, Xiaobaidongmai	249	32	Whole of China
Wang (1988)	Funo, Ulka/8cc, Era, Kavkaz, CI12632, Maris Huntsman, Kenaguil, Baimian3, Xiaobaidongmai	126	25	Henan

Although Hubei was the region affected by the first expansion of mildew, no survey of pathogen population structure and evolution has been conducted. Three batches of improved wheat varieties have been released in Hubei since the late 1970s. Some

were initially resistant to mildew and some were not. The resistance of newly released varieties was more or less rapidly overcome by the pathogen, with the interaction presumably following a typical gene-for-gene system.

Resistance breeding

Guizhou Agricultural College was the first institution to carry out resistance breeding to wheat powdery mildew in China because mildew has been a serious problem in Guizhou province. Several cultivars or lines, such as Dadongshan1 and 2 and their derivatives, showed a high level of resistance to mildew. Baimian3 is the most famous variety from Guizhou, and possesses a resistance gene located on the 2B chromosome different from the *Pm6* gene (Jin, 1996).

Other institutes have conducted wheat mildew resistance breeding from the early 1980s. Yang at Beijing Agricultural University introduced a set of resistant germplasm from University of Novi Sad, Yugoslavia, in 1978 and later wheat material from the International Winter Wheat Powdery Mildew Nursery (IWWPMN), USDA/ARS. He found line C39 (British) and 19 lines from the Sc series (US), Fr series (French) and Zg series (former Yugoslavia) resistant to mildew in China. These lines were very late maturing. He transferred resistance genes from this germplasm and obtained 26 advanced lines which showed good resistance to mildew (Yang, personal communications).

The Plant Protection Institute of the Chinese Academy of Agricultural Sciences identified many Chinese local landraces and found ten varieties, such as Xiaobaidongmai, highly resistant to mildew (Shi, *et al.*, 1987). None of these varieties or lines can be used directly in commercial production because of poor agronomic characters.

Traditionally, breeding for stem rust and yellow rust resistance was important mainly in northwest, northeast and north China respectively. In south China, especially along the Yangtze River reaches, wheat resistance breeding was mainly focused on wheat scab caused by *Gibberella zeae*. Powdery mildew resistance was not included as a target for breeding and no variety resistant to mildew was intentionally released for use on a large scale. *Pm8* is believed to be the most widely used resistance gene in China, but it was transferred from Lovrin varieties (from Romania) by chance as a consequence of breeding for resistance to yellow rust.

In central China, little is known about the resistance of cultivars to powdery mildew since no breeding for resistance to mildew has been carried out. As in other regions of China, newly developed cultivars were resistant to powdery mildew when

first released for field production. Within four to five years, their resistance was overcome by the change in the pathogen population. Cultivar E'an1 was a very clear example of this. It was highly resistant, even immune to mildew when first released in 1984 (Gan, 1985). Several years later, it was seriously affected by mildew, causing a substantial yield loss (Anon., 1990).

Although specific resistance is much easier to manipulate in breeding programmes, other types of resistance, including partial resistance, have proved durable to mildew in wheat (Shaner, 1973) and other cereals (Jones and Davies, 1985; Jones and Hayes, 1971). No research on durable resistance has been carried out in China so far.

Fungicide sensitivity in the pathogen population

Application of fungicides is an effective way of controlling mildew. Three decades ago, emulsion of carbendazim, a benzimidazole fungicide, or sulphur mixed with carbendazim, was often used to control mildew in areas where the disease occurred. These fungicides only have preventative properties. In the early 1980s, triadimefon, called Bayleton commercially, a systemic fungicide of the triazole family, was introduced into China for control of rusts and mildews in many crops. It was regarded as an ideal fungicide to control wheat mildew because it not only had preventative but also curative activity, and was easy to use as a seed treatment as well as a foliar spray.

Triazole fungicides have been widely used in Europe. Resistance of barley mildew to these compounds occurred in the United Kingdom in 1980, a few years after the introduction of triazole fungicides (Fletcher and Wolfe, 1981). Resistance of wheat mildew was first reported in 1986 (de Waard *et al.*, 1986). The frequency of resistance in the cereal mildew population in much of Europe increased rapidly after the resistance appeared (Limpert, 1987). At the time when triadimefon was first put into use in China, good control could be achieved throughout the cropping season by a single application at the booting stage with an active ingredient dose of 120g per hectare (Ge, 1984; Li, 1983; Wu, 1983a). Now two or three applications with an active ingredient dose of 135 to 150g per hectare are needed to achieve good control. It appears that the effectiveness of triadimefon has reduced, possibly because resistance to the fungicide in the mildew pathogen population is increasing.

Knowledge of the dynamics of fungicide resistance in the pathogen population is critical for the management of wheat mildew. There will be no means of controlling the disease if fungicide resistance in the pathogen populations has

developed at a time when there are few resistant varieties for wheat production and if mildew epidemics occur.

The aim and outline of the thesis

Before rational control strategies can be developed, a fuller understanding of the evolutionary processes in the population of the wheat mildew pathogen in central China must be obtained. The major objectives of this study were to provide relevant knowledge about aspects of disease epidemiology, pathogen population structure and evolution and host resistance in central China.

E. graminis f. sp. *tritici* is an obligate biotroph. During epidemics, it reproduces asexually and the sexual stage only occurs once a year. The importance of the sexual stage in epidemics of the disease and variation of the pathotypes in the population has been studied in some parts of the world. Little is known about the role of the sexual stage in central China. This is studied and discussed in Chapter 2, particularly in relation to the survival of mildew during the summer and the spread of mildew from highland to lowland regions.

To track the dynamics of the cereal mildew pathogen populations, virulence is often used as a marker (Limpert *et al.*, 1990). It is essential to select a set of cultivars and lines which can give a clear profile of the population structure, for monitoring the pathogen population. This is one of the main objectives of Chapter 3.

The use of resistant cultivars is the most economical and effective method of controlling disease. The resistance background of the varieties grown in a certain area should be identified for the effective deployment of the resistance, while resistant factors in the germplasm used for wheat breeding should be identified. In Chapter 3, the resistance of cultivars and germplasm used in central China is described and discussed.

The structure, dynamics and dispersal of pathogens are very important aspects of pathogen population biology and are crucial in relation to decisions about methods of disease control. Using the set of cultivars established in Chapter 3, populations of *E. graminis* f. sp. *tritici* were monitored on a large scale over two cropping seasons in Hubei province. The main purposes of this study were to test the hypothesis that mildew in the highlands provides initial inoculum for wheat grown in the lowlands, to test whether or not populations of *E. graminis* f. sp. *tritici* in the two zones where the pathogen can oversummer are in fact one single population and to test whether or not the population in highlands is an isolated population. The results of this study are reported and discussed in Chapter 4.

Application of fungicide will remain an important method of controlling wheat mildew. Therefore, information on the level and dynamics of fungicide resistance is crucial to achieving better use of fungicides. In Chapter 5, responses of *E graminis* f. sp. *tritici* populations to triadimefon in central China is studied.

Specific resistance, following the gene-for-gene model, is easily broken down by the evolution of virulence in the pathogen population. The long time required for to breed resistant varieties makes it difficult to follow the rapid changes in pathogen populations. So varieties with durable resistance are highly desirable. In principle, there is no single, ideal experimental method to select durable resistance, but it generally appears that partial resistance is durable. Partial resistance of varieties grown in central China is identified in Chapter 6.

In order to have more information about the characters of the partial resistance identified in varieties in fields in Chapter 6 and to facilitate resistance breeding using them as durable resistance sources, the components of partial resistance, shown as inhibition of stages of fungal development on leaves of partially resistant varieties, are described in Chapter 7.

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The role of the sexual stage in the over-summering of *Erysiphe graminis* f. sp. *tritici* in central China

Summary

In central China, the length of the period between successive wheat crops varies at different altitudes. Powdery mildew occurs every year throughout the region. The pathogen produces cleistothecia at the time that wheat ripens and wheat volunteers with mildew are often found in highland areas. Several important questions are as yet unanswered: Can the sexual stage tide the fungus over summer to cause epidemics each year or do only conidiospores on volunteers initiate epidemics; what is the initial source of mildew on volunteers, and whether or not there are volunteers in lowland areas. To test whether or not cleistothecia of *Erysiphe graminis* f. sp. *tritici* on wheat straw kept outside for domestic fuel can survive the summer, straw bearing cleistothecia was collected in the plains of Hubei province in 1996. Half was kept indoors and half outdoors. Straw kept indoors induced mildew on seedlings sown in July in the highlands and in October in both the highlands and lowlands, whereas straw kept outdoors did not induce any disease in either area in October. To clarify whether or not wheat volunteers can survive the summer only in the highlands but not in the lowlands, studies of volunteer plants were conducted in summer from 1996 to 1998. Volunteers could survive the summer in the highlands, but no volunteer was found to survive the summer in the lowlands. To test the effects of the source of cleistothecia and of natural conditions on the release of ascospores, straw collected from both the highlands and the lowlands was kept at typical highland or lowland summer temperatures and subjected to different periods of wetness by spraying daily with water. More ascospores were released from cleistothecia that were kept dry for several weeks before being wetted, while the rate of ascospore release declined over time. Straw source and temperature did not effect ascospore release. It is proposed that the sexual stage bridges the seasonal gap between wheat crops, either indirectly, by ascospores infecting volunteers in autumn, or directly, by ascospores infecting seedlings emerging early in autumn in the highlands. It is also proposed that wheat mildew in the lowlands is re-established each year by dissemination of conidia from highland areas or from other sources in spring.

Keywords: powdery mildew, *Blumeria graminis*, cleistothecia, ascospores, wheat volunteer.

Introduction

Unlike some temperate parts of the world, where winter wheat and spring wheat are grown in the same region, all wheat in central China is sown in autumn. The sowing time is from late August in high mountainous areas to the end of October in the lowland hilly areas and plains. Wheat is harvested in early July at high altitudes and at the end of May in the lowlands. Two weeks after harvest, volunteers are often found in areas at altitudes higher than 500m above sea level (Fig. 1). It has been proposed that conidiospores of the powdery mildew fungus, *Erysiphe graminis* f. sp. *tritici*, on wheat crops at altitudes higher than 1000m, disseminate to infect volunteers in areas at altitudes of 500-800m. Then, when volunteers appear above 1000m altitude, conidiospores from volunteers at 500-800m altitude move up to infect volunteers in higher regions (Wang, 1984). This proposal led to a suggestion that wheat powdery mildew survives the summer as asexual colonies and conidia and that the sexual stage plays no role in epidemics of the disease (Liu, 1989). Wang (1984) and Liu (1989) also suggested that conidia disseminated from the highlands cause epidemics of powdery mildew on wheat in the plains and hills.
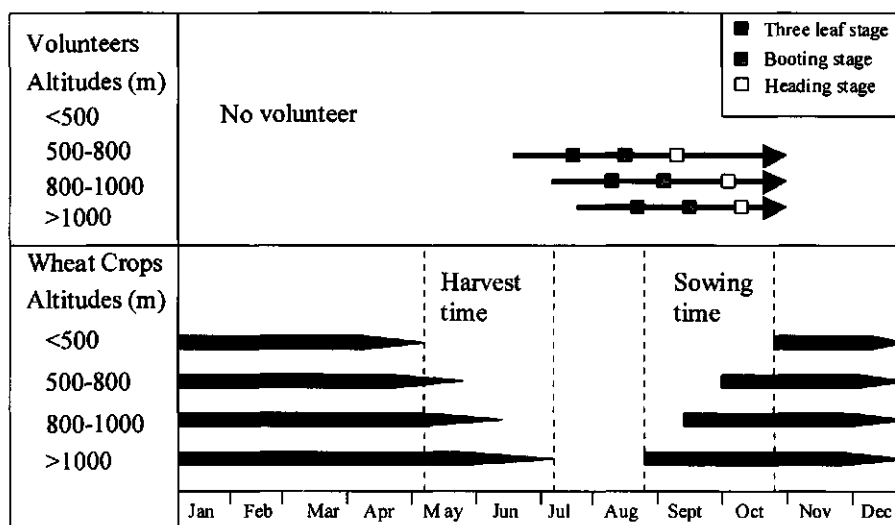
Figure 1. Sowing and harvest times of wheat crops and the growth stages of volunteers at different altitudes in central China.

This model of epidemiological cycle is not convincing, however, because it contradicts the true situation in several ways. Firstly, when volunteers emerge in the area at 500-800m altitude, wheat crops above 1000m have already begun to ripen, so

diseased upper leaves are senescing and mildew colonies have all entered the sexual phase. That makes it likely that there is, at most, negligible dissemination of conidiospores from crops in the highlands to volunteers at intermediate altitudes. Secondly, the temperature at the time that volunteers begin to appear is 25 to 28°C throughout Hubei, which is too high for conidia of *E. graminis* f. sp. *tritici* to infect volunteers. Thirdly, powdery mildew can be found on volunteers in areas above 1000m in late August, while volunteers in the region at 500-800m are free from mildew until mid-September, when the temperature falls.

When wheat plants begin to ripen, cleistothecia are formed within the mycelial mat on the surface of diseased leaves. This occurs in both the highlands and the lowlands. The objectives of the study reported here were to determine the role of the sexual stage as the causal agent of powdery mildew in autumn-sown wheat, to test the conditions in which ascospores may initiate epidemics and to discover if the life cycle of the pathogen is coordinated with that of the wheat crop and volunteers in central China.

Materials and Methods

Infection from cleistothecia in fields

This experiment investigated the potential of ascospores discharged from cleistothecia to infect volunteers in summer and seedlings in autumn in highland areas, and autumn seedlings in the lowlands, where no volunteers were thought to be available as hosts. At the time of the wheat harvest in 1996, straw and leaves with

Table 1. Summary of the design of field trials of cleistothecia as a source of inoculum

Treatment no.	Sowing time	Inoculum
Experiment in Yunyang County		
1	Mid-July	Straw with cleistothecia
2	Mid-July	No inoculation (control)
3	Mid-October	Straw with cleistothecia kept indoors
4	Mid-October	Straw with cleistothecia placed outdoors
5	Mid-October	No inoculation (control)
Experiment in Wuhan		
6	End of October	Straw with cleistothecia kept indoors
7	End of October	Straw with cleistothecia placed outdoors
8	End of October	No inoculation (control)

cleistothecia were collected from fields in Wuhan (lowlands). Half the straw and leaves collected were kept indoors at ambient temperature to protect them from rain, and half were put in a nylon net bag and placed outdoors in natural conditions. Trials were conducted in fields where there had been no wheat crop in the previous season in Yunyang County and Wuhan, representing high and low altitudes respectively. There were five treatments with three replicates in Yunyang and three treatments with three replicates in Wuhan. A universal susceptible cultivar Min169 (Chapter, 3 this thesis) was used as the host. The plot size was $2m^2$. After sowing seeds at a rate of 15 g m^{-2}, giving 350 seedlings m^{-2}, the plots were covered with four layers of cotton mosquito netting to prevent possible infection by airborne spores. When primary seedling leaves were fully expanded, plots were inoculated by spreading straw and leaves bearing cleistothecia on the ground. The treatments are summarised in Table 1. The percentage of diseased plants was scored and disease severity was assessed by a simple scoring system developed by CIMMYT (Anon. 1981), which has only six points, but which is easy to apply to a large number of plants (Table 2).

Table 2. Scale of assessment of powdery mildew severity (adopted from CIMMYT; Anon. 1981)

Score	Percentage cover of leaves by mildew [a]
0	No disease
1	< 5%
3	5 to 15%
5	15 to 25%
7	25 to 50%
9	> 50%

[a] Top three or four leaves were assessed

Survival of volunteers at different altitudes

Previous observations showed that no volunteers survived the summer in areas below 500m altitude, whereas in mountainous areas, volunteers did survive (unpublished data). The density of volunteers at different altitudes and the incidence of mildew on them was investigated. The study was conducted in mid-September in 1996, 1997 and 1998. In the mountainous area, four sites at different altitudes were surveyed (Fig. 2). At each site, thirty plots of $3m^2$ were studied at each of two altitudes. Twenty plots were in fields where wheat had been grown the previous year, ten on the sunny side of the mountain and ten on the shady side. Ten plots were
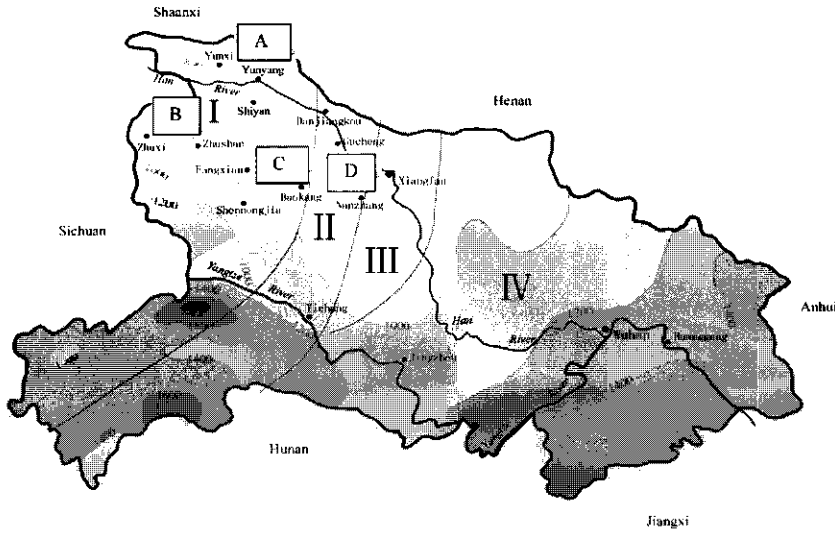
Figure 2. Map of Hubei province showing locations A, B, C and D where volunteer density was investigated through 1996 till 1998. Altitudes: **I**: 800-1200m above sea level, **II**: 500-800m, **III**: 500m, **IV**: <500 (hilly and plain area). The figures in the map are annual precipitation line.

established around farmers' threshing grounds or near farmers' houses where straw was kept for fuel. In the lowlands in 1996 and 1997, the investigation of volunteers was conducted around farmers' threshing grounds where many seeds were scattered.

In addition, to test whether or not volunteers could emerge in natural conditions, 20 plots, $2m^2$ in size, were sown with Min169 in Wuhan in June 1998. Ten plots were sown in fields, five of them around a threshing ground and five under trees to protect seedlings from direct sunshine to test whether or not volunteers could survive the summer in the lowland in relatively cool conditions. The number of volunteers per square meter and the incidence of mildew on volunteers were scored.

Conditions affecting ascospore discharge

This experiment tested whether or not cleistothecia from different altitudes had the same potential to eject viable ascospores and examined the effects of conditions at different altitudes on the release of ascospores. At harvest time in 1998, 2 kg of straw and leaves bearing cleistothecia were collected from each of two sources,

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Yunyang (highlands) and Wuhan (lowlands). As soon as the collections were made, the straw and leaves were spread out and kept at room temperature to dry. The average temperature from the second ten days of July to the first ten days of August is the highest for any period in the year in central China. The average temperature was 22°C in the highland and 28°C in the lowland, as obtained from the previous four years' data from local meteorological stations. These were therefore used as typical summer temperatures to store plant material bearing cleistothecia. The straw and leaves were divided into two parts. Half was kept at a temperature typical of the lowlands and the other half at a highland temperature in thermostat incubators.

The ascospore discharge experiment was performed following a design shown in Fig 3. The straw and leaves in each combination of straw source and temperature were divided into 6 batches as treatments. Batch (a) was kept wet by spraying daily with a mini-sprayer two weeks before ascospores were first sampled and kept wet continuously in the same way. In batch (b) wetness started two weeks later, batch (c) four weeks later, and so on. Batch (f) was kept dry for the whole period of the experiment. Four replicates of each batch were tested at each sampling time. The method of discharging ascospores described by Brown *et al.* (1992) was used with minor modifications. In each of four replicates, twenty large, black cleistothecia
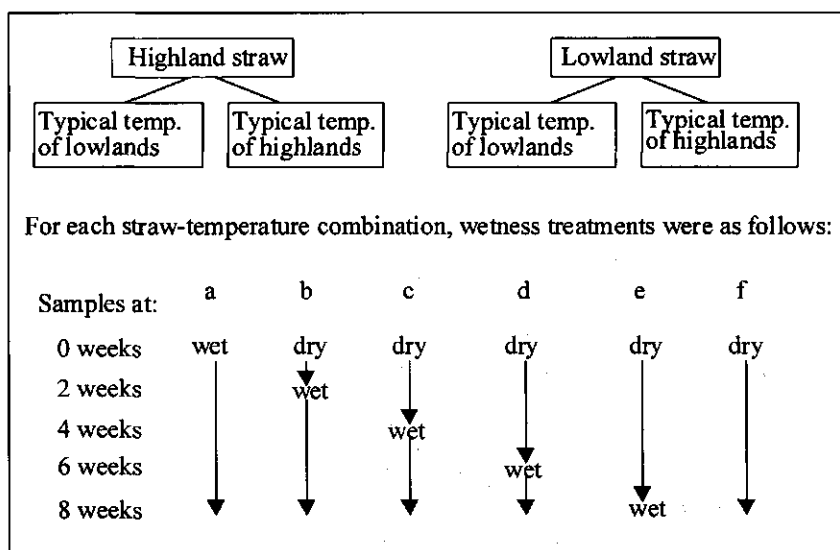
Figure 3. Design of experiment on ascospore discharge.

were sampled from each treatment. Primary leaf segments of 10-day old Min169 seedlings were placed in petri-dishes containing water agar supplemented with 50

mg L^{-1} benzimidazole. Cleistothecia were submerged in water for 3 days then put onto a sandwich of filter paper and cotton to retain wetness. The sandwich with cleistothecia was placed on the inner side of the lid of a petri dish, so suspending the cleistothecia over the leaf segments. The petri dishes were put in an incubator with continuous light (1000 lux cm^{-2}) at $18\pm1°C$. Mildew colonies were counted 15 days later. In the meantime, the cleistothecia were also checked with a microscope to see if asci or ascospores had formed (Smedegard-Petersen, 1967).

Results

Infection from cleistothecia in fields

In the highland area, Yunyang County, in the middle of July, cleistothecia released ascospores to infect seedlings (Table 3). Inoculum placed outdoors did not infect seedlings in the middle of October, whereas inoculum kept indoors retained the ability to infect. In Wuhan, in the lowland area, cleistothecia placed indoors were also able to infect seedlings in the middle of October, but those placed outdoors were not.

Table 3. Incidence and severity of powdery mildew in plots inoculated with straw bearing cleistothecia kept indoors or outdoors

Treatments[1] Replicates	Disease incidence[2]			Severity[3]			Total number of plants investigated
	I	II	III	I	II	III	
1	32.2	23.7	15.4	4.9	3.3	2.5	567
2	0.0	0.0	0.0	0.0	0.0	0.0	712
3	21.4	10.7	14.1	5.2	2.4	3.6	809
4	0.0	0.0	0.0	0.0	0.0	0.0	635
5	0.0	0.0	0.0	0.0	0.0	0.0	826
6	8.3	5.2	4.5	0.5	0.3	0.4	678
7	0.0	0.0	0.0	0.0	0.0	0.0	754
8	0.0	0.0	0.0	0.0	0.0	0.0	869

[1] See Table 1.

[2] Percentage plants infected.

[3] 1-9 scale (see Table 2).

Survival of volunteers at different altitudes

At four sample sites in the mountainous areas, volunteers grew at altitudes from

500m to 1200m (Fig. 4). The densities of volunteers shown are the mean of thirty samples. Volunteers were sparser at 500-800m than at altitudes higher than 800m. No volunteers were found at an altitude of 500m in 1997.

Annual fluctuations in disease incidence were evident at all altitudes where volunteers grew (Table 4). The higher the altitude, the higher the disease incidence on volunteers. Within the high altitude area, higher rainfall allowed more volunteers to develop and caused more disease on the volunteers. For example, both the incidence of disease and the density of volunteers were greater at 1000 m location at site C, where rainfall is higher, than at site B, which in turn, were greater than at site A, with the lowest rainfall.
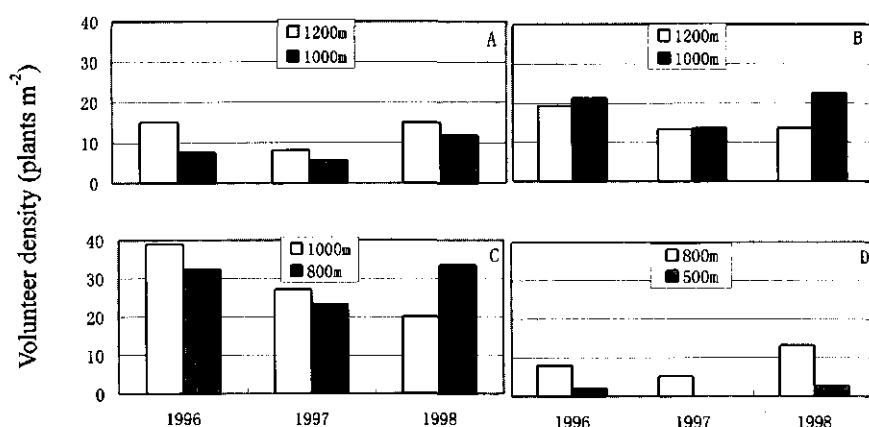
Figure 4. Volunteer density at four locations in the highlands in Hubei province through 1996 till 1998. The densities in the histograms are means of thirty samples at each altitude, A, B, C and D are corresponding to the locations shown in Figure 2.

Table 4. Powdery mildew incidence (% infected) on volunteer plants at different

altitudes during 1996-1998

Sample sites	Site in Fig. 2	Altitude(m)	1996	1997	1998	Mean
Yungxi and Yunyang	A	1200	24.5	8.3	15.1	16.0
	A	1000	18.5	5.6	9.4	11.2
Zhuxi and Zhushan	B	1200	38.2	21.4	22.7	27.4
	B	1000	26.3	11.7	15.2	17.7
Fangxian and Baokang	C	1000	34.1	13.6	16.8	21.5
	C	800	12.8	3.6	5.0	7.1
Guzheng and Nanzhang	D	800	6.4	2.3	10.1	6.3
	D	500	0.5	0.0	0.9	0.5

In the lowlands, 30 threshing grounds in 22 villages were investigated around Wuhan in mid-August. No volunteers were found. In summer 1998, 20 plots of $3m^2$ were sown with Min169 on 10th June. Seedlings emerged 10 days after sowing in all plots. As the temperature rose, the seedlings in the plots, both in the field and around threshing grounds, became yellow and finally senescent in the first ten days of July, when they had reached the two leaf stage. Seedlings in plots under trees survived longer, but died in the second ten days of July. Although there were abundant straw and leaves with cleistothecia around the plots, no mildew was found on the seedlings in any plot before they died.

Conditions affecting ascospore discharge

The ascospore discharge experiment was started on 20 July 1998, as soon as the straw and leaves became dry at room temperature. Analysis of variance (ANOVA) was performed on the number of ascospores released with a square root transformation. The linear effects of both the duration of wetness and the time of sampling on the rate of ascospore release and on the total number of ascospores released were highly significant ($p < 0.001$). However, there was no significant difference between either the sources of straw or the incubation temperature on the discharge of ascospores. Straw kept under continuous dry conditions or with eight weeks of dryness released viable ascospores for 8 weeks until mid-September, while those kept in continuously wet conditions ceased releasing ascospores by 6 weeks, at the end of August (Table 5). The total number of ascospores released was greater from straw kept in dry conditions (Fig. 5). The rate of ascospore release was higher at early sampling times, but then decreased quickly (Fig. 6).

Table 5. Mean number of ascospores released in an experiment to test the effects of wetness on ascospore release

Treatment	Time after cleistothecia first wetted				
(period of dryness)	0 weeks	2 weeks	4 weeks	6 weeks	8 weeks
0 week	5.2	1.9	1.6	0.0	0.0
2 weeks	5.4	4.6	2.3	1.0	0.0
4 weeks	5.0	5.4	2.1	1.0	0.0
6 weeks	4.3	4.3	4.5	1.4	0.0
8 weeks	6.3	5.0	2.8	3.0	1.0
10 weeks	5.6	5.6	3.6	3.5	2.5
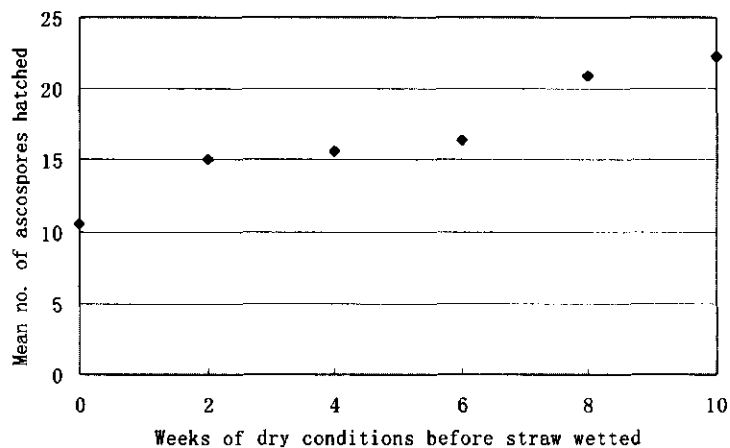
Figure 5. Mean number of ascospores hatched from straw.
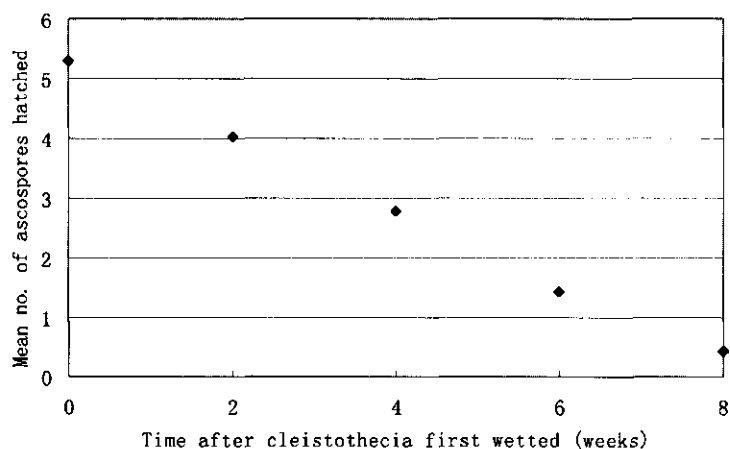
Figure 6. Mean number of ascospores hatched over time.

Discussion

Cleistothecia formed in both mountainous highlands and hilly or plain lowland areas in central China are capable of releasing viable ascospores. Straw bearing cleistothecia kept under dry conditions indoors could release ascospores to infect wheat seedlings in mid-October in both the highland and the plains, but those kept under natural conditions outside could not (Table 3). From shortly after the wheat harvest until the emergence of the next wheat crop, volunteers were present in the

mountainous highlands at altitudes above 500m (Fig. 4), but not in the lowland hills and plains. Seedlings sown in summer in the lowlands died, probably because the conditions were too hot and damp. The difference in temperature between the highlands and lowlands in summer had no significant effect on the rate or duration of ascospore ejection. However, the longer the straw was kept dry, the longer the period over which cleistothecia remained viable with the potential to release ascospores (Fig. 5, Table 5).

This study reveals that the sexual stage plays an important role in transmitting mildew between cropping seasons in central China. Both the density of volunteers and incidence of mildewed volunteer plants in the highlands increased with increasing altitude (Fig. 4, Table 4). This may indicate that volunteers at altitudes over 1000m were not infected by conidia from volunteers in the 500-800m area as Wang (1984) suggested. There are also no overlapping wheat crops anywhere in China less than 3000 km north of Hubei. So the only remaining possible source of inoculum is ascospores released from cleistothecia onto volunteers. These bridge the gap between crops and transmit mildew between seasons.

The rate and duration of ascospore ejection depends greatly on the amount and time of wetness. Precipitation in the northwest mountainous area is lower than in the rest of the province (Fig. 2), which may allow a longer duration of ascospore ejection (Fig. 6, Table 5). The higher the altitude, the smaller the gap between wheat crops (Fig. 1). Seedlings emerging early in autumn in fields may be hosts for ascospores liberated later. So ascospores may also infect autumn seedlings directly in the highlands at 1000m. In the area at 500-800m, the gap between crops is larger. The initial inoculum for the volunteers can only be from ejected ascospores. The co-ordination of the growth of volunteers and field seedlings with the pathogen life cycle shows that the sexual stage is responsible for the recurrence of wheat powdery mildew in the highlands of central China.

Cleistothecia cannot transmit mildew between seasons in the lowlands, although cleistothecia on straw collected from lowlands of Hubei province were also capable of discharging viable ascospores. No volunteers survive the summer because it is too hot and too damp. The subtropical monsoon in the hilly lowlands and plains of Hubei province brings a month of "plum rain" (the name given to the early monsoon rains that fall when plums are ripening), causing high humidity after the wheat harvest each year. This may provide a long period of continuous wet conditions and so shorten the duration of ascospore ejection (Fig. 5, Table 5). Wheat mildew in the lowlands of central China must therefore be re-established each year because of the early ejection of ascospores and the absence of the host in summer.

Several reports have documented cleistothecium formation, ascospore ejection and the role of the sexual stage in transmitting cereal mildew between seasons. Smedegard-Petersen (1967) reported a close relationship between the time of cleistothecium formation and that of the first infection of cereal mildew in Denmark. He demonstrated that cleistothecia were always formed one month after the first infection. This is not the case for wheat mildew in central China. Cleistothecia are never found until the last ten days of April in Wuhan (lowlands) or until the second ten days of May in Yunyang (highlands), no matter when the first infection occurs (Yu, personal observations). When wheat begins to ripen and diseased tissues become senescent as the temperature rises, cleistothecia are formed. Aging of the host was proposed to be the cause of cleistothecium formation (Graf-Marin 1934, cited by Yarwood, 1957). If infection occurs earlier, lower leaves are diseased. The fungus on these early diseased leaves produces few fruiting bodies because the temperature is too low for cleistothecia to form rapidly and the leaves die before the plant ripens. This implies that warm, but not hot temperatures and host senescence are essential for cleistothecium formation.

Cherewick (1944) and Moseman & Powers (1957) reported that cleistothecia on wheat and barley were still viable after storage for two years at room temperature, in a refrigerator, or at alternating temperatures, even for seven to eight years at room temperature. In our experiments, the temperature used had no significant influence on the viability of cleistothecia in dry conditions. Smedegard-Petersen (1967) pointed out that humidity is of decisive importance for the formation and release of ascospores. Turner (1956) reported that long wet periods initiated liberation of ascospores, causing a heavy shower of ascospores. In cleistothecia kept under continuously wet conditions for 4 to 6 weeks, which formed no mildew colonies on detached leaf segments, both empty asci and rotted cleistothecia were often found. This suggests that ascospores had already been released and that the cleistothecia had been rotted by other microorganisms found on the cleistothecia-bearing straws. The period of "plum rains" with high temperature in the lowlands of central China creates favourable conditions for saprotrophic fungi to grow on straw kept outside (these rains are also called "mould rains"; as the words "plum" and "mould" have the same pronunciation in Chinese). This may be a further reason why cleistothecia cannot tide the fungus over the whole summer in the lowlands.

Some authors in China (Wu, 1984 and He, 1985, in Guizhou Province, southwest China; Du, 1983 and Liu, 1985, in Jiangsu Province, east China; and Zhang and Li, 1986, in Shaanxi Province, northwest China) reported that cleistothecia could not survive the whole summer and therefore did not believe that

the sexual stage played any role in the recurrence of mildew from one year to the next. Koltin and Kenneth (1970) reported that, in Israel, cleistothecia of *Erysiphe graminis* f. sp. *hordei* tide the fungus over the summer and produce inoculum for newly emerging hosts after the first rains of late autumn. Smedegard-Petersen (1967) showed that cleistothecia of cereal mildew allowed the fungus to survive the summer under the natural conditions in Denmark. Turner (1956) reported that an ascospore shower took place from middle July to middle October in England.

We propose a model of the epidemiology of wheat powdery mildew in central China that involves both ascospores and conidiospores (Fig. 7). In the highlands, cleistothecia bridge the gap between wheat crops and volunteers or between the last crop and the next. The pathogen then produces asexual conidiospores on volunteers to cause mildew in the next crop by dissemination of conidia onto seedlings in autumn. In the lowlands, mildew does not survive the summer, either as cleistothecia or as asexual colonies. In the spring, conidia from wheat in the highlands disperse onto wheat in the lowland to cause mildew epidemics. Other sources from neighbouring provinces, where the situation may be similar to the highlands of central China, may also contribute to epidemics in the lowland of central China. These sources of inoculum cannot be excluded by this study.
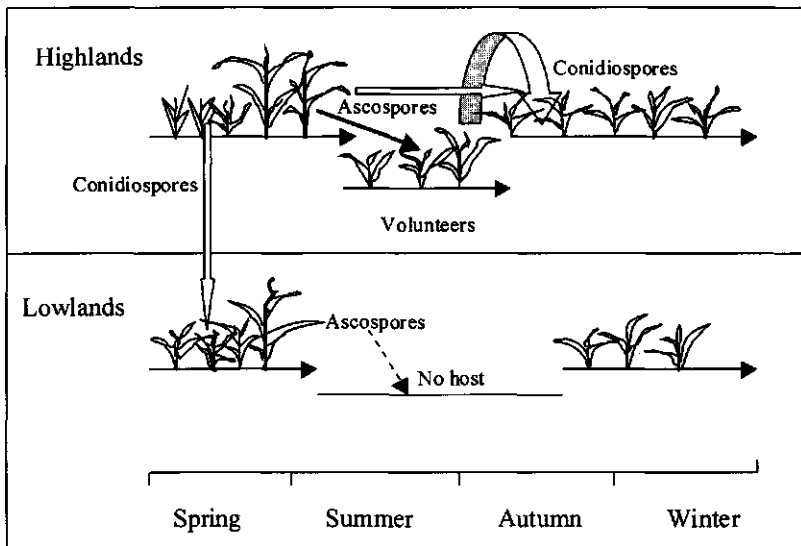
Figure 7. A model of the epidemiological cycle of *Erysiphe graminis* f. sp. *tritici* in central China, emphasizing the role of the sexual stage

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Identification of Specific Resistances to Powdery Mildew in Chinese Wheat varieties and Selection of a Set of Cultivars for Monitoring the Structure and Movement of *Erysiphe graminis* f. sp. *tritici* Populations in Central China

Summary

Twenty single-conidium isolates of *Erysiphe graminis* f. sp. *tritici* from central China were characterised for virulence to 14 known *Pm* genes. These isolates were used to postulate mildew resistance genes in 37 commercial wheat varieties, mainly from Yangtze valley provinces, and breeding lines from the Yangtze valley and other parts of China. 36 varieties possessed resistance effective against some or all of the 20 isolates, 19 varieties had resistances that were not controlled by any of the 14 known resistance genes tested. The resistances of these 19 varieties were controlled by unknown genes that could not be identified with these isolates. In 17 varieties, *Pm*1, *Pm*3b, *Pm*3c, *Pm*5 or *Pm*8 were postulated to be present, but were associated, in every case, with other, unknown resistance factors that differed from one variety to another. The variety Min169 was susceptible to all isolates. These results indicate that the resistance of many Chinese wheat varieties is largely controlled by unknown genes, different from those in the European and North American differential sets. Frequencies of virulence matching the 14 known *Pm* genes ranged from 0% to 80%. High frequencies of virulence were detected matching the genes *Pm*1, *Pm*3b, *Pm*3c, *Pm*5, *Pm*6, *Pm*8 and *Pm*17, most of which were postulated to be present in the commercial wheat varieties. Frequencies of virulence matching *Pm*3d, *Pm*4b and *Pm*1+2+9 were rare in all regions, with overall frequencies less than 1%. These genes should be of value in wheat breeding. The frequencies of virulences to six of the 14 known *Pm* genes varied among regions, but only two of the corresponding resistance genes (*Pm*1 and *Pm*8) may be present in the wheat varieties tested. The differential set with known *Pm* genes used in Europe and North America is therefore not suitable for monitoring the population structure of *E. graminis* f. sp. *tritici* in central China. The frequencies of virulence on the 37 commercial wheat varieties or breeding lines tested varied from 0% to 100%. Frequencies of virulence on 14 cultivars varied significantly between regions of Hubei province. Of these 14 varieties, nine were chosen as a differential set for monitoring the population structure and migration of *E. graminis* f. sp. *tritici* in central China.

Key words: Powdery mildew, *Blumeria graminis* f. sp. *tritici*, wheat, specific resistance, differential set.

Introduction

Powdery mildew of wheat, caused by *Erysiphe graminis* f. sp. *tritici*, is one of the most important diseases of wheat worldwide, causing yield losses and reducing grain quality. Host resistance is the most economical and effective method of controlling the disease. In Hubei province, in central China, with 1.6 million hectares of wheat cultivation, powdery mildew has been an important disease since the late 1970s. Owing to its vast wheat growing area and diverse environmental conditions, numerous varieties are grown in this province, but little is known about their resistance.

Evolution of matching virulence has caused the "breakdown" of host resistance. For example, the resistance of E'an1 became ineffective 5 years after its release in Hubei province. Conidia of *E. graminis* f. sp. *tritici* can be dispersed by wind over long distances (Hermansen *et al.*, 1978). Migration of pathogen populations is important in the dynamics of the population structure of cereal mildew pathogens (Wolfe and McDermott, 1994). Two years after *Mla*13, a gene for resistance to mildew, was introduced in barley varieties in the UK, three clones of *E. graminis* f. sp. *hordei* virulent to this gene were found in locations several hundred kilometers apart in the British Isles (Brown *et al.*, 1991). At least one of these clones may have originated in the former Czechoslovakia (Wolfe *et al.*, 1992). Information on virulence frequencies in pathogen populations, the directional movement of these populations and the presence and frequency of resistance genes in host varieties is therefore indispensable for the improvement of the efficiency of host resistance in controlling powdery mildew.

The wheat growing area of Hubei province can be divided into three zones in relation to the epidemiology of wheat powdery mildew, because of different geographical and topographical features (Yu, 2000a). In the mountainous highlands, where the weather in summer is cool and dry, ascospores discharged from cleistothecia transmit the disease from season to season by infecting autumn seedlings directly or by infecting volunteers. In areas of intermediate altitude, ascospores transmit the disease only by infecting volunteers. In the lowlands, such as the Jianghan Plain, in the southeastern part of the province, however, neither the pathogen nor wheat volunteers survive the summer at all because the weather is too hot and wet. Wheat mildew must therefore be reestablished in the lowlands each year.

Information about the movement of the pathogen population may assist the temporal and spatial deployment of resistance in the province. Virulence has been used as a marker to track the structure, dynamics and movement of populations of cereal mildew pathogens (Limpert *et al.*, 1990; Wolfe *et al.*, 1992) and cereal rust pathogens (Kolmer, 1997; Wellings and McIntosh, 1990). Briggle (1969) developed a set of

near-isogenic lines with genes for specific resistance to powdery mildew, which has been widely used as a differential set. Since the late 1970s, this set has been used to investigate the population structure of *E. graminis* f. sp. *tritici* in parts of China (Li, 1985; Lu, 1986; Wang, 1988; Zhu, 1986). Data from investigations of pathogen populations are useful for breeders to improve the resistance of wheat varieties to mildew by transferring known *Pm* genes that are not overcome by virulence in the pathogen population.

In research on the resistance of local varieties, however, the use of the same differential set as that used in Europe and North America has not been satisfactory because the differential set may not be relevant to local varieties. Wang *et al.* (1992) tested isolates of five physiological races, as defined by the European and North American differential set, on 19 varieties grown in Henan province and found no difference in virulence on the cultivars among the isolates. They also tested 15 cultivars with different isolates from the same physiological race, defined as above, and found clear differences between the isolates in virulence on the varieties. In this case, the races identified with the differential set do not provide information on virulence on the local varieties in the pathogen population. Furthermore, for tracking directional movement of the pathogen population, it is necessary to select a set of varieties that can reveal regional variation.

The research reported here had three objectives. The first was to establish a set of differential isolates, each derived from a single conidium, with which the resistance factors in wheat varieties grown in the Yangtze valley and some other parts of China could be identified on the assumption of a gene-for-gene relationship (Flor, 1955). Secondly, by investigating frequencies of virulences in the pathogen population to known *Pm* genes and to local varieties, information on resistance genes and virulence frequencies could be obtained to assist resistance breeding programmes. Thirdly, to investigate the population of *E. graminis* f. sp. *tritici,* particularly to track its movement across regions, a set of varieties or lines suitable for monitoring the pathogen throughout central China were selected.

Materials and Methods

Wheat varieties

Near-isogenic lines and lines with known *Pm* genes (Table 1) were kindly provided by Dr X. Duan (Plant Protection Institute, Chinese Academy of Agricultural Sciences, Beijing, China) and Dr E. Limpert (ETH, Zurich, Switzerland). Varieties mainly from

the provinces along the Yangtze valley were collected, as well as lines from both the provinces along the Yangtze valley and some provincial breeding centres in other parts of China (Yu, 2000b). From a total of 60 varieties and lines, 37 varieties were chosen for this study (Table 2) according to their importance in wheat production and breeding.

Pathogen isolates

Three different wheat growing regions of Hubei province, defined by altitudes, were selected as target areas for collections (Fig. 1). Single colony isolations were made from diseased leaves and taken to the laboratory in plastic boxes with small wells containing 5 g L^{-1} agar with 50 mg L^{-1} benzimidazole. At first, we did not know which wheat varieties were universally susceptible to *E. graminis* f. sp. *tritici* genotypes in Hubei province and would be suitable for maintaining and multiplying all isolates.
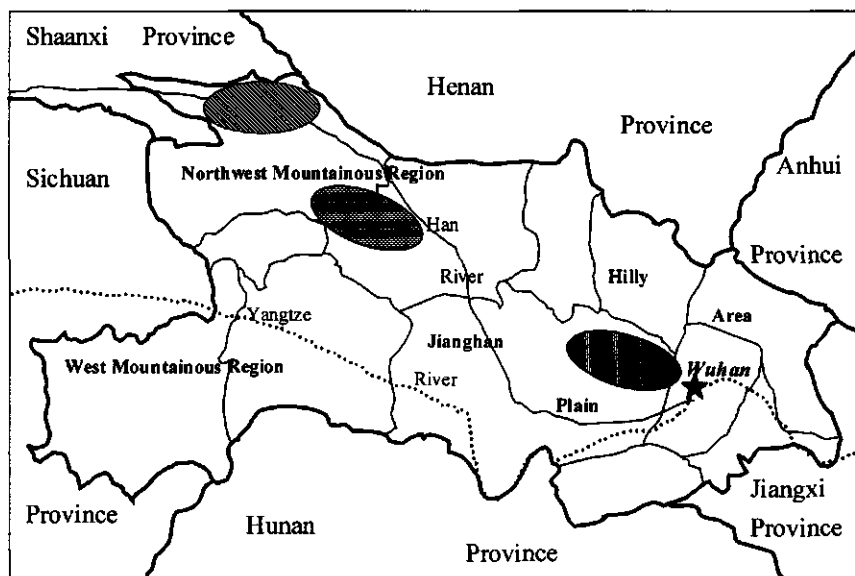
Figure 1. Map of Hubei Province with three different regions in which wheat powdery mildew isolates were collected. Shading: from bottom right to top left = altitude over 1000m, brickwork pattern = altitude between 500-800m, vertical = lowlands.

Several different varieties, which were susceptible in field conditions, were therefore used for multiplication and maintenance of isolates. Seedlings were grown under contamination-proof conditions in a growth chamber with constant light and

temperature (20°C). Leaf segments of 10-12 day old seedlings, cut from the middle parts of primary leaves of susceptible varieties, were put in 6cm-diameter petri dishes containing 5 g L^{-1} agar with 50 mg L^{-1} benzimidazole. Single colony isolates were transferred with autoclaved tooth-sticks to the leaf segments for multiplication and maintenance.

Selection of differential isolates

From tests of 351 single colony isolates on lines with known *Pm* genes, 43 were chosen as candidate differential isolates according to their virulence spectra. These candidate isolates were tested on the lines with known *Pm* genes several times and showed consistent reactions in all tests. To ensure purity of the isolates, they were then re- isolated as mono-conidium isolates and tested onto the lines with known *Pm* genes again to check the concordance of their virulence spectra with those of the mother isolates. Seventeen differential isolates were finally chosen. Since frequencies of virulence on *Pm*3d, *Pm*4b and *Pm*1+2+9 in the pathogen population in Hubei were low, all isolates with virulence to these *Pm* genes were included in the differential set. Three isolates, Gui-88, Gui-413 and Gui61, which were virulent on *Pm*3d, *Pm*4b and *Pm*1+2+9 were kindly provided by Professor Xiang (Guizhou Agricultural College, Guiyang, China).

Virulence tests

Near-isogenic lines, the lines with known *Pm* genes and 37 Chinese wheat varieties were grown in contamination-proof conditions. Leaf segments, 3 cm in length, cut from the middle parts of primary leaves of 10-day old seedlings, were placed in petri dishes containing 5 g L^{-1} agar with 50 mg L^{-1} benzimidazole, using three segments of each variety for each isolate. Plastic beakers with a hole in the bottom were inverted as settling towers to cover the petri dish and conidia of isolates were blown into the tower at a density of 250-400 conidia cm^{-2} and allowed to settle for three minutes. Leaf segments were incubated under conditions of constant light at 17°C for 12 days. Infection types (IT) were recorded by the system of Moseman (1984), modified by Shi (1987):

0 = immune: no visible sign of infection or necrosis, no mycelium.

1 = resistant: increasing from no mycelium to little mycelium, sometimes with necrosis.

2 = moderately resistant: increasing amount of mycelium, little conidiospore

production with necrosis.

3 = moderately compatible: large amount of mycelium, moderate conidiospore production.

4 = completely compatible: large amount of mycelium, substantial conidiospore production.

Postulation of resistance genes

Varieties were classified as resistant (IT 0-2) or susceptible (IT 3-4) to each differential isolate. Two steps (le Boulc'h *et al.*, 1995) were taken to postulate the resistant genes present in a variety on the assumption of a gene-for-gene relationship (Flor 1955). The first step was to eliminate resistance genes that are not present in a variety. When a compatible reaction has been recorded for a particular variety-isolate combination, the variety cannot possess resistance genes to which the isolate is avirulent. The second step was to postulate resistance genes present in the variety. When an incompatible reaction was recorded for a variety-isolate combination and the isolate possesses a known gene for avirulence against one of the resistance genes not excluded by the first step, then the variety could be postulated to carry that gene. Finally, comparisons were made between the reactions of lines with known resistance genes and those of varieties postulated to carry the same resistance genes, to check if the variety has additional resistance factors.

Results

Identification of specific resistances in wheat varieties

The virulence spectra on 14 *Pm* genes of twenty isolates, used as differential isolates to postulate resistance genes present in 37 wheat varieties grown in central China, were recorded (Table 1). Results of reactions between the varieties and the differential mildew isolates are shown in Table 2. Variety Min169 was susceptible to all isolates, suggesting that this variety does not possess race-specific resistance to isolates from central China.

The resistance genes present in the varieties were postulated only according to virulent or avirulent reactions. Nineteen varieties possessed resistance effective against some or all of the 20 isolates, but the gene or genes they carry could not be identified with these isolates. They cannot have any of the 14 *Pm* genes present in the European/North American differential set of wheat cultivars. Among the 19 varieties

Table 1. Infection types of a set of *Erysiphe graminis* f. sp. *tritici* isolates on near-isogenic lines of wheat and other lines with known resistance genes.

Near isogenic lines or cultivars	Pm-gene	Powdery mildew isolates																			
		W-1057	G-14	G-236	G-262	G-204	Y-631	Gui-88	G-396	Gui-413	Y-619	Y-670	G-943	G-894	W-911	W-1011	Gui-61	Y-723	G-374	G-281	Y-489
Chancellor	none	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Axminster/8cc	1	3	1	4	4	4	0	3	4	4	0	3	3	3	0	3	3	3	0	0	4
Ulka/8cc	2	0	1	0	4	0	0	0	4	3	0	3	0	0	0	0	3	0	0	0	3
Asosan/8cc	3a	0	2	4	3	3	4	2	0	0	3	3	0	2	1	4	4	3	0	3	2
Chul/8cc	3b	4	3	4	4	4	4	3	4	4	0	3	3	4	3	4	3	3	0	3	1
Sonora/8cc	3c	3	3	4	4	4	4	3	4	4	3	3	3	3	4	4	0	3	4	3	2
Kolibri	3d	0	3	1	1	1	0	3	0	1	1	0	1	1	1	1	0	0	0	1	3
Khapli/8cc	4a	0	0	4	0	4	0	0	3	0	0	0	0	0	0	0	0	0	3	4	3
Armada	4b	0	0	2	1	0	3	0	0	2	0	0	0	0	0	4	4	0	0	0	3
Hope	5	3	4	4	4	0	4	3	3	2	4	4	4	4	3	4	0	3	2	4	2
Timgalen	6	2	3	4	1	1	0	3	0	1	1	3	2	1	0	1	1	3	0	2	1
Transec	7	3	0	0	0	0	0	1	3	0	4	0	4	4	4	3	0	2	0	2	4
Kavkaz	8	3	3	4	3	4	4	0	4	0	3	3	3	4	1	4	3	4	0	2	1
Amigo	17	1	1	4	4	4	4	3	1	4	4	1	1	3	1	4	2	3	4	4	0
Normandie	1+2+9	0	0	0	4	0	0	0	4	4	0	3	0	0	0	0	3	0	0	0	0

Table 2. Infection types of 37 Chinese wheat cultivars with 20 differential isolates of *Erysiphe graminis* f. sp. *tritici* [a]

Cultivars	Differential Isolates																				Postulated genes
	W-1057	G-14	G-236	G-262	G-204	Y-631	Gui-88	G-396	Gui-413	Y-619	Y-670	G-943	G-894	W-911	W-1011	Gui-61	Y-723	G-374	G-281	Y-489	
1 E8911	3	3	2	3	3	2	0	3	3	4	2	3	3	3	3	4	3	3	4	1	?
2 E81513	3	4	2	2/3[b]	2	3	3	3	3	3	3	3	4	3	3	3	4	3	4	3	?
3 Emai6	1	2	3	1	1	0	3	3	2	4	1	3	3	3	1	2	4	0	1	1	3c+5+?
4 Emai11	1	3	3	3	2	4	4	3	3	4	1	3	2	3	3	3	1	3	4	4	?
5 S048	3	3	2	3	1	3	3	1	0	3	1	3	4	0	3	0	3	1	4	2	3c+?
6 Jin66	2	2	2	3	0	3	0	0	3	2	2	3	3	3	3	3	3	1	0	2	3b+?
7 Hua8	2	0	2	2	1	0	0	3	0	2	4	3	3	1	2	2	2	1	0	3	1+?
8 E28547	4	3	3	3	3	3	4	3	3	4	4	4	3	3	3	2	2	4	4	3	?
9 Zhen831	0	2	2	0	0	2	0	1	0	2	0	0	0	0	0	0	0	0	3	0	?
10 Su3	2	2	1	2/3	1	2	4	3	3	4	2	2	3	2	2/3	2	0	0	3	2	3c+?
11 Yang9363	4	3	1	4	1	3	2	3	3	4	4	3	3	3	3	3	3	3	4	3	?
12 Jinong215	2	2	2	2	1	4	3	1	0	3	1	2	1	0	0	0	2	2	4	3	?
13 Dian633	0	0	0	1	0	0	0	1	2	1	1	2	1	0	0	1	0	1	1	1	?
14 Lin5064	2	3	2	3	3	3	3	3	3	1	2	4	2	3	2/3	3	3	1	1	2	3b+?
15 Dian832	2	0	1	0	0	0	3	0	0	1	1	1	0	0	0	0	3	3	0	0	?
16 Dh182	1	3	2	3	1	3	4	3	2	4	0	2	3	3	2	2/3	2	3	1	1	?
17 Chun1066	1	3	1	3	3	3	2	3	3	3	3	3	2	3	3	2/3	3	4	1	1	3c+?
18 Mfian8724	4	3	1	3	3	4	4	0	0	4	1	3	4	2	4	0	4	0	1	1	3c+?
19 E22072	2	3	2	2	2	3	1	4	1	2	4	4	3	4	3	3	4	3	2	1	?
20 E8143	2	3	0	0	2	0	0	3	0	4	2	0	3	0	2	0	0	1	4	1	3c+5+?
21 E'an1	1	2	2	2	3	3	2	3	2	3	4	3	4	2	4	2	1	2	2	1	3c+8+?

Table. 2. (continued)

Cultivars	Differential Isolates																				Postulated genes
	W-1057	G-14	G-236	G-262	G-204	Y-631	Gui-88	G-396	Gui-413	Y-619	Y-670	G-943	G-894	W-911	W-1011	Gui-61	Y-723	G-374	G-281	Y-489	
22 Dian51152	2	2	2	3	3	3	2	4	4	4	4	3	4	3	4	2	3	3	3	1	3c+?
23 Dian30955	0	2	2	2	3	2	4	3	1	4	4	3	2	3	4	4	3	3	4	0	?
24 Ziza1028	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?
25 Hx8541	2	1	3	3	4	1	1	3	3	1	1	2	1	4	4	2	3	2	2	0	3b+8+?
26 Ning7840	1	2	2	0	3	2	1	3	0	2	1	2	4	2	3	2	4	3	1	0	3c+?
27 Ningzi21	0	0	0	0	0	0	0	0	1	0	4	0	0	1	0	2	0	0	0	0	?
28 Chun10577	1	2	2	2	1	2	3	2	3	3	4	3	4	2	3	3	3	4	4	0	?
29 Chun35050	0	1	0	0	4	2	0	2	0	0	4	2	2	1	3	0	2	1	1	1	8+?
30 Mian8855	0	1	0	2	0	1	1	1	0	0	1	0	1	0	2	1	0	1	0	0	?
31 Dian20007	1	0	0	0	1	2	1	1	0	1	1	0	0	1	2	2	0	0	1	0	?
32 Dian675-1	2	0	4	0	3	0	1	0	0	0	0	2	0	0	4	0	2	0	0	1	1+8+3a/b/c+? [c]
33 Lin90-35	1	0	0	2	1	1	0	0	0	1	0	0	0	0	1	0	0	0	0	0	?
34 Nenk6	1	2	2	0	2	3	1	1	0	3	3	3	1	0	3	1	2	0	3	1	3c+5+?
35 Ze88pin6	1	2	3	3	2	2	4	2	1	3	3	0	2	2	4	3	3	3	3	1	?
36 85Zon33	1	3	4	0	4	3	3	2	0	4	3	3	2	1	3	0	3	0	0	1	3c+?
37 Min169	3	3	4	3	4	3	3	3	3	4	3	4	4	3	4	3	4	3	4	3	No resistance

[a] Resistance genes present in the cultivars were postulated only according to virulent or avirulent reactions; some infection types may not be as expected from this classification.

[b] Infection type varied from IT2 to IT3.

[c] May have Pm3a, Pm3b or Pm3c.

four, Dian633, Mian8855, Ziza1028 and Lin90-35, expressed resistance to all 20 isolates. Three varieties, Zhen831, Ningzi21 and Dian2007, were susceptible to only one isolate of the 20, the virulent isolate being different in each case. E81513 and E28547 gave a susceptible reaction to 18 of the 20 isolates, the two virulent isolates, again, differing for the two varieties. Other varieties with unidentified resistance had narrow or broad resistance spectra.

Five resistance alleles were postulated as possibly being present in the varieties tested. *Pm*1 may be present in Hua8 because it was resistant to the same six isolates to which Axminster/8cc was resistant. However, it was also resistant to a further nine isolates and so has one or more additional resistance factors. Jin66 and Lin5064 were resistant to the same three isolates as those to which Chul/8cc was resistant, so they may carry *Pm*3b. However, Jin66 was resistant to eight other isolates to which Chul/8cc was susceptible, so it must carry other resistance factors as well as or instead of *Pm*3b. Lin5064 had a narrower spectrum than Jin66 although both of them were postulated to possess *Pm*3b. It too must have additional resistance. The reactions of 11 varieties were consistent with presence of *Pm*3c. Among these, three, Emai6, E8143 and Nenk6, may have *Pm*3c plus *Pm*5 as well as other unknown resistance factor(s). However, the additional resistance factors they carry cannot be the same because they responded differently to certain isolates. Seven varieties, postulated to possess *Pm*3c without *Pm*5, also varied in their reactions to other isolates. E'an1 may possess *Pm*3c+8 combined with unidentifiable resistance factors. Three other varieties were postulated to possess *Pm*8. Hx8541 may have *Pm*3b+8 associated with unknown resistance factors. Dian675-1 carried *Pm*1+8 plus either *Pm*3a, *Pm*3b or *Pm*3c, again with unknown resistance factors. Chun35050 may possess *Pm*8, but its resistance spectrum was not in complete accord with that of Kavkaz so it too may contain other resistance genes.

Frequency of virulence matching known *Pm* genes

A total of 351 isolates, collected from three different wheat growing regions, were tested on near-isogenic lines and other lines with known *Pm* genes (Table 3). Frequencies of virulence matching *Pm*3d, *Pm*4b, and *Pm*1+2+9 were rare in all regions and were less than 1% overall. These resistances are therefore effective against the mildew pathogen populations in central China. The overall frequencies of virulence matching *Pm*2, *Pm*4a and *Pm*7 were also low, less than 10%, so these genes may also provide useful resistance. The frequencies of virulence matching other *Pm* genes, however, were higher, surpassing 60% for *Pm*3b and *Pm*3c, and 80% for *Pm*5. A chi-squared test of independence was done to test the significance of variation in virulence

frequencies among the three regions. Of the 14 virulences studied, six, matching $Pm1$, $Pm3a$, $Pm4a$, $Pm6$, $Pm8$ and $Pm17$, varied significantly in frequency across regions (p ≤ 0.05).

Table 3. Virulence frequencies (%) in populations of *Erysiphe graminis* f. sp. *tritici* collected from three different wheat growing regions of Hubei, on near-isogenic lines of wheat or lines with known resistance genes

Lines or cultivars	Pm-gene	Highland	500-800m	Lowland	Overall	Probability for X^2 test
Axminster/8cc	1	48.2	23.4	50.9	43.6	0.00
Ulka/8cc	2	4.1	7.8	4.8	5.1	0.48
Asosan/8cc	3a	19.4	35.1	17.3	22.2	0.01
Chul/8cc	3b	62.4	66.2	61.5	62.9	0.76
Sonora/8cc	3c	68.8	77.9	78.8	73.8	0.19
Kolibri	3d	1.2	0.0	0.0	0.6	0.30
Khapli/8cc	4a	8.2	1.3	8.7	6.8	0.05
Armada	4b	0.0	0.0	0.9	0.3	0.38
Hope	5	77.1	83.1	80.8	79.5	0.55
Timgalen	6	31.2	67.5	41.4	42.2	0.00
Transec	7	7.7	7.8	9.6	8.3	0.86
Kavkaz	8	48.8	69.7	53.9	54.7	0.01
Amigo	17	41.8	61.0	48.1	47.9	0.02
Normandie	1+2+9	0.6	0.0	1.9	0.9	0.31
Number of isolates tested		170	77	104	351	

Frequencies of virulence on Chinese varieties, their spatial distributions and development of a differential set of varieties

A total of 201 single colony isolates collected from three different regions were tested on 37 varieties (Table 4). All isolates were virulent on variety Min169 (no. 37) and the frequency of virulence on E28547 (no. 8) was next highest among all varieties tested in the three regions. Virulence frequencies on E89-11 (no. 1), E81513 (no. 2), Yang9363 (no. 11) and Dian51152 (no. 22) were also high. The lowest virulence frequencies were on Dian633 (no. 13) and Lin90-35 (no. 33). On Ziza1028 (no. 24), Ningzi21 (no. 27) and Mian8855 (no. 30) the matching virulence frequencies were less than 10% in all

three regions and the overall frequencies did not reach 5%. On other varieties, the matching virulence frequencies varied from less than 10% to more than 70%.

Table 4. Virulence frequencies (%) in populations of *Erysiphe graminis* f. sp. *tritici* collected from three different wheat growing regions in Hubei on 37 wheat varieties and breeding lines

Cultivar no.	Cultivar name	Highland	500-800m	Lowland	Overall	Probability for X^2 test
1	E89-11	60.4	72.1	69.2	62.2	0.09
2	E81513	66.7	76.7	56.5	65.7	0.01*
3	Emai6	38.5	37.2	27.4	34.8	0.19
4	Emai11	44.8	44.2	22.6	37.8	0.00*
5	S048	42.7	32.6	33.9	37.8	0.27
6	Jin66	24.0	18.6	34.4	21.4	0.03*
7	Hua8	20.8	32.6	12.9	20.9	0.00*
8	E28547	83.3	88.4	83.9	84.6	0.55
9	Zhen831	12.5	28.9	12.9	15.4	0.00*
10	Su3	40.6	37.2	24.2	34.8	0.04*
11	Yang9363	67.7	69.8	58.1	65.2	0.18
12	Jinong215	47.9	41.9	33.9	42.3	0.13
13	Dian633	1.0	4.7	0.0	1.5	0.04
14	Lin5064	53.1	55.8	45.2	51.2	0.29
15	Tian832	14.6	11.6	4.8	10.9	0.07
16	Dh182	42.7	48.8	37.1	42.0	0.25
17	Chun1066	40.6	41.9	35.5	39.3	0.62
18	Mian8724	59.4	73.8	43.6	57.2	0.00*
19	E22072	52.1	60.5	70.9	59.7	0.02
20	E8143	28.1	25.6	35.5	29.9	0.28
21	E'an1	35.4	62.8	50.0	45.8	0.00*
22	Dian51152	61.5	69.8	66.1	64.7	0.46
23	Dian30955	46.9	48.8	33.9	43.3	0.07
24	Ziza1028	1.0	0.0	9.7	3.5	0.00
25	Hx8541	51.0	34.9	21.0	38.6	0.00*
26	Ning7840	18.8	32.6	22.6	22.9	0.07
27	Ningzi21	2.1	2.3	1.6	2.0	0.94
28	Chu10577	57.3	55.8	64.5	59.2	0.41
29	Chu35050	26.0	39.5	29.0	29.9	0.10
30	Mian8855	2.1	2.3	1.6	2.0	0.94
31	Dian20007	10.4	11.6	14.5	11.9	0.66
32	Dian675-1	7.3	6.7	11.3	8.5	0.48
33	Lin90-35	0.0	4.7	1.6	1.5	0.07
34	Nenk6	28.1	46.5	37.1	34.8	0.03
35	Ze88pin6	41.7	51.2	41.9	43.8	0.31
36	85Zon33	42.7	58.1	43.6	46.3	0.05
37	Min169	100	100	100	100	
Number of isolates tested		96	43	62	201	

* Varieties chosen as differentials.

Frequencies of virulence on 14 of the 37 varieties differed significantly among regions (Table 4). A set of varieties for monitoring the pathogen population was selected only from these fourteen varieties because they showed the greatest regional differences in virulences. Of these 14, nine were eventually chosen on the basis of virulence frequencies in the pathogen population and of the importance of the varieties in commercial production and breeding. Overall virulence frequencies varied in the fourteen varieties from 1.5% (Dian633) to 65.7% (E81513). Virulence frequencies on Dian633 and Ziza1028 were very low, so these two varieties were not considered to be suitable for inclusion in a set to be used for investigating pathogen population structure. Virulence on the varieties 85Zon33 and E'an1 had similar overall frequencies and in the three different regions, the virulence frequencies were also similar. However, E'an1 is widely grown not only in Hubei province but also in neighbouring provinces, so E'an1 was chosen in preference to 85Zon33. Virulence frequencies on Nenk6 and Su3, known as Sumai3 in European and American literature (Waldron *et al.* 1999), were very similar and showed distinct regional differences. However, Su3 is very important in breeding for resistance to *Fusarium* head blight and was therefore included in the set instead of Nenk6. Overall virulence frequencies on Mian8724 and E22072 were also similar, but Mian8724 is very popular in the highlands of Hubei province and in Sichuan and some parts of Shaanxi, the neighbouring provinces. Inclusion of Mian8724 in the set may assist tracking the population on a larger scale so this variety was chosen for the set. The variety Min169 was susceptible to all isolates tested, so it was chosen as a susceptible control.

Discussion

A total of 37 commercial varieties mainly from Yangtze valley provinces, as well as breeding lines from the Yangtze valley and other parts of China, were tested with 20 differential isolates. Of the 37 varieties, 17 were postulated to carry five known specific resistance genes. Of the five *Pm* genes, the one most commonly postulated to be present was *Pm3c*, detected in 11 varieties. *Pm8* may be present in 4 varieties, *Pm3b* in 3, *Pm5* in 3 and *Pm1* in 2 (Table 2). In none of the 17 varieties does the postulated known resistance gene or genes account fully for the reaction to all 20 differential isolates. These varieties must also carry unknown resistance factors that differ from one variety to another. It is possible that some varieties may have complex resistance, controlled by more than one gene. The presence of known *Pm* genes has been studied previously in 26 Chinese advanced wheat breeding lines, using *E. graminis* f. sp. *tritici* isolates from Germany (Xia *et al.*, 1995). Among the 26 lines, 23 were developed by the Beijing

Agricultural University, two by Gansu Academy of Agricultural Sciences, Lanzhou, and one by the Chinese Academy of Agricultural Sciences, Beijing. No varieties have been studied previously for their mildew resistance from the Yangtze valley or from provinces outside Beijing, other than Gansu. Most of the breeding lines investigated by Xia *et al.* (1995) were postulated to carry more than one mildew resistance gene, some of which were unknown resistances. Together, the work reported here and that of Xia *et al.* (1995) indicates that the resistance to mildew of many wheat varieties grown in the Yangtze valley and other parts of China is controlled by genes other than those that can be identified by the European and North American differential sets.

The *Pm* genes in the varieties studied here were only postulated according to virulence and avirulence, and some infection types were not as expected from the infection types for these *Pm* genes, shown in Table 1. So the gene or genes postulated to be present in these varieties is tentative. For example, *Pm*1, *Pm*3b, *Pm*3c and *Pm*8 were postulated to be present in some varieties with some unknown resistances, but the infection types were not completely in accord with those on the lines with known *Pm* genes (Table 1). These varieties in fact may not possess these *Pm* genes or carry genes that modify the infection types of the *Pm* genes.
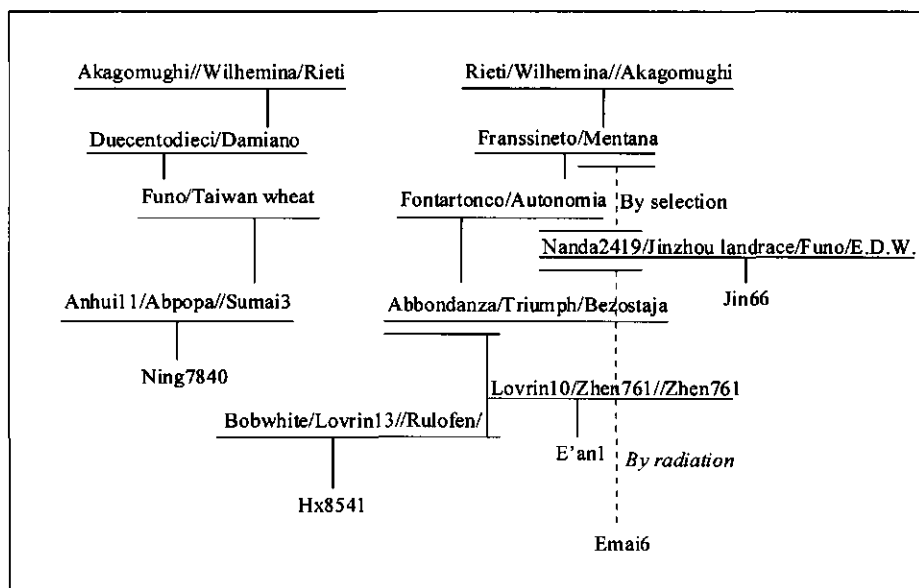

Figure 2. Pedigrees of important wheat cultivars grown in central China.

It is not surprising that Chinese wheat varieties share resistance genes with European wheat varieties because several important commercial varieties are descendants of Rieti,

an Italian landrace, Wilhelmina, a Dutch landrace, and Akagomughi, a Japanese landrace (Jin, 1983, Fig. 2). A famous variety, Nanda2419 was widely grown and was the first improved variety developed by selection from an Italian cultivar, Mentana, in China. Many varieties currently grown are derived from Mentana. The cultivars Abbondanza and Funo were widely used for wheat breeding not only in central China, but also in many other parts of the country and can also be traced back to the three original cultivars (Fig. 2). We can speculate that several Chinese varieties possess *Pm*3b, *Pm*3c and *Pm*5 inherited from Rieti, Wilhelmina or Akagomughi, because all varieties possessing these genes with known pedigrees in this study are derived from those three ancestral cultivars. However, the mildew resistance genes, if any, in Rieti, Wilhelmina and Akagomughi are unknown. Cultivars Lovrin10 and Lovrin13 were introduced into China from Romania, originally for yellow rust resistance breeding, and have the 1BL-1RS wheat-rye chromosome translocation (Pang *et al.*, 1993). *Pm*8, on the rye segment of 1BL-1RS chromosome (Briggle, 1966; McIntosh, 1978), is present in such Chinese varieties as E'an1 and Hx8541, and is believed to derive from the Lovrin varieties.

Frequencies of virulence matching the known *Pm* genes in the *E. graminis* f. sp. *tritici* population in central China ranged from 0% to over 80%. High frequencies of virulence were detected for the genes *Pm*1, *Pm*3b, *Pm*3c, *Pm*5, *Pm*6, *Pm*8 and *Pm*17. Except for *Pm*6 and *Pm*17, the possible presence of these genes in varieties grown in central China and high frequencies of virulence to them indicates that the pathogen population may have been subject to selection by host resistance genes. This is further evidence, albeit indirect, that genes *Pm*1, *Pm*3b, *Pm*3c, *Pm*5 and *Pm*8 are present in Chinese varieties

Twenty-eight genes for resistance to wheat powdery mildew, named from *Pm*1 to *Pm*21 (including multiple alleles at *Pm*3 and *Pm*4 loci), have so far been identified in wheat (McIntosh, 1993). The majority of these genes originated in wild relatives of cultivated wheat, including *Triticum dicoccum, T. dicoccoides, T. timopheevi, T. carthlicum, Haynaldia villossa, Aegilops spp.* and *Secale cereale* (Jin, 1996). Among them, *Pm*10, *Pm*11, *Pm*14 and *Pm*15 confer resistance to mildew on *Elytrigia* Desv (Jia, 1990) but not to wheat mildew. *Pm*12, *Pm*13, *Pm*16, *Pm*18, *Pm*19, *Pm*20 and *Pm*21 have only recently been transferred from related species into wheat and have not yet been released in wheat varieties. The 14 known resistance genes used in the work reported here are therefore those most widely used in wheat breeding.

Among the virulences matching the five known *Pm* genes, only virulence on *Pm*1 and *Pm*8 showed significant variation between regions. Others, such as those matching *Pm*4a and *Pm*6, revealed regional differences, but the corresponding resistance genes

were not identified in local varieties. In order to analyse the structure and dynamics of the *E. graminis* f. sp. *tritici* population in central China, therefore, it is better to select an appropriate set of local varieties. This set has been used to investigate the initiation of wheat mildew epidemics and the movement of pathogen populations (Yu, 2000c). The purposes of many pathogen surveys, in which a set of differential varieties is used to identify virulences, are the early detection of the risk of host resistance being overcome by new, virulent pathogens and the monitoring of changes in the frequencies of virulences, as in the UK Cereal Pathogen Virulence Survey (UKCPVS; Bayles *et al.*, 1997). The differential varieties in the UKCPVS either have identified specific resistance genes or resistances which are not identified, but are relevant to current cultivars or to breeding programmes. The set of varieties established in this study is not appropriate for these purposes, as it cannot provide information about risks of newly introduced resistances because no new resistance is included in the set and because of the paucity of information about the resistance genetics of Chinese wheat varieties. *E. graminis* f. sp. *tritici* can survive the summer in the highland epidemic zones but not in the lowlands (Yu, 2000a). This may lead to more efficient use of most resistance, for example in regional deployment of resistance genes. The differential set established here may therefore assist the strategies of using resistance to control wheat mildew in central China by tracking the regional variation and dynamics of the pathogen population.

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Structure, Evolution and Movement of Wheat Powdery Mildew Population in Hubei Province, China

Summary

A total of 1750 single colony isolates of *Erysiphe graminis* f. sp. *tritici* was collected at different altitudes in central China in spring and late summer in 1996 and 1997. Their virulence patterns were determined on a set of ten wheat cultivars. The structure of the pathogen populations at both high and intermediate altitudes were similar to each other in spring and in September in both years, suggesting that the populations at these two altitudes can be treated as a single population. However, the structure of the pathogen population in spring was significantly different from that in late summer in the highlands in both years. In the lowlands where the pathogen cannot oversummer, the structure of the population in 1996 was significantly different from that in 1997, which implies that wheat mildew in the lowlands is re-established each year. The structure of the pathogen population in the lowlands was similar to that in the highlands in spring 1996. This is consistent with the hypothesis that the highlands provide initial inoculum of powdery mildew for the wheat crop in the lowlands. In 1997, however, the structure of the pathogen population in the lowlands was very different from that in the highlands in spring, indicating that the highland population is not the only the initial source of wheat powdery mildew in the lowlands. Other, external sources must also be involved in initiating the epidemics of wheat mildew in the lowlands, These may include neighbouring regions of Sichuan province and Shaanxi province. Pathotypes were very diverse. Only simple pathotypes 0 and 1 were frequent in the late summer population, but they were not dominant in spring. No consistent associations between virulences were detected over time or regions. This is consistent with sexual reproduction playing a very important role in the structure and evolution of the population of *Erysiphe graminis* f. sp. *tritici* in central China.

Key words: Powdery mildew, *Erysiphe graminis* f. sp. *tritici*, *Blumeria graminis* f. sp. *tritici*, structure, recombination, wheat.

Introduction

Powdery mildew, caused by the obligate fungus *Erysiphe graminis f. sp tritici*, is one of the most important diseases of wheat. It causes significant losses in production in many parts of the world (Herbert *et al.*, 1948; Large and Doling, 1962; Smith and Smith, 1974; Liu 1986). *E. graminis* f. sp. *tritici* is a wind-dispersed pathogen. Evidence for long-distance dispersal of spores and its epidemiological consequences have been recognized for many obligate wind-dispersed pathogens, including rust (Zadoks 1967; Hermansen *et al.*, 1976, 1978; Roelfs 1985), downy mildew (Popular 1981; Davis *et al.*, 1986) and powdery mildew fungi (Schnathorst, 1959, 1965). Analysis of European barley mildew indicated that the entire population of the pathogen may move across the European continent in an easterly direction and that large parts of Europe should be considered as a single epidemiological unit (Andrivon and Limpert 1992). Although the general tendency is for the barley mildew pathogen to move from west to east in Europe, wind dispersal allows epidemics to be initiated by spores moving in all directions (Brown, 1994, 1995a,).

Various strategies for the control of plant diseases caused by wind-dispersed pathogens have been investigated. One common strategy has been to deploy varietal resistance. In order to make the effective rate of dispersal of pathogens slower and to reduce the rate of breakdown of resistance, the distribution of different resistance genes in different regions or countries on the pathogen dispersal pathway has been suggested (Person *et al.*, 1976). Understanding pathogen populations, especially their structure, evolution and movement is particularly important for effective use and maintenance of crop resistance.

Hubei province is located in central China. Geographically, the land slopes down from the northwest to the east. The northwest region is mountainous. The Qinling Mountain range forms a barrier from west to east along the northern boundary of the region, reducing the frequency and strength of cold fronts moving into the area in winter. The Daba, Wudang and Jinshan mountain ranges bound the region in the south, and protect the area from hot, wet weather coming from the south in summer (Fig. 1). These geographical characteristics give the region a relatively mild climate. Wheat volunteers can therefore grow in this area in summer and powdery mildew colonies can be found on them in late summer as well as on seedlings in autumn. By contrast, in the lower hills and lowland plains, such as the Jianghan plain, it is very hot and damp in the summer, no volunteer can survive and wheat mildew is never found on

seedlings in autumn (Yu, 2000a). The start of epidemics of mildew has always been in late February and early March, one month later than in the mountainous area in spring. This suggests that the volunteers bridge the gap between wheat crops in the northwest region of the province, and that mildew in the highlands initiates epidemics in the lowlands.
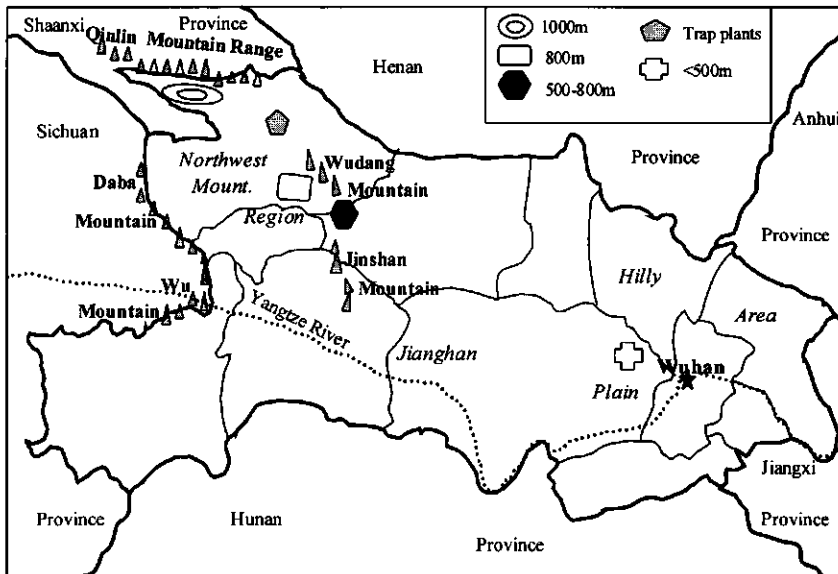
Figure 1. Map of Hubei Province showing collection sites of isolate of *Erysiphe graminis* f. sp. *tritici*.

The objectives of this study are to test this hypothesis about the movement of *E. graminis* f. sp. *tritici* population and to investigate whether mildew from other areas contributes to epidemic initiation in the lowlands. This analysis of the structure and evolution of the wheat powdery mildew population is of particular importance for developing a strategy for disease control in central China.

Material and Methods

Isolate collection

Isolates of powdery mildew were collected from wheat growing areas at different altitudes (Fig. 1) and at different times (Table 1) during 1996 and 1997. Single colony

isolates were directly sampled at random from diseased leaves. Leaf pieces with single colonies were put in plastic boxes with small wells filled with 5 g L^{-1} agar containing 50 mg L^{-1} benzimidazole in order to keep the isolates alive while being brought to the laboratory. In late summer, isolates were collected from diseased leaves of volunteers at high altitudes (800-1200m above sea level). To ensure a successful collection from the oversummering population, seeds of a universal susceptible variety, Min169 (Yu, 2000b) were sown before the wheat harvest in the highlands to trap the pathogen and colonies were collected as described above. In order to avoid high temperatures during transport from the location where isolates were collected to the laboratory, the plastic boxes were kept in cooler box with ice.

Table 1. Collections of isolates of *E. graminis* f. sp. *tritici* in Hubei in1996 and 1997

Locations according to altitudes	Time		
	Feb.	April	Sept.
<500m		Collection	
500-800m	Collection	Collection	
800m			Collection
1000m		Collection	Collection

Virulence tests

Virulences of the isolates were tested on a differential set of 10 cultivars (Table 2; Yu, 2000b). 10-day old seedlings were sown in contamination-proof conditions in a

Table 2. Differential cultivars and their order for binary coding of pathotypes

Cultivars	Order
E81513	1
Emai11	2
Jin66	3
Hua8	4
Zhen831	5
Su3	6
Mian8724	7
E'an1	8
Hx8541	9
Min169	Susceptible control

growth chamber. Subsequently 3cm sections were cut from the middle of primary leaves and placed in petri dishes containing 5 g L^{-1} agar amended with 50 mg L^{-1} benzimidazole. They were inoculated with conidia of *E graminis* f. sp. *tritici* isolates in an inoculation tower made of plastic beakers at an inoculum density of 250-400 conidia cm^{-2} of leaf segment (Limpert *et al.*, 1990). Two replicates of each cultivar were used per isolate. The petri dishes were incubated in constant light (960 lux) at a temperature of 17°C for 10 days.

Scoring and pathotype coding

Infection types were assessed after 10 days incubation. To avoid scoring errors, all batches were scored by a single person. The scale of Moseman (1984), modified by Shi *et al.* (1987), was used:

0 = immune, no visible sign of infection or necrosis and no mycelium;

1 = resistant, increasing from no mycelium to little mycelium, sometimes with necrosis;

2 = moderately resistant, with an increased amount of mycelium, little conidiospore production, no necrosis;

3 = moderately compatible, with a large amount of mycelium, moderate conidiospore production, no necrosis or chlorosis.

4 = completely compatible, with a large amount of mycelium and substantial conidiospore production.

Infection types of 0, 1 or 2 were classified as avirulent and 3 and 4 as virulent on a given cultivar. Pathotypes were coded by the binary system developed by Habgood (1970), by the following equation:

$$\text{Pathotype} = \Sigma_{i=1-9} \text{ reaction}_i \times 2^{(i-1)}$$

where *i* is the order number of the cultivars in the set (Table 2), reaction is the infection type on differential cultivar, a compatible reaction (IT3 or 4) is given a value of 1, and an incompatible reaction (IT0-2) a value of 0.

Result

Virulence frequency

Virulences are denoted by V-cultivar (Table 3). In order to compare virulence frequencies in the lowlands and highlands, the frequencies of samples from the highlands were considered as a whole (columns five and column eight in Table 3). Virulence frequencies of successive pairs of samples and between highlands and lowlands in spring were compared by X^2 tests.

The frequencies of all virulences sampled in the 500-800m area were stable between February and April in both years except for V-Su3 in 1996 ($p < 0.05$) and V-E81513 in 1997 ($p < 0.01$).

There were few significant differences in the frequencies of virulences between intermediate and high altitudes in either spring or summer in either year. The frequency of V-Mian8724 at high altitudes was significantly higher than at intermediate altitudes in 1996, but significantly lower in 1997. There were also significant differences in the frequency of V-Hua8 and V-E'an1 between the two zones in 1996 and in V-Su3 in 1997. All these effects were relatively small ($0.05 > p > 0.01$). In September samples, only one virulence frequency, that of V-Email1, differed between high and intermediate altitudes in 1996.

There was no significant difference in the frequencies of virulences between the lowlands and highlands in April 1996 except for V-Su3 and V-Hx8541 which had slightly lower frequencies ($0.05>p>0.01$) in the lowlands in 1997, presenting a complete contrast to 1996. Frequencies of V-Jin66, V-Hua8 and V-Mian8724 were significantly higher ($p<0.001$) in the lowlands than in the highlands, while frequencies of V-E81513, V-E'an1 and V-Hx8541 were also higher.

There were no significant differences in virulence frequencies for samples from volunteers or from trap plants in either year except for those of V-E81513 ($p<0.01$) and V-Jin66 ($p<0.05$) in 1997.

There were dramatic seasonal changes in the frequencies of most virulences in the highlands in both years. Frequencies of all virulences decreased from spring to summer in 1996. All except V-Hua8 were restored to a higher level in the next spring, then all except for V-Zhen831 decreased again in summer. In the lowlands, frequencies of most virulences increased significantly between 1996 and 1997 except for those of V-Zhen831, V-Su3 and V-E'an1.

Table 3. Frequencies of virulences (%) in populations of *Erysiphe graminis* f. sp. *tritici* in Hubei during 1996 and 1997

Virulence	500-800m	500-800m	1000m	500-1000m	<500m	800m	1000m	800-1000m	Trap plants
	Feb.	April	April	April	April	Sept.	Sept.	Sept.	Sept.
					1996				
V-E81513	64.5	66.7	73.0	69.7	62.6	64.3	55.4	60.6	59.7
V-Email1	27.4	41.9	31.1	36.7	29.8	13.6	5.9*d	10.4****e	10.4
V-Jin66	27.4	27.2	23.0	25.2	32.7	22.9	14.9	19.5	25.4
V-Hua8	46.8	43.2	25.7*b	34.8	28.1	27.9	26.7	27.4	35.8
V-Zhen831	3.2	6.2	8.1	7.1	8.8	4.3	5.0	4.6	4.5
V-Su3	22.6	42.0*a	27.0	34.8	24.0*c	12.1	5.9	9.5***	10.4
V-Mian8724	58.1	50.6	66.2*	58.1	55.6	15.7	8.9	12.9***	22.4
V-E'an1	48.4	40.7	56.8*	48.4	48.5	23.6	24.8	24.1***	14.9
V-Hx8541	58.1	58.0	44.6	51.6	40.4*	37.1	38.6	37.7**	28.4
Sample size	62	81	74	155	171	140	101	241	67

Table 3. (Continued)

Virulence	500-800m Feb.	500-800m April	1000m April	500-1000m April	<500m April 1997		800m Sept.	1000m Sept.	800-1000m Sept.	Trap plants Sept.
V-E81513	84.5	71.0**	71.8	71.9***[g]	83.9*	(***[h])	56.5	60.1	58.7***	72.4**[f]
V-Emai11	49.2	39.8	28.2	34.6***	44.3	(*)	13.1	13.5	13.3***	20.0
V-Jin66	37.0	31.3	38.8	34.6***	55.7***	(***)	8.9	6.3	7.4***	13.8*
V-Hua8	30.4	28.9	25.2	27.3	58.5***	(***)	30.4	24.7	27.1	31.7
V-Zhen831	9.9	4.7	8.7	6.5	10.4		10.7	6.3	8.2	9.7
V-Su3	23.2	32.8	20.4*	27.3***	28.3		14.3	18.4	16.6**	23.5
V-Mian8724	17.7	25.8	12.6*	19.9*	68.9***	(*)	14.3	12.1	13.0*	17.9
V-E'an1	55.2	51.6	40.8	46.8***	57.5*		22.6	16.6	19.2***	24.8
V-Hx8541	64.1	55.5	62.1	58.4***	71.7*	(***)	39.6	35.4	37.1***	45.5
Sample size	181	128	103	231	106		168	223	391	145

Significance of X^2 test for comparisons of virulence frequencies.[a] at 500-800m between Feb. and April; [b] 500-800m and 1000m in April; [c] 500-1000m and <500m in April; [d] 800m and 1000m in Sept.; [e] 500-1000m in April (column five) and 800-1000m (column nine) in Sept.; [f] trap plants and volunteers at 800-1000m in Sept.; [g] Sept. 1996 and April 1997 in highlands; [h] 1996 and 1997 in lowlands.   *: $0.05 \geq P > 0.01$, **: $0.01 \geq p > 0.001$, ***: $P < 0.001$.

Pathotype composition and its dynamics

Each sample was composed of many, diverse pathotypes. A total of 696 isolates from the seven samples were tested in 1996. Among these isolates, 204 pathotypes identified with the differential set of cultivars were detected. 241 pathotypes were detected in a total of 1054 isolates from seven samples in 1997. Only eight pathotypes had frequencies higher than 5% in any one sample (Table 4). In the highlands, pathotypes 0 and 1 were frequent in September populations in both years, but not in spring populations. In the lowlands, only pathotype 0 had a frequency higher than 5% in 1996 while no pathotype reached a frequency higher than 5% in 1997.

Table 4. Regional frequencies of the major pathotypes of *Erysiphe graminis* f. sp. *tritici* at three sampling times during two years [a]

Code	Pathotypes Virulence	500-800m Feb	500-800m April	1000m April	<500m April	800m Sept	1000m Sept	Trap plants Sept
				1996				
0		1.6	6.2	1.4	5.8	10.7	13.9	16.4
1	V-E81513	4.8	2.5	1.4	2.3	14.3	21.8	16.4
9	V-E81513 V-Hua8	0.2	2.5	1.4	0.6	2.1	2.0	9.0
201	V-E81513, V-Hua8 V-Mian8724, V-E'an1	9.7	0.0	0.1	0.0	0.0	0.0	1.5
256	V-Hx8541	6.5	6.2	1.4	1.2	2.9	7.9	1.5
384	V-E'an1 V-Hx8541	6.5	1.2	0.0	3.5	0.0	3.0	0.0
				1997				
0		0.6	3.9	1.0	0.9	18.4	20.6	10.3
1	V-E81513	2.2	1.6	4.8	1.9	11.3	13.4	11.7
256	V-Hx8541	1.1	0.0	3.9	0.0	7.7	5.8	2.8
257	V-E81513, V-Hx8541	1.1	3.1	5.8	0.0	5.4	5.4	6.2
260	V-Jin66, V-Hx8541	0.0	0.6	5.8	0.0	0.0	0.0	0.0

[a] Major pathotypes present in at least one sample at a frequency higher than 5% are listed. Each pathotype is denoted by the code and the virulences it carries.

Association of virulences

Associations between virulence phenotypes in the *E. graminis* f. sp. *tritici* population were investigated by means of the covariance of their frequencies. Mathematically, the covariance is equal to the gametic disequilibrium statistic when each virulence is controlled by a single gene. In the present case, the number of genes controlling virulence on each differential variety is not known; more than one virulence gene is probably involved in each case (Yu, 2000b). As *E. graminis* f. sp. *tritici* is haploid, the covariance (*D*) can be calculated as follows:

$$D = p \ (VxVy) - p(Vx)p(Vy).$$

Where, p (VxVy) is the frequency of isolates possessing both virulences, and p (Vx) and p(Vy) are the frequencies of the individual virulences. Contingency tables were constructed with the four phenotypes VxVy, VxAy, AxVy and AxAy, where V denotes virulence and A avirulence. The independence of the two virulences was examined by a X^2 test.

Table 5.Covariances (*D*) between pairs of virulences in population of *E. graminis* f. sp. *tritici* in Hubei (figure = *D* x 10) [a]

Pairs of virulences	5-800m Feb.	5-800m April	1000m April	<500m April	800m Sept.	1000m Sept.	Trap Sept.
1996							
V-E81513-V-Emai11	0.327	0.658**	0.435	0.648***	0.056	0.166	0.123
V-E81513-V-Jin66	0.489	0.412	0.351	0.758***	0.017	0.266	0.575*
V-E81513-V-Hua8	1.015***	0.699**	0.153	0.407*	-0.138	0.201	1.353***
V-E81513-V-Hx8541	-0.494	0.206	-0.042	0.224	0.666***	-0.260	0.695**
V-Jin66-V-Hua8	0.052	1.296***	0.356	0.133	0.363*	0.296	0.285
V-Jin66-V-Hx8541	-0.330	0.276	-0.214	0.433*	0.151	0.219	0.922***
V-Su3-V-Mian8724	0.624*	0.962***	-0.033	0.071	-0.119	0.046	0.363*
V-E'an1-V-Hx8541	0.255	0.022	0.172	0.381*	0.196	0.925***	0.025
1997							
V-E81513-V-Su3	0.027	0.220	0.186	0.360*	0.323*	0.465***	0.095
V-E81513-V-Mian8724	-0.058	0.056	0.064	0.255	0.145	0.349***	-0.057
V-E81513-V-E'an1	-0.029	0.278	0.177	0.263	0.482***	0.348**	0.271
V-Jin66-V-Hua8	-0.186	0.112	0.088	-0.028	0.384***	0.159*	0.321*
V-Hua8-V-E'an1	-0.077	0.306	0.233	0.501*	0.623***	0.174	0.178
V-Hu3-V-Mian8724	0.0327	0.022	-0.063	0.221	0.213*	0.450***	0.131

[a] Among 36 pairs of virulences, only associations that were significant with *p*<0.001 in at least one sample are shown in the Table.

There were no consistent associations between virulences over regions or time. V-E81513 showed more positive associations with other virulences in different locations and at different times in both years (Table 5). In the lowlands, two pairs, V-E81513 with V-Emai11 and with V-Jin66, showed highly significant, positive associations in

1996, but no significant positive association in 1997. In the highlands, significant positive association occurred in spring 1996, but not in summer 1996. The reverse was the case in 1997.

Discussion

Population sampling

In population studies of cereal mildew in Europe, pathogens are usually sampled from the air spora by a car-mounted wind impaction spore trap (Wolfe and Knott, 1982; Limpert *et al.*, 1990), or by trap plants exposed on the roofs of tall buildings at a reasonable distance from any host crop (Brown and Wolfe, 1990). In this study, the isolates were randomly sampled directly from diseased leaves. The source varieties were unknown, but, given the large number of wheat varieties grown in Hubei, these were almost certainly very diverse. There was no significant difference between virulence frequencies in the samples from trap plants and those from volunteers in September. This indicates that the samples obtained in this way were representative of the local populations even though they were not random samples from an aerial spore populations.

Structure and evolution of the pathogen population

The frequencies of virulences were stable from February to April in both years at intermediate altitudes. There were few significant differences between the *E. graminis* f. sp. *tritici* samples from intermediate altitudes or high altitudes in April or September in either year. This indicates that the powdery mildew fungus in the highlands of Hubei province can be considered as forming a single population.

The pathogen population in the lowlands differed significantly in the frequencies of most virulences between the two years. This is consistent with the hypothesis that wheat mildew does not survive the summer in the lowlands of Hubei province and must be re-established each year, as discussed later.

E. graminis f. sp. *tritici* pathotypes are very diverse in Hubei. No common pathotype was detected among the samples except for pathotype 0, avirulent on all differential varieties, and pathotype 1, virulent only on E81513, in summer

populations in highlands (Table 4). There were statistically significant ($p < 0.001$) associations between some virulences, but they were not consistent over regions or time (Table 5). These data are consistent with the hypothesis that *E. graminis* f. sp. *tritici* in the highlands of Hubei province passes through a sexual cycle each year (Yu, 2000a).

Sexual reproduction increases the genetic diversity of pathogen populations by disrupting associations between alleles and phenotypes (Brown and Wolfe, 1990). In the UK in 1985, sexual reproduction was roughly estimated to contribute a quarter of initial inoculum of *E. graminis* f. sp. *hordei* on winter barley (Brown and Wolfe, 1990). The absence of consistent gametic disequilibria and the high diversity of pathotypes in Hubei in 1996-7 confirms that the sexual stage plays an important role, not only in transmitting wheat powdery mildew between crops (Yu, 2000a), but also in the structure and dynamics of the population of *E. graminis* f. sp. *tritici* in Hubei.

The simple pathotypes 0 and 1 had much lower frequencies in spring samples than that in late summer (Table 4). The seasonal fluctuations in the frequencies of the simple pathotypes were paralleled by contrary fluctuations in the frequencies of seven of the nine virulence phenotypes studied, the exceptions being V-Hua8 and V-Zhen831. The frequencies of V-E81513, V-Email1, V-Jin66, V-Su3, V-Mian8724, V-E'an1 and V-Hx8541 fell in highland samples between April and September 1996, had risen again by February 1997 and then fell once more between April and September 1997.

The dynamics of the two simple pathotypes are similar to that of a pathotype of *E. graminis* f. sp. *hordei* with very few virulences in France which dominated the population in September (Caffier *et al.*, 1996). A possible explanation for these changes in virulence and pathotype frequencies lies in the role of recombination in the population biology of *E. graminis* f. sp. *tritici* in central China. The resistance of the nine differential varieties are genetically complex (Yu, 2000b), which implies that a phenotype of virulence on any of these varieties involves the loss of several avirulence alleles. If so, when avirulence genes are recombined in the sexual, over-summering population of *E. graminis* f. sp. *tritici* in the highlands of Hubei, the frequency of virulent phenotypes in the population will fall. This is because a mating between a virulent individual and an avirulent one will produce 25% or fewer virulent progeny if avirulence is conferred by two or more genes. Subsequently, cultivation of resistant varieties, sown in autumn, may cause selection for the corresponding

virulence phenotypes during the growing season of wheat, from September to April. These dynamics may account for the fall in the frequencies of most virulence phenotypes between April and September in both years and their rise between September 1996 and February 1997.

Gametic disequilibrium may arise from selection, migration, random genetic drift and mutation (Wolfe and Knott, 1982; Ostergard and Hovmoller, 1991; Brown, 1995b). Unlike barley mildew in the UK, where very few clones may dominate the population and gametic disequilibrium between virulences is a consequence of host selection for these clones (Brown, 1994), the associations observed in Hubei could not have been caused by one common clone or a few clones because the population of *E. graminis* f. sp. *tritici* in this province is so diverse. Other factors, such as those discussed by Wolfe and Knott (1982) and Ostergard and Hovmoller (1991), may have generated the associations observed in Hubei. Virulences other than those identified by our set of differential varieties may be involved in these population dynamics.

The frequencies of differential cultivars in regions of Hubei from 1995 to 1997 are listed in Table 6 (Anon. 1996, 1997, 1998). E81513 was released recently in

Table 6. Relative area (%) of cultivars grown at different altitudes

Cultivars	1995			1996			1997		
	1000m	500-800m	<500m	1000m	500-800m	<500m	1000m	500-800m	<500m
E81513	0	0	0	0	0	1.5	0	0	3.1
Emai11	0	0	12.8	0	0	7.6	0	0	4.5
Jin66	0	0	0	0	0	0.6	0	0	1.6
Hua8	0	2.3	1.6	0	9.1	5.2	0	2.9	11.4
Zhen831	0	0	0	0	0	0	0	0	0
Su3	0	0	0	0	0	0	0	0	0
Mian8724	24.9	23.7	0	11.2	8.9	0	8.7	4.9	0
E'an1	17.4	46.8	53.9	12.5	37.0	37.0	8.3	41.2	39.6
Hx8541	0	1.9	0.6	0	0	0.38	0	0	0

Hubei and is only grown in the lowlands over a relatively small acreage. However, in the population of *E. graminis* f. sp. *tritici*, the frequency of V-E81513 was the highest of those tested in all samples in both years. This may be explained as follows. E81513 was derived from an old, local cultivar, Emai6, which was important in Hubei during

the 1970s and is still grown now. E81513 may have inherited resistance genes from Emai6, so virulence on E81513 may have been selected indirectly. This hypothesis could be tested by genetic analysis of the mildew resistance of these varieties. However, such analysis has not been done for any wheat variety in central China.

V-Hua8 was selected by the resistant host variety. In 1996, although the fraction of the total area sown with Hua8 at 500-800m altitudes was much lower than in the lowlands, the frequency is marginally higher than in the lowlands, as were the frequencies of V-Hua8 though not significantly. In 1997, the area frequency of the cultivar increased substantially in the lowlands, and V-Hua8 subsequently rose to a frequency of 58.5%.

The cultivar Su3 has never been grown as a commercial cultivar, but has been used as germplasm in breeding for resistance to *Fusarium* head blight along the Yangtze River valley. Many cultivars grown in the valley are derived from Su3, so V-Su3 may not be an unnecessary virulence and may have been maintained at a fairly high frequency as a consequence.

Changes in the frequency of V-Mian8724 have important implications for the structure of wheat mildew pathogen populations in Hubei. V-Mian8724 was at high frequencies in the four samples collected in spring 1996, but decreased dramatically in summer at high altitudes. In the following spring, it increased slightly at high altitudes, but in the lowlands, it was even higher than in the highlands in 1996. These changes may be attributed partly to selection for the virulence exerted by the matching host and partly by immigration. Mian8724 was a very popular cultivar in the mountainous area before 1995, occupying almost a quarter of the wheat area (Table 6). This would have provided a large population of volunteers for oversummering mildew in 1995. It was therefore at a high frequency in the following spring. However, there would have been fewer volunteers of Mian8724 in 1996 because the area cultivated decreased to 10%. The frequency of V-Mian8724 therefore followed the frequency of the matching host closely. It was notable that in the lowlands V-Mian8724 had a high frequency although the corresponding host had been never grown there (Table 6). This may be attributed to migration of *E. graminis* f. sp. *tritici* from the highlands to the lowlands, as discussed below.

Movement of wheat mildew populations

The wheat mildew pathogen cannot oversummer in the lowlands of Hubei, but does so in the highlands of the province (Wang, 1984; Yu, 2000a). This led to the hypothesis that the initial inoculum for epidemics of wheat mildew in the lowlands of Hubei originates in the highlands. There were no significant differences in the frequencies of virulences between the highlands and the lowlands in spring 1996 (Table 3) and this is consistent with the hypothesis about the movement of *E. graminis* f. sp. *tritici* from highlands to the lowlands. However, frequencies of pathotypes and of individual virulences were very different between the highlands and the lowlands in spring 1997. This indicates that other sources must contribute to wheat mildew epidemics in the lowlands. One possible source is suggested by the changes observed in the frequency of V-Mian8724. In the lowlands of Hubei, Mian8724 and related cultivars have never been grown (Table 6), but the frequency of the corresponding virulence was high in both years (Table 3). In the neighbouring provinces of Sichuan and Shaanxi, southwest and northwest of Hubei respectively, Mian8724 and related cultivars covered 1.5 million hectares from 1990 to 1997 (Anon. 1991-1998). This would surely have caused selection for the V-Mian8724 phenotype. Neighbouring areas of Sichuan and Shaanxi may therefore have contributed initial inoculum for the lowlands of Hubei. Such movement of the mildew pathogen population was demonstrated for *E. graminis* f. sp. *hordei* in Europe (Andrivon and Limpert 1992). The frequency of Va13 was nil in eastern Lower Austria in 1984, but rose to 7% in 1985 and to 30% in 1986. Prior to 1986, cultivars with the corresponding resistance gene, Mla13, covered only 2-4% of the barley area in this part of Austria, but were very popular in neighbouring Czechoslovakia, where they covered about 75% of the spring barley area. Immigration of Czechoslovak pathotypes was therefore postulated to be responsible for the dramatic increase of Va13 in northeastern Austria. This is very similar to the situation regarding V-Mian8724 in Hubei.

Although the population of *E. graminis* f. sp. *tritici* in the highlands of Hubei may not be isolated from those in other regions, it is likely that the local, oversummering population is sufficiently large that immigrant *E. graminis* f. sp. *tritici* from neighbouring provinces has relatively little influence on the structure of population in the highlands of Hubei.

Hubei province is located in the centre of the subtropical monsoon in China. In the spring before the monsoon season, the predominant winds in Hubei are northwesterlies, governed by the high pressure in the Bay of Bengal and partly obstructed by the Himalaya mountain range. This characteristic of the climate may promote migration of pathogen populations from west to east in central China. The movement of *E. graminis* f. sp. *tritici* contributes not only inoculum to epidemics, but also diversity of virulences to the population structure.

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Variation in the Responses to Triadimefon of the Population of *Erysiphe graminis* f. sp. *tritici* in Hubei, China

Summary

Variation in responses to triadimefon of *Erysiphe graminis* f. sp. *tritici* in central China was studied during 1996 and 1997. In 1996, the mean ED50 was stable from February to April but was lower in the oversummering populations in September with an increased proportion of sensitive isolates in the mountainous highlands. This may have been caused by immigration of the pathogen population into Hubei from areas where less triadimefon was used or by a cost of reduced sensitivity in terms of fitness. In 1997, the mean ED50 continually increased from February to September. Sensitive isolates were almost eliminated or were at very low frequencies in the spring population and the oversummering population. Wheat mildew was severe throughout the province in 1997, which led to a large amount of triadimefon being applied. Isolates with reduced sensitivity may therefore have been selected by fungicides applied to crops. The mean ED50 stayed stable in the lowlands in Hubei in both years. Variation of responses of *Erysiphe graminis* f. sp. *tritici* to the fungicide was greater in oversummering population than in spring population in the highland in both 1996 and 1997. It was larger in 1996 than in 1997 in both the highlands and the lowlands. There was wide variation of responses to the fungicide in the pathogen population, covering a 370-fold range of estimated ED50s. This indicates there has been a reduced performance of triadimefon in controlling wheat powdery mildew in Hubei although one cannot definitely conclude that there has been a shift towards resistance in the pathogen population because of a lack of wild type sensitive isolates.

Key words: *Erysiphe graminis* f. sp. *tritici*, triadimefon, fungicide responses, variation of responses

Introduction

Powdery mildew of wheat, caused by the obligate biotrophic fungus *Erysiphe graminis* f. sp. *tritici*, is now a major disease in large parts of China. Fungicide application is one of the main methods used to control this disease. In the 1970s, emulsion of carbendazim, a benzimidazole fungicide, or sulphur mixed with carbendazim, were the fungicides most commonly used to control wheat powdery mildew. In the early 1980s, triadimefon, a systemic fungicide of the triazole family, was introduced into China under the commercial label of Bayleton to control rusts and powdery mildews of many crops. Chinese agrochemical companies produced triadimefon immediately after it was registered in China and it became a major fungicide for protection of wheat against disease, as in other crops. In Hubei province it is used not only as a seed treatment to control initial infections of mildew and rusts of wheat, but also as a foliar spray when disease epidemics occur. Owing to its broad spectrum of anti-fungal activity, it is now used in Hubei to control not only powdery mildew and rusts but also used to control wheat sheath blight, *Pellicularia gramineum*, when applied either as a single compound or mixed with other fungicides. Triadimefon is therefore applied quite frequently to wheat even in years when mildew is not the main target disease. This may exert strong selection for resistance to triadimefon on the population of *E. graminis* f. sp. *tritici.*

Triazoles are members of the demethylation inhibitor (DMI) group of compounds, which inhibit the sterol C-14 demethylation step in the synthesis of ergosterol (Dekker, 1985). Resistance of the barley mildew pathogen, *E. graminis* f. sp. *hordei*, to a closely related fungicide, triadimenol, was detected in the UK in 1980, three years after its introduction into use in agriculture (Fletcher and Wolfe, 1981). Resistance of wheat mildew pathogen to triazole fungicides was detected in Europe in 1986 (de Waard *et al.*, 1986). After resistance to triazole appeared, the frequency of resistance in cereal mildew populations increased in much of Europe in the years following (Limpert, 1987).

At the time when triadimefon was first put into use in China, good control could be achieved throughout the cropping season by a single application at the booting stage with an active ingredient dose of 120g per hectare (Ge, 1984; Li, 1983; Wu, 1983). Now two or three applications with an active ingredient dose of 135 to 150g per hectare are needed to achieve good control. It appears that the effectiveness of triadimefon has reduced, possibly because resistance to the fungicide in the mildew pathogen population has developed. This paper describes variation in responses of *E. graminis* f. sp. *tritici* to triadimefon in Hubei province, central China.

Materials and Methods

Mildew isolate sources

Isolates were collected from various parts of Hubei province in regions defined by altitudes, <500m, 500-800m and 800-1000m above sea level, during 1996-1997. Details of the samples are given in Table 1. In spring, isolates were collected directly from diseased leaves in wheat fields. Leaf pieces, each with a single colony, were placed in plastic boxes with small wells filled with 5 g L^{-1} agar amended with 50 mg L^{-1} benzimidazole and transported to the laboratory. Each colony was put in an individual well to avoid cross-contamination. Isolates from oversummering populations at high altitude were collected directly from diseased leaves of wheat volunteers as above. In addition, trap seedlings of a universally susceptible variety, Min169 (Yu, 2000) were exposed in the mountainous area in northwestern Hubei province, 800-1000m above sea level. When the isolates had been taken to the laboratory, they were multiplied on fresh leaf segments of Min169 under continuous light of 1000 lux at 17 ± 1°C to produce enough inocula for tests of fungicide responses.

Table 1. Details of samples of single colony isolates of *E. graminis* f. sp. *tritici* from Hubei province in 1996 and 1997

Altitudes	500-800m	800-1000m	<500m	800-1000m	Trap plants 800-1000m
Sampling time	Feb	April	April	Sept	Sept
1996	54	124	80	189	52
1997	51	108	53	278	88

Fungicide response tests

Fifty seeds of Min169 were sown in 9cm-diameter pots in a spore-proof growth chamber at 20°C. Triadimefon powder, 98% active ingredient, made by Jianhu agro-chemical company and kindly provided by Prof. Liu, Institute of Agrochemical Inspection, Ministry of Agriculture of China, was dissolved in acetone as a stock solution. A dosage series of 0.0, 0.15, 0.3, 0.6, 2.4, 4.8 and 9.6 mg L^{-1} was used, following preliminary experiments. In each batch, five pots of 10-day old seedlings were placed in a box, 40 x 40 x 65cm, on top of which a mini-sprayer was mounted

for applying the fungicide. 35 ml of the fungicide solution of each dose, to which two drops of Tween80 were added as a surfactant, were applied to the seedlings. The seedlings were then left overnight. The following day, 4cm segments, cut from the middle of primary leaves, were placed in petri dishes containing water agar with benzimidazole. Five leaf segments treated with each dose were used to test each isolate. The leaf segments were inoculated at a density of 250-400 conidia cm^{-2} under a settling tower. The petri dishes were incubated in constant light at 18° C. Three isolates were included in all tests as controls to check variation between batches.

Scoring and data analysis

Visible colonies were counted after 10 days incubation. A median effective dose was estimated for each isolate by probit analysis, using Wadley's method, which is appropriate when only surviving colonies can be counted but not those that have been killed. Colony numbers were fitted to the logarithm of the dose. ED50s were calculated by the probit analysis procedure in Genstat 5 (Payne, 1993). Student's t-tests of differences between samples were carried out on log (ED50) values. Samples from 1000m zone and the 500-800m zone were pooled as a single highland sample for the purpose of this analysis.

Results

Dynamics of response to triadimefon

A total of 1077 isolates (Table 1) were tested for responses to triadimefon during 1996-7. In 1996, there was no significant difference between the mean ED50s of populations sampled in the highlands in February and in April or between those in the highlands and the lowlands in April (Table 2). The mean ED50 decreased from April to September in the highlands. The mean ED50 of isolates sampled from volunteers was highly significantly higher than that of isolates sampled from trap plants.

The mean ED50 in the highland sample in February 1997 did not differ significantly from that in the highlands in September 1996, but that in the sample in April was significantly higher than in September 1996. In 1997, the mean ED50 increased from February to April and continued to increase from April to September in the highlands. However, there was no significant difference between the highland and the lowland samples in April. The mean ED50 of volunteer sample was similar to

that of trap plant sample. From September 1996 to February 1997, the mean ED50 in the highlands kept stable. In the lowlands, the mean responses were similar in April 1996 and April 1997.

Table 2. Mean ED50s of triadimefon between samples of *E. graminis* f. sp. *tritici* in Hubei[§]

	Highland February	Highland April	Lowland April	Highland September	Trap plants September
1996	1.0806	0.8917 ns [a]	0.7159 ns [b]	0.6759 * [c]	0.4114 ** [d]
Ck	0.6646	0.8209	0.8115	0.6431	0.9069
1997	0.6742 ns [e]	0.8372 * [a] (*) [f]	0.7599 ns [b] (ns) [g]	1.4105 ** [c]	1.0850 ns[d]
Ck	0.7399	0.7853	0.8047	0.7491	0.7019

[§] Student's t-tests. [a] Between February and April in highlands; [b] Between highlands and lowlands in April; [c] Between April and September in highlands; [d] Between highlands and trap plants in September; [e] Between highlands Sept 96 and highlands Feb 97; [f] Between highlands Sept 1996 and April 1997; [g] Between lowlands April 1996 and April 1997. ns: no significant difference; *: $0.05 > p > 0.01$; **: $p < 0.01$.

As no wild type isolates were maintained before triadimefon was introduced into practical use in China, three isolates were arbitrarily selected from domestic populations as control isolates to check variation among batches of tests. The mean ED50s of the control isolates for the tests of populations sampled in February and September in the highlands were smaller and for the population sampled from trap plants larger in 1996 than those in the tests for other samples in which the total mean ED50s were similar among one other in both 1996 and 1997 (Table 2). The standard deviations of Ed50s in these control isolates in all batches are 0.386, 0.514 and 0.450 respectively. Although the variation between batches was small, the extreme variation was six-fold in the mean ED50s of control isolate 1 and nine-fold for control isolate 3 in the tests.

Variation in responses to triadimefon

Responses of *E. graminis* f. sp. *tritici* to triadimefon were more variable in both spring and summer in 1996 than in 1997 (Table 3, Figure 1). In the highlands in 1996, the proportions of sensitive isolates slightly increased from February to April and then
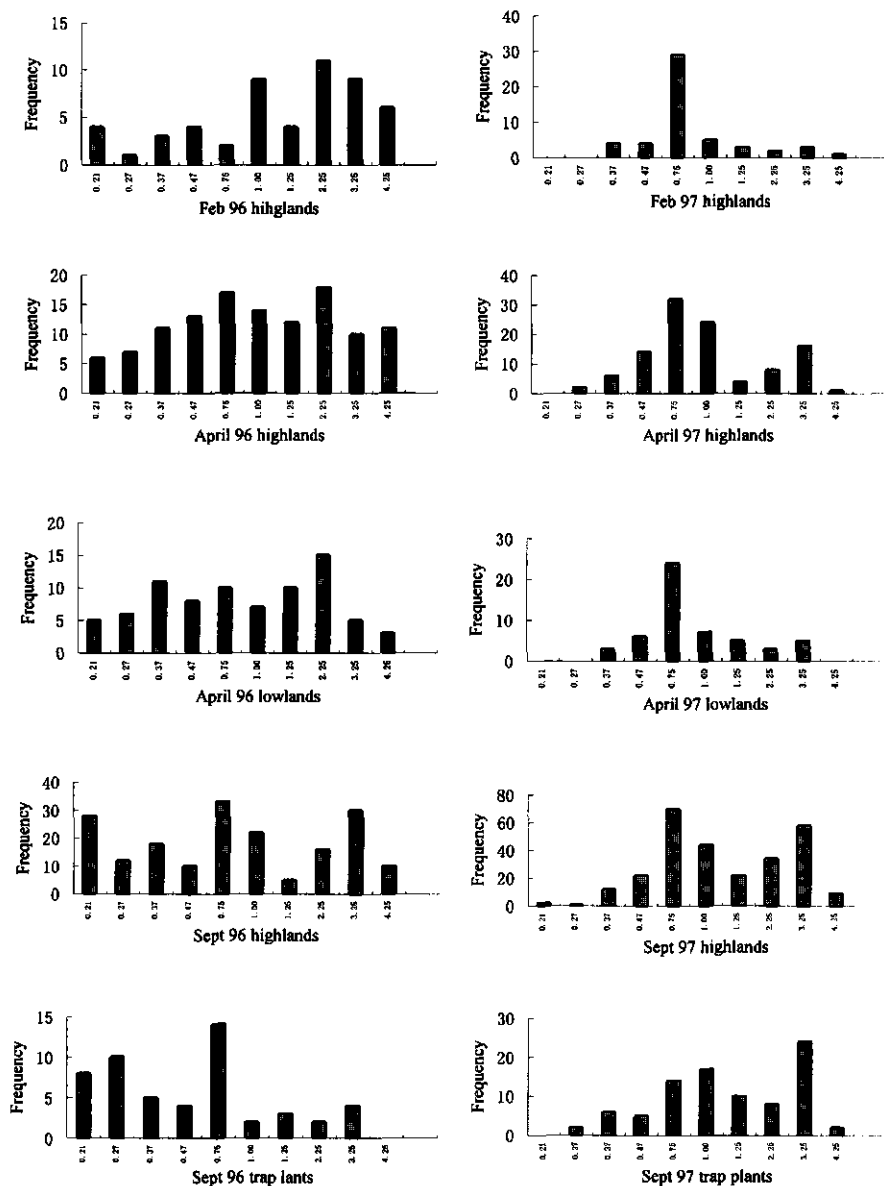
Figure 1. ED50 frequencies of triadimefon in *E. graminis* f. sp. *tritici* populations in Hubei province in 1996 and 1997.

increased further in September. Variation in the lowlands was similar to that in the highlands in April 1996. In 1997 from February to September, the proportions of sensitive isolates were small in the highlands compared to those in 1996. Although some very sensitive isolates were detected in September population in the highlands, the proportions of more resistant isolates increased. In the lowlands, variation of the response of *E. graminis* f. sp. *tritici* was also great in 1996 compared to that in 1997.

Table 3. Variation in responses of *E. graminis* f. sp. *tritici* to triadimefon in Hubei

	1996			1997		
	Minimum	Maximum	s. d. of log (ED50)	Minimum	Maximum	s. d. of log (ED50)
Highlands Feb	0.0503	4.5722	1.035	0.2871	3.2511	0.545
Highlands Apr	0.0615	4.6927	0.922	0.2511	4.6739	0.657
Lowlands Apr	0.0720	3.7285	0.906	0.2991	2.9037	0.566
Highlands Sept	0.0133	4.9234	1.224	0.1782	4.7731	0.919
Trap plant Sept	0.0476	2.6829	0.948	0.2357	3.6111	0.726

Discussion

There has been a reduced performance of triadimefon in controlling wheat powdery mildew in Hubei province. Variation in responses of *E. graminis* f. sp. *tritici* to the fungicide was very wide, covering a 370-fold range of estimated ED50s (Table 3). The results in this paper give insights into the variation in the responses to the fungicide of *E. graminis* f. sp. *tritici* population in central China. Any conclusions about whether there has been a shift towards resistance to triadimefon in *E. graminis* f. sp. *tritici* populations since introduction of the fungicide cannot not be drawn in this paper because of a lack of wild-type control isolates. To determine the resistance level of cereal mildew pathogens to a fungicide, many authors (Limpert and Fischbeck, 1987, Brown and Wolfe, 1991) used wild-type sensitive isolates as standard control isolates in all batches of tests of target populations. Some authors used ratios of responses of target isolates to standard isolates as an indication of the level of resistance (Limpert, 1987). Sensitive isolates of *E. graminis* f. sp. *tritici* are available in Europe, further studies must be carried out to determine the fungicide resistance level by using European wild-type isolates as controls.

In the highlands, the mean ED50s increased continuously from February to September in 1997. The continual rise in mean ED50 can be attributed to two factors.

Firstly, sensitive isolates were almost eliminated (ED50 < 0.27 in Feb. and < 0.21 in April) from the spring populations, or were at very low frequencies (< 0.27 in April). Secondly, the proportion of insensitive isolates increased in the pathogen population in September although sensitive isolates, which were eliminated in spring, were detected (Fig 1). In 1997, wheat powdery mildew was severe throughout Hubei province. This situation led to a large amount of triadimefon being applied in March and April (Shi Shangbo, General Station for Plant Protection of Hubei Province, personal communication). The insensitive proportion of the pathogen population might be selected in spring 1997. This suggests that the reduced-sensitivity isolates continually dominated the pathogen population in September when a large amount of triadimefon was applied in late April.

By contrast, in 1996, the mean ED50s decreased from February to September in the highlands although there was no significant difference between the mean ED50 in February population and April population. The proportion of isolates which were more sensitive increased from February to April and the proportion of less sensitive isolates was stable (Fig 1). This is consistent with the change of mean ED50. This change in the response of the pathogen population might be caused by migration of population into Hubei from areas where less triadimefon was used. From April to September, the mean ED50 decreased significantly in the highlands. The proportion of insensitive isolates increased in September. Reduced fitness of *E. graminis* f. sp. *tritici* isolates with reduced sensitivity to fungicides has been reported (Al-Mughrabi and Gray, 1995, 1996; Engels and de Waard, 1996). The increase proportion of sensitive isolates in the oversummering population in the highlands in 1996 suggests that such a cost of resistance may also be responsible for the dynamics of responses to triadimefon in the wheat mildew pathogen population in central China.

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Assessment of Partial Resistance to Powdery Mildew in Chinese Wheat Varieties

Summary

Field trials in two cropping seasons and two locations were conducted on 60 Chinese autumn-sown wheat varieties to assess their partial resistance to powdery mildew. Mean levels of disease ranged from close to 0% to more than 90%. The method of inoculation and the location in which trials were conducted affected the relative performance of the varieties, but were much smaller than the main effect of variety. The area under the disease progress curve was highly correlated with final disease severity, but both were poorly correlated with apparent infection rate. Disease severity was regressed against frequencies of virulence in the *Erysiphe graminis* f. sp. *tritici* populations in the trial plots. A vertical distance (D) from the mean mildew severity to the fitted line was calculated for each variety. The D value can be used for classifying partial resistance. Five of the 60 varieties, Hx8541, E28547, Chun1066, Ze88pin6 and Lin5064, consistently expressed relatively low disease despite high frequencies of virulence to them and had consistently high D values. They may therefore, have good levels of partial resistance.

Key words: Wheat, *Erysiphe graminis* f. sp. *tritici,* partial resistance, virulence frequency, vertical distance.

Introduction

Plant resistance is one of the most important methods of controlling disease. Specific resistance genes have been extensively used in breeding. In plant pathogen, only one mutation is needed to change a pathogen from avirulent to virulent. Thus, race-specific resistance is readily overcome by matching virulent pathotypes when varieties containing these resistances are used on a large scale for a sufficient length of time. To prolong the effectiveness of specific resistance, several methods, such as "pyramiding", i.e. combining several resistance genes into a variety (Vanderplank, 1968), multilines (Browning and Frey 1969) and cultivar mixtures (Wolfe and Barrett 1977, Wolfe 1985) have been proposed or used. Another type of resistance, partial resistance, which is characterized by a compatible interaction in all growth stages, but lower infection frequency, a longer latent period, or a lower rate or a shorter period of spore production (Parlevliet and Van Ommeren, 1975; Kranz, 1983), has provided durable control of mildew in wheat (Shaner, 1973), barley (Jones and Davies 1985) and oats (Jones and Hayes 1985).

Wheat powdery mildew was a minor disease in China before the 1970s in terms of proportion of the acreage affected and the frequency of epidemics. It only caused yield losses occasionally in the southwest plateau of China. Since the early 1980s, wheat powdery mildew has been a serious disease because of changes in methods of cultivating wheat. Higher inputs of nitrogen fertilizer, expansion of the irrigated acreage and a shift of varieties from those of tall stature to semi-dwarf cultivars were significant changes. These improved yields, but also caused favourable conditions for powdery mildew. For instance, semi-dwarf varieties allow much denser plant populations to be grown and this high density provides favourable conditions for the powdery mildew fungus (Bennett, 1984). Since then, through the efforts of breeders and phytopathologists, several resistant varieties have been released, intentionally or otherwise, for commercial production in China. However, these varieties became susceptible to mildew when they were grown over a large area after four or five years, as in other countries (Wolfe, 1984; Bennett 1984; Brown, 1994). Hubei province has experienced such a situation. When the high yielding variety E'an1 was released in 1984, it was highly resistant, even immune, to mildew in the field (Gan, 1985). Five years after its release, the acreage grown with this variety reached about 800 thousand hectares in the province and neighbouring regions (Anon., 1990), and it became susceptible to mildew, probably because of changes in the pathogen population structure. It was seriously attacked by mildew and an estimated 5,000 tonnes of yield loss occurred in 1989 when favourable weather conditions also caused a high level of mildew. Farmers could not find suitable varieties to replace E'an1 because

progress in breeding was not sufficiently rapid to meet demand.

Although identified specific resistance genes are available for breeding, this situation would arise again if the lessons were not learnt from this experience. China has a vast wheat acreage and many diverse varieties. So far however, little attention has been paid to investigating durability of resistance to mildew. The objective of this study was to assess partial resistance to mildew in autumn-sown wheat varieties grown in central China.

Materials and Methods

60 varieties in commercial production and breeding lines (Table 1) were collected from autumn-sowing wheat growing parts of China, especially from the area along the Yangtze River valley. Field trials were conducted during the 1996-1998 growing seasons. In 1996-1997, only one trial was conducted, at the experimental station of Hubei Academy of Agricultural Sciences, Wuchang, Wuhan. Varieties were grown in 6-row plots, each 1.5m long with 25cm between rows. The plots were arranged in a randomized design with one replicate. No wheat was grown for 2km around the trial site. A very susceptible variety, Min169 (Yu, 2000), was grown in a drill along the aisles perpendicular to the rows of the plots, as a mildew spreader. In order to have enough disease to differentiate resistance and susceptible varieties, the trial was inoculated twice. The first inoculation of the spreader row was done in late November when the seedlings had reached the three-leaf stage with two leaves fully expanded. The inoculum consisted of 43 isolates collected in the summer of 1996 from the highlands in the mountainous region in the northwest of Hubei. The second inoculation was done in the middle of February with 45 isolates collected from the Jianghan Plain, in the southeast part of Hubei. The isolates were multiplied on detached primary leaves of Min169 placed in 9cm plates filled with 5 g L^{-1} agar supplemented with 50 mg L^{-1} benzimidazole and incubated in continuous light (1000 lux cm^{-2}) at 17°C. Each isolate was multiplied on two plates. Conidia from all isolates were harvested by tapping them into a plastic box. The mixed conidia were inoculated onto the leaves of the spreader with a fine brush.

In 1997-1998, field trials were conducted at the experimental station of Hubei Academy of Agricultural Sciences and in Yunyang County, in the mountainous highlands in the northwest of Hubei province. The varieties were grown in 8-row plots, each 1.5m long with 25cm between rows. The plots were arranged in a completely randomized block design with three replicates. A 10 m belt of oilseed rape was grown between replicates to isolate them from each other, to avoid interference between replicates. In order to prevent lodging and to provide favourable condition for differentiating

Table 1. List of varieties, their provinces of origin and year of collection

Code	Variety	Origin	Collection year	Code	Variety	Origin	Collection year
1	E81513	Hubei	1993	31	Mian1855	Sichuan	1993
2	Emai11	Hubei	1989	32	Yun2007	Yunnan	1988
3	Jin66	Hubei	1984	33	Yun675	Yunnan	1988
4	Hua8	Hubei	1992	34	Lin90jan35	Shanxi	1987
5	Zhen831	Henan	1985	35	Nenk6	Peking	1980
6	Su3	Jiangsu	1980	36	Ze88pin6	Zejiang	1984
7	Mian8724	Sichuan	1986	37	85zon33	Hebei	1985
8	E'an1	Hubei	1984	38	Wen4	Henan	1994
9	Hx8541	Hubei	1986	39	Nany82505	Henan	1995
10	Min169	Peking	1980	40	Ren81-5	Hubei	1993
11	E8911	Hubei	1993	41	Su8060	Jiangsu	1995
12	Emai6	Hubei	1972	42	Shan89150	Shaanxi	1992
13	S048	Hubei	1988	43	Yang158	Jiangsu	1987
14	E28547	Hubei	1994	44	R6(158)	Jiangsu	1995
15	Yang9363	Jiangsu	1994	45	Yang9506	Jiangsu	1994
16	Heb215	Hebei	1987	46	Yu18	Henan	1994
17	Yun633	Yunnan	1988	47	Xiang1003	Hunan	1989
18	Lin5064	Shanxi	1985	48	Mian26	Sichuan	1995
19	Yun832	Yunnan	1988	49	Jin35	Hubei	1995
20	Dh8222	unknown	1987	50	Zhen0889	Henan	1994
21	Chun1066	Sichuan	1996	51	Ning9531	Jiangsu	1988
22	E22072	Hubei	1987	52	Ning9544	Jiangsu	1988
23	E8143	Hubei	1990	53	Ning9546	Jiangsu	1988
24	Yun51152	Yunnan	1987	54	Ning9547	Jiangsu	1988
25	Lin30955	Shanxi	1985	55	Ning9558	Jiangsu	1988
26	Ziza1028	Zejiang	1996	56	Ning9144	Jiangsu	1988
27	Ning7840	Jiangsu	1985	57	Linyuan92	Shanxi	1987
28	Ningzi21	Jiangsu	1993	58	Linyuan93	Shanxi	1987
29	Chun10577	Sichuan	1996	59	Linyuan94	Shanxi	1987
30	Chun35050	Sichuan	1996	60	Linyuan95	Shanxi	1987

resistance, 750 kg ha^{-1} of compound fertilizer was applied in trial fields. Min169 was grown as a mildew spreader as above. Two replicates were inoculated while one replicate was not inoculated, to study the influence of the local pathogen population on the experiment. In the trial in Wuchang, inoculation of the spreaders was done with 100 lowland isolates in late February. The trial in Yunyang County was inoculated with 33 highland isolates and 33 lowland isolates in early March, as described above.

Before field inoculations, the isolates were tested on detached primary leaves of the 60 varieties on water agar with benzimidazole and the virulence and avirulence phenotypes recorded on the scale described elsewhere (Yu, 2000). As mildew also occurred on the uninoculated replicates, frequencies of virulence on the 60 varieties in local pathogen populations were estimated in early April when the aerial conidiospore population was at its highest. 7-day old seedlings of Min169 were exposed to the air spora in a place adjacent to the trial fields for 5 days and were then moved to an incubation compartment for 4 days. Leaves with single colonies were cut into pieces and put into plates containing water agar supplemented with benzimidazole. The single colony isolates were tested on primary leaves of 10-day old seedlings of the 60 varieties and virulence frequencies in local pathogen populations were estimated.

Disease severity was only scored once, on the three upper leaves, on 6th May 1997 in the trial in Wuchang in 1996-1997. At that time, most varieties were at growth stage (GS) 73 (Zadoks *et al*, 1974). In 1997-1998, five assessments of disease severity were made from GS45 to GS73 in Wuchang at intervals of one week and four times in Yunyang at intervals of 10 days. Thirty leading tillers were randomly sampled from the two middle rows to assess mildew severity on each variety by a scale developed by CIMMYT (Anon. 1981, Table 2), which has only six points, but which is easy to apply to large number of plants. Mildew was assessed on the four upper leaves until senescence allowed only the upper three to be scored, then eventually only one for some varieties or two for the rest. In such cases, the previous score for the senescent leaf was used in calculating cumulative mildew cover for the upper four leaves. As the flag leaves, in some cases, had not developed at the first scoring, the score for this leaf at this time was set to zero.

Disease severity was expressed by a disease index (DI), calculated as follows:
DI = 100 x Σ (score x number of leaves with that score) / (9 x total number of leaves scored).

As a criterion of resistance, the apparent infection rate (r) (Vanderplank, 1963) was estimated for each variety for the trial in 1997-8. r was estimated as the slope of the regression line of log [DI / (100-DI)] against time (Vanderplank, 1963).

Table 2. Scale of assessment of powdery mildew severity (adapted from CIMMYT; Anon. 1981)

Scores	Percentage cover of leaves by mildew
0	No disease
1	< 5%
3	5 to 15%
5	15 to 25%
7	25 to 50%
9	> 50%

The area under the disease progress curve (AUDPC) is also used as measure of resistance. The AUDPC was calculated as the total area under the graph of DI against time, from the first scoring to the last (Shaner and Finney, 1977).

It is difficult to classify quantitative resistance, such as partial resistance, by the methods used to evaluate specific resistance. It is especially so for a large collection of varieties that may possess both specific and quantitative resistance in field trials inoculated with a set of isolates with wide virulence spectra or allowed to become naturally infected. Varieties with effective specific resistance may have low disease severity whether they have good quantitative resistance or not. In this case, classification of resistance by disease severity level, AUDPC or apparent infection rate will fail to detect partial resistance.

The main purpose of assessing partial resistance is to identify resistance that may be durable over a large area. Varieties which suffer relatively low disease levels under a high pressure of corresponding virulence exerted by the pathogen population may have such durable resistance. A method of assessing partial resistance which takes account both of the level of disease on a variety and the frequency of matching virulence in the pathogen population was devised. Final disease indices were regressed against matching virulence frequencies; the intercept was placed at the origin because, when the frequency of corresponding virulence is zero, the disease severity on a variety should also be zero. The regression equation is:

$$Y' = Ax,$$

where Y' is the expected final disease of a variety at a matching virulence frequency of x. The vertical distance D (from the data point of a variety to the fitted line) was used to

quantify partial resistance:

$$D = Y'-Y$$

where Y is the final disease index. When Y'-Y is positive, the variety is relatively resistant, given the matching virulence frequency. When Y'-Y is negative, the variety is relatively susceptible. Varieties with positive D may therefore have useful partial resistance. In biological terms, D is the additional amount of protection conferred by the partial resistance compared to the value expected given the frequency of matching virulence in the pathogen population.

Results

Trial in 1996/97

The disease severity, expressed as disease indices (DI), of the 60 varieties was regressed on the virulence frequencies of the isolates used as inoculum (Fig. 1). Six varieties were uninfected by mildew probably because of very low virulence frequencies among the
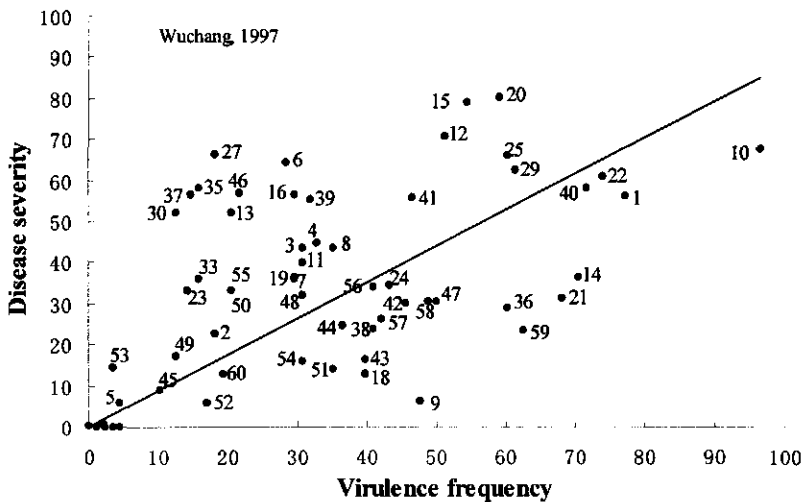
Figure 1. Disease index against virulence frequency in the field trial in 1996-97 in Wuchang to assess partial resistance of sixty Chinese autumn-sown wheat varieties (the figures are codes for varieties corresponding to Table 1. Varieties plotted at left-hand bottom are varieties coded 17, 26, 28, 31, 32 and 34).

pathogen isolates. Varieties Hx8541 (coded 9), Linyuan94 (59), Ze88pin6 (36), Chun1066 (21) and E28547 (14) were compatible with a large proportion of the isolates, but had relatively low disease severities compared to those of other varieties under similar pressure of virulence.

Trials in 1997/98

Scoring disease severity four or five times made it possible to calculate two parameters believed to be important for assessing partial resistance: apparent infection rate (r) and area under the disease progress curve (AUDPC). AUDPC was highly correlated with final disease index (R=0.96), but r was poorly correlated with both final disease index (R=-0.12) and AUDPC (R=-0.26) (Fig. 2). Analysis of variance of AUDPC (Table 3) showed that there were highly significant differences between the performance of different varieties. There was also a highly significant difference between the two locations. There were significant variety x inoculation and variety x inoculation x location interactions, but these effects were much smaller than the main effect of variety. There was a very high correlation between mildew severity in inoculated and uninoculated blocks (R=0.96).

Table 3. Analysis of variance of area under disease progress curve (AUDPC) in trial in 1997-8 [a]

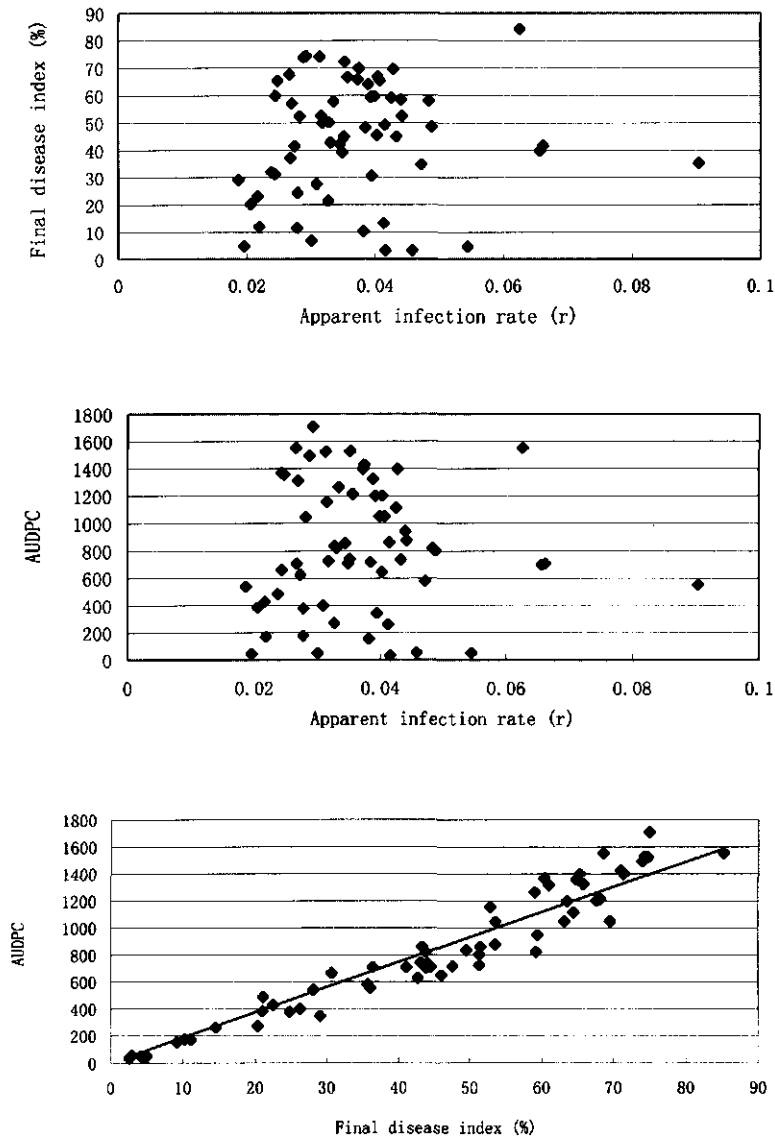| Source of variance	d.f.	Variance	F	p
Cultivar	59	1.338	176.37	<0.001
Location	1	15.99	2107.98	<0.001
Cultivar x Inoculateion	59	0.044	5.81	<0.001
Cultivar x Location	59	0.1314	17.32	<0.001
Inoculation x location	1	0.1179	15.55	<0.001
Cultivar x Inoculation x Location	59	0.0236	3.12	<0.001
Residual	119	0.0076		
Total	359			

[a] d.f.: degrees of freedom; p: probability.

Figure 2. Relationship between apparent infection rate (r) and both AUDPC and final disease index, and between AUDPC and final disease index

The final disease index, for each variety averaged over replicates, is plotted against the frequency of corresponding virulence among the isolates inoculated onto the trials (Fig 3). Disease severity in the trial in Wuchang was more severe than that in Yunyang; the grand
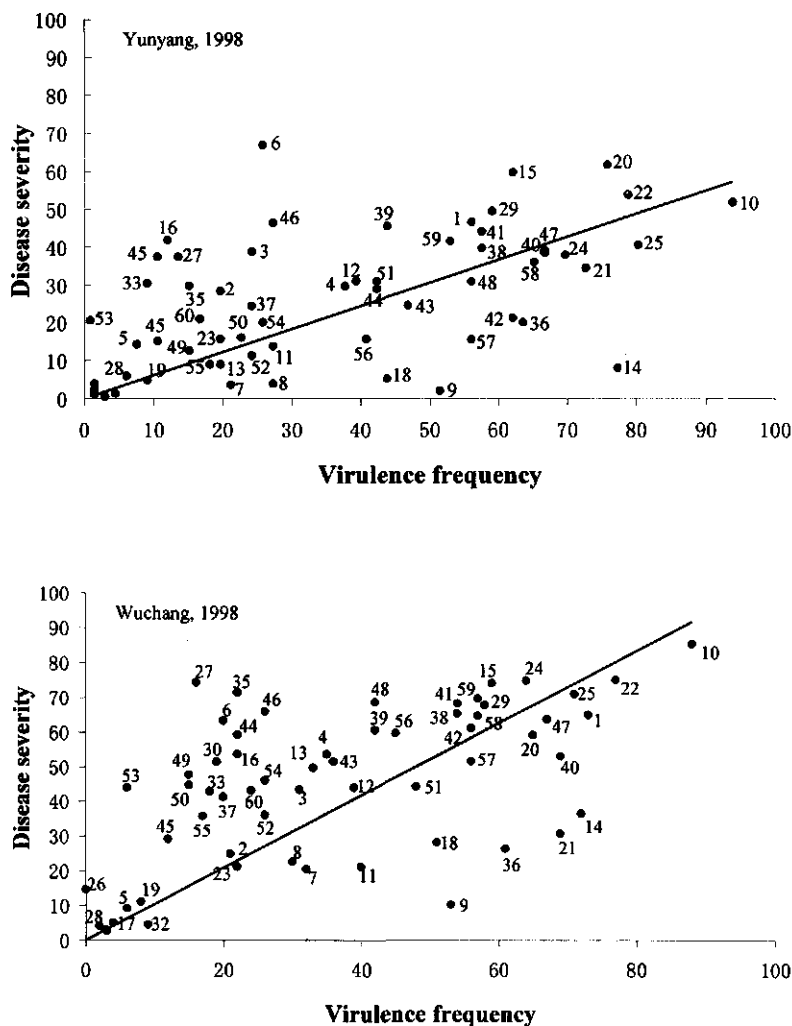
Figure 3. Disease index against virulence frequency in the field trials in 1997-98 in Yunyang and Wuchang to assess partial resistance of sixty Chinese autumn-sown wheat varieties (the figures are code for varieties corresponding to Table 1. Five varieties plotted at left-hand bottom for the trial in Yunyang are varieties coded 16, 26, 31, 32 and 34, and two for the trial in Wuchang are 31 and 34).
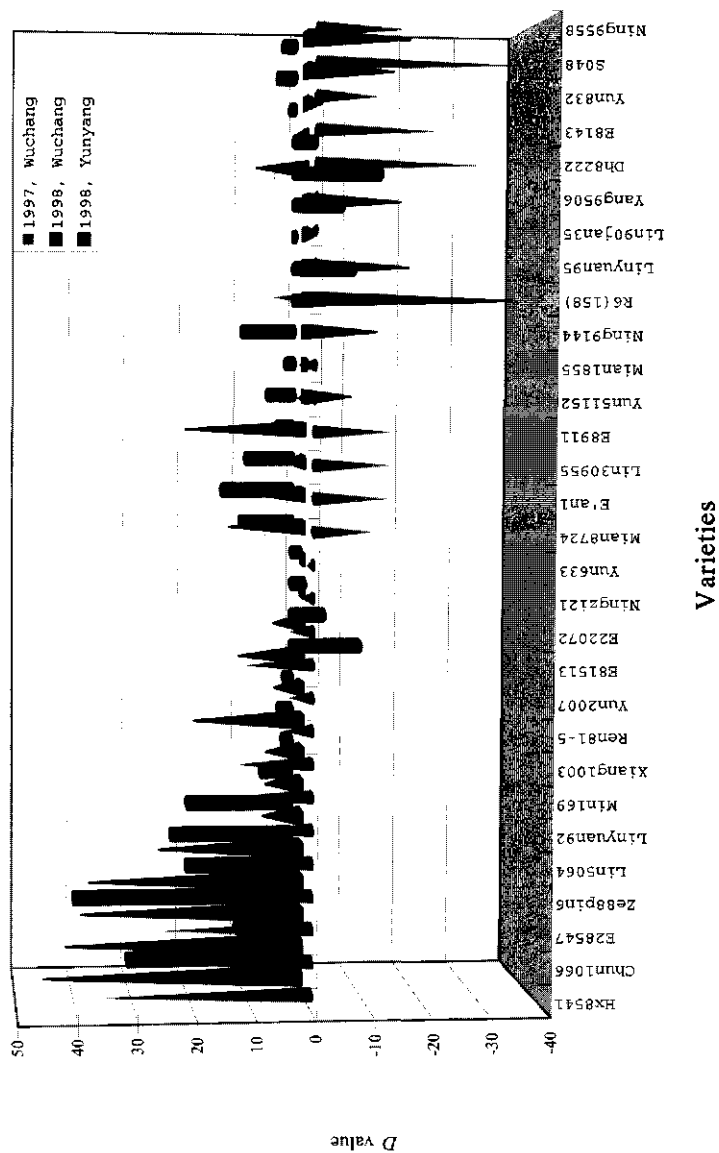
Figure 4. D values of varieties which had a positive D in at least one location or year.

means of final DI were 44.8% and 26.2% respectively. At both sites, varieties Yun633 (coded 17), Ziza1028 (26), Ningzi21 (28), Yun2007 (32) and Lin90jian35 (34) had very low AUDPC, as in the 1996-7 trial in Wuchang. These varieties also had very low frequencies of matching virulence, which explains their low level of infection. Despite high pressure from virulent isolates, either in the inoculum or in the local pathogen populations, varieties Hx8541 (coded 9), E28547 (14), Lin5064 (18) and Ze88pin6 (36) had relatively low disease levels in both sites. Shan89150 (42) and Linyuan92 (57) had relatively low mildew in Yunyang but not in Wuchang. Chun1066 (21) expressed a low disease level in Wuchang but somewhat higher level in Yunyang.

Evaluation of partial resistance

Varieties with positive D values in at least one trial are shown in Fig. 4. Ten varieties, Hx8541, Chun1066, E28547, Ze88pin6, Lin5064, Linyuan92, Min169, Xiang1003, Ren81-5, and Yun2007 had positive D in all three trials. The first five had consistently high D values, which implies they had partial resistance which conferred good protection against mildew despite high frequencies of matching virulence. Other varieties that had one or two positive D values were very variable from year to year or from location to location.

Discussion

The sixty Chinese wheat varieties tested here varied greatly in partial resistance to mildew. Among the varieties, Hx8541, E28547, Chun1066, Ze88pin6 and Lin5064 performed consistently well in all three trials in two years and two locations. They were susceptible to a large proportion of the *E. graminis* f. sp. *tritici* isolates collected from different places and different years, but had relatively low mildew severity in field trials. They therefore have good partial resistance to mildew.

Inoculation increased disease severity compared to the levels on uninoculated plots, but the correlation between scores on inoculated and uninoculated plots was very high. This indicates on one hand that the inoculum used was representative of the local pathogen virulence spectra, but on the other hand, that breeders may be able to select resistant cultivars and lines without needing to inoculate trials, provided that disease severity reaches a sufficiently high level.

Parameters that may be used to quantify partial resistance are infection frequency, latent period and sporulation intensity (Kranz, 1983). In field trials to assess partial resistance these parameters may be estimated by the process of epidemic development.

Vanderplank's (1963, 1968) concept of disease as a function of time, such as apparent infection rate (r), is used to investigate this type of resistance. However, in these trials r was very poorly correlated with both final disease index and AUDPC. The value of the apparent infection rate (r) was strongly influenced by minor differences in disease severity early in the season when severity was low (Shaner and Finney, 1977). Also, Knudsen et al. (1986) pointed out that the rapid development of new leaves at the time of stem elongation frequently results in a decrease in the percentage of diseased leaf area, which causes disease progress curves often to lie far from the theoretical sigmoid curves. The poor correlation between r and disease severity in our results demonstrates the difficulty of evaluating levels of partial resistance by r. Varieties with the same value of r could be very resistant or very susceptible.

By contrast, AUDPC was highly correlated with final disease index. Investigation of AUDPC is very time-consuming and labourious, so this high correlation implies that wheat breeders may be able to assess their lines by a single scoring at an appropriate time. Although it is useful in characterizing partial resistance, the AUDPC values cannot be the only parameter used to evaluate partial resistance when both partial resistance and effective race-specific resistance are present in a large set of varieties. Varieties with specific resistance also have lower AUDPC values when pathogen virulence matching the varieties is at a very low frequency.

Partial resistance allows some epidemic development of disease, but at a reduced level (Knudsen et al., 1986). The quantitative nature of the resistance means that it is more difficult to identify than race-specific resistance, but it may be apparent as relatively low disease severity under a high pressure of pathogen virulence. When disease severity is plotted against virulence frequency, the existence of good partial resistance is indicated by a high positive D value. The biological meaning of the D value is appropriate to the concept of partial resistance, as high D values imply that the rate of epidemic development is reduced.

Resistance characterized by a susceptible reaction in the seedling stage and a resistant reaction in the adult plant stage is described as adult-plant resistance. Bennett (1981) pointed out that, in the wheat mildew pathosystem, some varieties are susceptible as seedlings but relatively resistant as adult plants, some behave in the opposite way and others correspond closely in seedling and adult stages. In the present study, it is possible that varieties with high D, which are susceptible as seedling to a large proportion of $E.$ $graminis$ f. sp. $tritici$ genotypes but relatively resistant in the field, have effective, race-specific, adult plant resistance, as known in wheat yellow rust (Johnson and Taylor, 1972), rather than partial resistance. These two possibilities cannot be distinguished with these data alone.

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Five varieties, Hx8541, E28547, Chun1066 and Ze88pin6 and Lin5064 appear to have consistently effective partial resistance to mildew and may therefore be of value in breeding for durable resistance. Although partial resistance has proved to be durable, the concept of durability contains two elements. One is that the resistance should remain effective for a long time, and the other is that it should be effective in widespread use in an environment favourable to the disease (Johnson, 1984). Consequently, no experimental method can provide as strong a test of durability as growing varieties in an agricultural situation.

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Components of Partial Resistance to *Erysiphe graminis* f. sp. *tritici* in Four Chinese Wheat Varieties

Summary

Components of partial resistance have been studied in five Chinese wheat varieties, four of which, Hx8541, E28547, Chun1066 and Ze88pin6, had good resistance, while the fifth, E22072, was susceptible. All factors investigated contributed to the resistance of the varieties. Latent period of colony formation, number of colonies formed and sporulation capacity were the most common and important components that differentiated all four resistant varieties from E22072. Germination success, formation of appressoria and haustorium and haustorium size also contributed to partial resistance. There was minor but significant variation in the importance of these components among the varieties. Hx8541 had the longest latent period, E28547 had the smallest haustoria and Chun1066 had the lowest level of conidium germination and lower percentage of appressorium and haustorium formation. On later formed leaves, fewer conidia germinated, fewer appressoria, haustoria and colonies formed and fewer conidia were produced per colony than on earlier-formed leaves. In addition, the latent period was longer on later formed leaves than on early formed leaves of all varieties, particularly on the resistant varieties. The fact that all important components are expressed in third leaves suggests that the resistance of the four varieties is partial resistance rather than adult plant resistance.

Keywords: *Erysiphe graminis* f. sp. *tritici*, Powdery mildew, wheat, partial resistance, components.

Introduction

Sixty Chinese wheat varieties, including commercial cultivars and breeding germplasm, were evaluated for partial resistance in a series of field trials (Yu, 2000a). Of these varieties, Hx8541, Chun1066, E28547 and Ze88pin6 consistently expressed compatible reactions to a major proportion of the mildew pathogen population, but had relatively lower disease severity in the field. Partial resistance allows some epidemic development, but at a reduced level (Knudsen *et al.*, 1986). It is therefore suggested that these four varieties may possess good levels of partial resistance. Selection for partial resistance is difficult as it is best done on a large scale (Parlevliet and Van Ommeren, 1975). However, another approach to the analysis of resistance that is partial in its expression is to examine the individual contribution of the components of such resistance (Asher and Thomas, 1984; Carver, 1986; Knudsen *et al.*, 1986).

Different aspects of pathogen development and host responses, such as mechanisms of resistance at the cellular level, infection frequency, latent period and sporulation capacity, have been analysised as possible components of partial resistance to powdery mildew in wheat (Shaner, 1973; Rouse *et al.*, 1980; Nass et al., 1981), oats (Jones, 1978) and barley (Asher & Thomas, 1983, 1984). However, they have not so far been studied in wheat varieties in China, which has the largest cultivated area and production of wheat in the world. The objectives of the experiments reported here were to investigate the components of partial resistance and to evaluate their contributions to the mildew resistance of the four selected Chinese wheat varieties.

Materials and Methods

Inoculum and plant preparation

To chose suitable isolates for this study, 30 isolates recovered from cleistothecia collected at the wheat harvest time in Wuhan in 1998 were tested on primary leaf segments of a differential set of 10 varieties, including Hx8541 (Yu, 2000b) to check the virulence spectra. A further test was done with these isolates on the primary leaf segments of the four partially resistant varieties and the susceptible variety E22072. Three isolates, C-14, W-9, and E-5, had different virulence spectra with the nine differential varieties and a uniformly compatible reaction (infection type 4) with the varieties used in this study (Table 1). They were therefore selected for use in this

study. Three isolates were used to ensure that the host responses studied were not influenced excessively by any unknown or unusual characteristic of any one isolates. The isolates were maintained and multiplied on detached leaf segments of a very susceptible variety Min169 (Yu, 2000b).

Seedlings of the five varieties were grown in 30cm diameter pots in a spore-proof greenhouse at the ambient temperature. To minimise variation between replicate plants, when they began to tiller, all secondary tillers were removed and only the main tillers retained for the experiments.

Table 1. The infection type of three *E. graminis* f. sp. *tritici* isolates on a differential set of Chinese wheat varieties and the five varieties analysed in this paper.

	Cultivar [a]	C-14	W-9	E-5
1	E81513	4	2	1
2	Email	4	4	3
3	Jin66	2	1	4
4	Hua8	2	3	3
5	Zhen831	0	4	2
6	Su3	3	3	4
7	Mian8724	3	4	2
8	E'an1	4	2	4
9	Hx8541	4	4	4
10	Min169	4	4	4
11	E28547	4	4	4
12	Chun1066	4	4	4
13	Ze88pin6	4	4	4
14	E22072	4	4	4

[a] Varieties 1-9 are differential varieties for testing virulence spectra, 10 is a susceptible control variety and 9 and 11-14 are the varieties used in this study.

Conidium germination and latent period.

This experiment was to test whether or not reduced conidium germination is a component of partial resistance. When the second, fourth and sixth leaves were fully expanded, 10 leaf segments of the primary, third and fifth leaves, each 3cm long,

were cut from each variety and placed in petri dishes containing 5 g L^{-1} agar supplemented with 50 mg L^{-1} benzimidazole. Detached leaves were inoculated with mixed conidiospores of the three isolates (the same amount of each) by blowing conidia into a settling tower made of a plastic beaker. Immediately prior to inoculation, the infected leaf segments were gently shaken to remove old conidia (Carver, 1986). The inoculation density was 400 spores cm^{-2}. Germinated spores with obvious primary germ tubes and ungerminated spores were counted in 10 randomly selected microscope fields at 10x magnification on each of two segments at 4, 8, 24, 48 and 72 hours post-inoculation (hpi).

The experiment on latent period was conducted on five segments of primary, third and fifth leaves of each variety. The inoculum and the inoculation procedure was the same as in the conidium germination experiment. The number of colonies on leaf segments was recorded in the morning every day from 3 days post-inoculation (dpi) onwards, using a dissecting lens, until no more colonies appeared. The final number of colonies was set as 100 percent and the colony numbers recorded on each day were converted to percentages of the final colony number. The proportions were then transformed to logits and regressed on time (hours post-inoculation). The latent period was estimated as the predicted time at which 50% of colonies were formed (Parlevliet, 1975).

Appressorium and haustorium formation

The appressorium of powdery mildew fungi is the structure from which the infection peg, which penetrates wheat cells, grows and the haustorium assimilates nutrients from host epidermal cells. An experiment was done to discover whether or not reduced formation of these two structures could be a component of partial resistance. This experiment was also performed on the first, third and fifth leaves of the five varieties. Only isolate W-9 was used. Leaf segments were inoculated at a density of 200 spores cm^{-2} and thereafter incubated at 17°C under continuous light (1000 lux). The method described by Carver and Carr (1977) was used for fixation, clearing and staining of the leaves. Leaf segments were fixed and bleached by immersion in 5 ml 7:3 ethanol : glacial acetic acid solution overnight, then washed thoroughly in water, cleared by immersion in lactophenol for 10 hours at 60°C, stained by immersion in cotton blue/lactophenol for 3 hours at 60°C, then partially destained by immersion in pure lactophenol for 12 hours at 60°C. Appressorium and haustorium formation were scored in 10 randomly selected fields under 10x magnification for each leaf segment. Two leaf segments of each variety were sampled to score appressorium

formation at 24, 48 and 72 hpi and two for haustorium formation at 48 and 72 hpi. Haustorium size was measured using a micrometer at 100x magnification at 72 hpi and the length from the tip of the longest digitate process on the right to the tip of longest on the left recorded (Carver and Carr, 1978). 10 to 15 haustoria were chosen randomly on the basis of their visibility and orientation for measuring the length of haustoria because the arrangement of digitate processes is not planar beneath host tissues.

Colony formation and sporulation

Colony formation and sporulation were measured on three densities of inoculation, 100, 200 and 400 spores cm^{-2}. To determine the inoculation density, six microscope slides were placed in a plum-petal-like arrangement under an inoculation tower of 45cm height and 30cm diameter. Different numbers of primary leaf segments of Min169 of the same size and uniform sporulation were put in an inoculating pipe and the conidiospores were blown into the tower and allowed to settle for 15 minutes. The number of spores cm^{-2} on the slides was recorded under a binocular microscope to determine how many leaf segments were required to achieve the desired density.

When the second, fourth and sixth leaves were fully expanded, the first, third and fifth leaves, respectively, were cut into 25 mm segments and placed in petri dishes containing water agar supplemented with 50 mg L^{-1} benzimidazole and inoculated with mixed conidia of equal amounts of the three isolates. Each combination of variety and inoculation density had four replicates. Petridishes were incubated as described above. Colonies were counted with a magnifying lens 7 to 10 dpi. The final colony number was recorded on each leaf segment and the colony number cm^{-2} was calculated.

To measure sporulation, the same inoculation procedure as for the experiment on colony formation was used with four replicates of each variety at each inoculation density. The colony number was recorded on each leaf segment at 10 dpi when all colonies had been formed. Sporulation was scored from 12 to 21 dpi at intervals of 3 days. For each replicate observation, three leaf segments were sampled at each time. Each leaf segment was put into a tube containing 2.5ml distilled water to which two drops of Tween 80 had been added. Then the tube was shaken on a mini shaker to dislodge the conidiospores and suspend the spores. The concentration of conidia was estimated with a haemocytometer under $10\times$ magnification and sporulation was calculated (Knudsen, 1984). The experiment was done on the first,

third and fifth leaves as above.

Field experiment

A field trial was conducted in the growing season of 1998-1999 to confirm previous results (Chapter 6, this thesis). The four resistant varieties Hx8541, E28547, Chun1066, Ze88pin6 and the susceptible control variety E22072 were sown in the autumn in Wuchang in plots of 60 m². The seed rate was 1 kg per plot giving 18,000 seedlings per plot. A randomised complete block design was used, with three blocks and one plot of each variety per block. The plants were allowed to become naturally infected by mildew and the disease severity was scored five times from 23rd March at an interval of one week using a six point scoring system (Anon., 1981).

Results

Conidium germination and latent period

Conidiospores germinated more quickly on primary leaves of all the five varieties
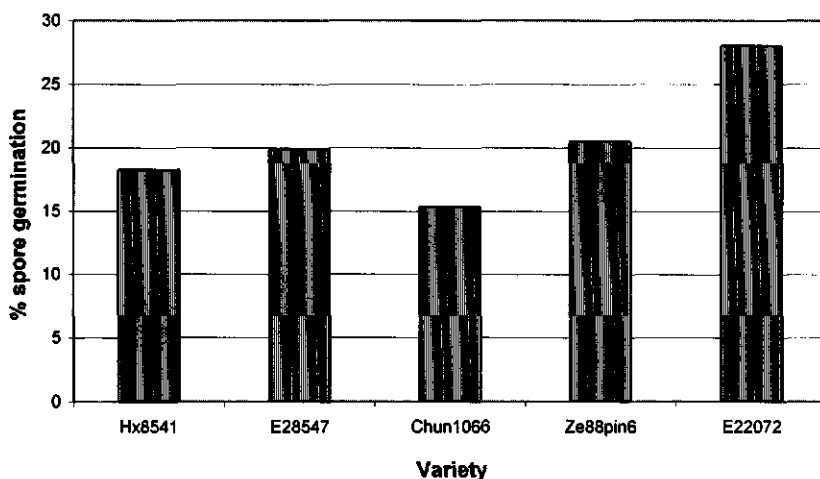

Figure 1. Mean percentage of conidiospore germination of *Erysiphe graminis* f. sp. *tritici* on three leaf positions of five Chinese wheat varieties.

than on third or fifth leaves. On primary leaves of the five varieties, some conidiospores germinated at 4 hpi, but on later formed leaves, no germination had occurred by 4 hpi. Analysis of variance was performed on percentages of spores germinated, using an angular transformation. The proportion of germinated spores was also greater on the primary leaves than on third or fifth leaves ($p<0.001$). Among the varieties, the germination rate was lowest on Chun1066 from primary leaves to later formed leaves and highest on the susceptible control variety E22072. Differences among varieties were statistically significant ($p<0.001$) but not very great (Fig. 1).

Latent period on later formed leaves of all varieties was longer than on early-formed leaves (Table 2). On primary leaves, Hx8541 had a significantly longer latent period than the other varieties. Latent period on the other resistant varieties was not significantly different from that on E22072. On third and fifth leaves, latent period on all four resistant varieties was significantly longer than on E22072. Latent period on Hx8541 remained the longest in later formed leaves. Compared to E28547 and Ze88pin6, Chun1066 had a longer latent period on primary leaves, but a shorter one on later formed leaves. The change of latent period from the primary leaf to the third leaf was much greater on resistant varieties than on the susceptible variety, especially on Hx8541 and Ze88pin6. Those that had the largest change from primary leaves to third leaves had a smaller increase from third to fifth leaves, especially Ze88pin6. This might imply that Ze88pin6 had already expressed a longer latent period as a component of partial resistance at the third leaf stage whereas the other varieties may not express this character fully until the fifth leaf.

Table 2. Latent period (estimated time at which 50% of colonies formed, hours post-inoculation) of *Erysiphe graminis* f. sp. *tritici* on the primary, third and fifth leaves of five Chinese wheat varieties

Varieties	Primary leaf	Third leaf	Fifth leaf
Hx8541	127.04c*	167.53e	176.59d
E28547	120.50a	152.09c	165.52c
Chun1066	124.30b	148.51b	160.58b
Ze88pin6	120.36a	162.08d	167.94c
E22072	122.42ab	141.25a	155.16a

* Significant differences ($p<0.05$) within columns are indicated by different letters.

Table 3. Percentage of appressoria of *E. graminis* f. sp. *tritici* on leaves of five Chinese wheat varieties

Variety	After 24h incubation				After 48h incubation				After 72h incubation			
	Leaf 1	Leaf3	Leaf5	Mean	Leaf 1	Leaf3	Leaf5	Mean	Leaf 1	Leaf3	Leaf5	Mean
Hx8541	31.7	14.0	8.9	18.2ab*	48.7	30.3	23.8	34.3b	56.8	34.9	25.9	39.2a
E28547	32.5	16.7	12.7	20.6b	48.8	31.1	25.4	35.1b	59.6	37.7	30.1	42.5ab
Chun1066	26.2	10.8	4.3	13.8a	42.2	26.6	18.2	29.0a	50.0	31.3	21.0	34.1a
Ze88pin6	28.2	15.9	12.3	18.8b	50.0	30.1	27.1	35.7b	58.0	40.7	32.8	43.8ab
E22072	46.2	27.6	25	32.9c	64.9	40.7	38.6	48.1c	65.8	49.1	42.9	52.6b
Mean	32.9c	17.0b	12.6a		50.9c	31.8b	26.6a		58.0c	38.7b	30.5a	

*Significant differences (p<0.05) within rows or within columns are indicated by different letters.

Appressorium and haustorium formation

The percentages of appressoria and haustoria were transformed to angles for analysis of the data. On later formed leaves fewer appressoria formed than on earlier formed leaves (Table 3). More appressoria were formed on E22072 than on the resistant varieties and they were formed more rapidly. Fewer appressoria were formed on Chun1066 than on the other resistant varieties at all times and on all leaves.

The percentage of haustoria formed decreased significantly from primary leaves to fifth leaves. More haustoria were formed on the susceptible control variety E22072, while Chun1066 had the lowest level of haustorium formation. There was no difference in haustorium formation among the other three partially resistant varieties at either sampling time (Table 4).

Table 4. Percentage of haustoria of *E. graminis* f. sp. *tritici* on leave of five Chinese wheat varieties.

Varieties	After 48h. incubation				After 72h. incubation			
	Leaf 1	Leaf 3	Leaf 5	Mean	Leaf 1	Leaf 3	Leaf 5	Mean
Hx8541	23.1	8.3	3.2	11.5b	31.8	13.9	10.3	18.7ab*
E28547	26.8	8.2	5.1	13.4b	48.9	18.1	7.9	25.0b
Chun1066	17.8	4.7	3.0	8.5a	22.9	10.4	8.1	13.8a
Ze88pin6	25.0	6.5	5.1	12.2b	32.6	16.9	13.1	20.9ab
E22072	37.8	15.3	12.3	21.8c	56.1	29.8	23.2	36.4c
Mean	26.1c	8.6b	5.7a		38.5b	17.8a	12.5a	

*Significant differences (p<0.05) within rows or within columns are indicated by different letters.

Analysis of variance was done on log-transformed data on haustorium size. Haustoria on primary leaves were significantly larger than on third and fifth leaves, but there was no significant difference between third and fifth leaves (Table 5). Mean haustorium size was smallest on E28547 and largest on the susceptible variety E22072.

Table 5. Haustorium size (μ) of *E. graminis* f. sp. *tritici* on leaves of five Chinese wheat varieties

Variety	Leaf			Mean
	Primary	Third	Fifth	
Hx8541	39.48	19.44	24.22	27.71b*
E28547	26.50	24.06	24.80	25.12a
Chun1066	37.74	29.40	23.57	30.24c
Ze88pin6	33.08	28.75	27.79	29.88bc
E22072	71.83	46.87	42.18	53.63d
Mean	41.73b	29.70a	28.51a	

* Significant differences (p<0.05) within rows or within columns are indicated by different letters.

Colony formation and sporulation capacity

Colony numbers were subjected to a square root transformation for analysis of variance. As inoculum density increased, colony numbers on all five varieties and all three leaves increased. Leaf number had a highly significant effect on the colony formation ($P<0.001$). More colonies were formed on primary leaves of all five varieties than on later formed leaves and colony numbers on primary leaves were similar among five varieties (Fig. 2). On later formed leaves, colony number
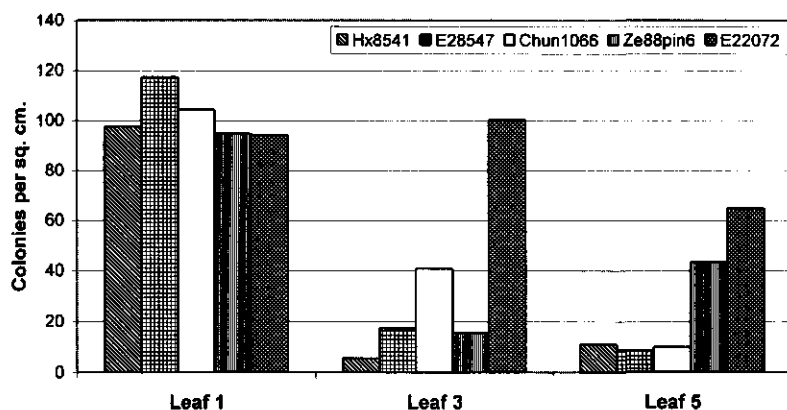

Figure 2. Mean colony number of *Erysiphe graminis* f. sp. *tritici* on the primary, third and fifth leaves of Chinese wheat varieties.

decreased sharply on Hx8541, E28547, Chun1066 and Ze88pin6, but not on the third leaf of E22072 and only slightly on the fifth leaf.

The number of conidiospores produced per colony was subjected to a logarithmic transformation for analysis of variance. Sporulation decreased on older leaves in all varieties (Fig. 3). The number of conidia produced on primary leaves of Hx8541 was lower than on other varieties and continued to be lower on the later formed leaves. On third and fifth leaves, sporulation capacity decreased sharply on the resistant varieties, but less so on E22072. There was a significant interaction between variety and sampling time (P<0.001) on conidium production. At earlier sampling times, the number of conidia produced by some of the resistant varieties was similar to that on E22072, but E22072 produced two to three times as many conidia as the other varieties at 21dpi.
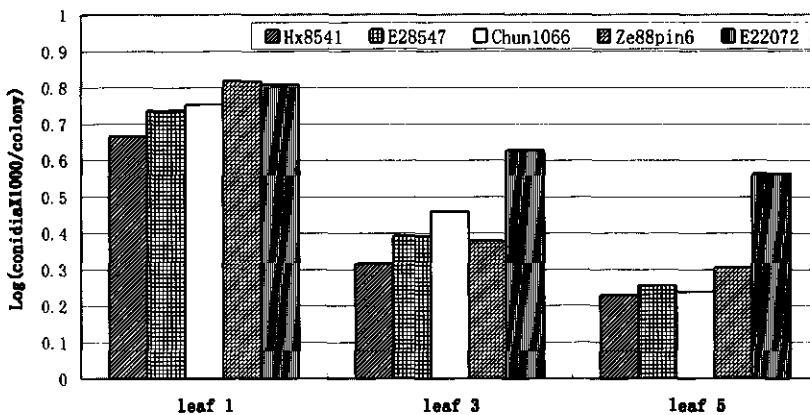

Figure 3. Sporulation capacity of Erysiphe graminis f. sp. tritici on three leaf position of five Chinese wheat varieties

Field trial of mildew resistance

The mean disease severity at the first scoring was similar among the five varieties, but E22072 had three times as much mildew as Hx8541 and more than twice as much as the other varieties at later scorings (Fig 4). This is consistent with results of three previous trials in which varieties were grown in smaller plots (Yu, 2000a).
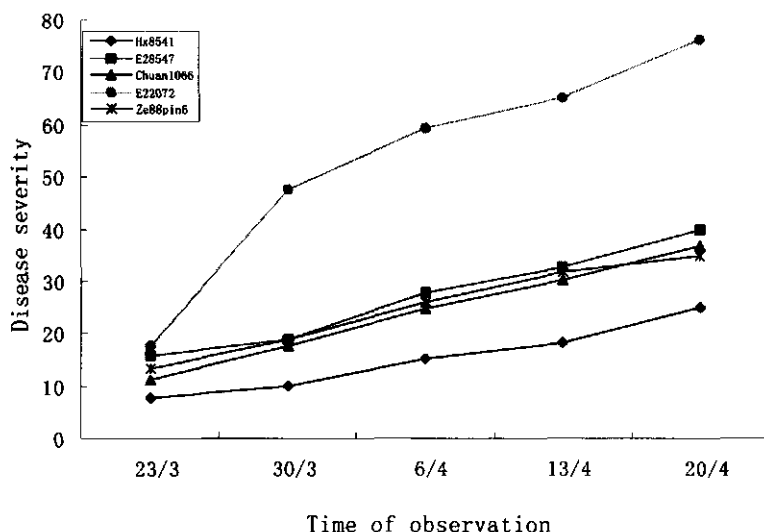
Figure 4. Field performance of four Chinese wheat varieties with partial resistance.

Discussion

From conidium germination to conidiospore production, all components studied in this paper contributed to the partial resistance of the four resistant varieties (Table 6). There was minor, but statistically significant variation in components between resistant varieties. Latent period, colony formation and sporulation capacity were the most common and important components in all four varieties. Hx8541 had the longest latent period, fewer colonies and least sporulation. E28547 had the smallest haustoria size, while Chun1066 had the lowest conidia germination rate, the fewest appressoria and fewest haustoria (Table 6). All components in Ze88pin6 were at an intermediate level. The net benefit of these components for the performance of partially resistant varieties in the field is slower development of disease and much lower disease severity (Fig. 4).

These results account for the low disease severity on the partially resistant varieties, especially Hx8541, in the field. It takes one week or so for powdery mildew to go through one infection cycle in a warm month (Wolfe and McDermott, 1994). The longer latent period of the resistant varieties, compared to E22072, increases the time required for one cycle. Fewer colonies and fewer conidiospores produced on these varieties generate less inoculum for the next cycle of infection. So, from an epidemiological point of view, even small initial differences in these

components will result in large differences in disease severity in the field.

This appears to be the first report of the possible involvement of conidium germination in partial resistance to a powdery mildew. Nicholson *et al.* (1988) reported that the enzyme exuded by the pathogen itself is a prerequisite to the process of conidium germination, while the type of material on which a conidiospore lands is reported not to affect germination (Carver *et al.*, 1989, Gay *et al.*, 1985, Nicholson *et al.*, 1993). Germination does not occur while conidia remain in the spore chain attached to the mother conidiophore. Removal of an unknown suppressive factor produced by the mother colony was therefore suggested to be the only stimulus for germination (Carver *et al.*, 1995). However, the primary germ tube of *Erysiphe graminis* is believed to have three functions in the early stages of the plant-fungus interaction. The first is rapidly attaching the fungal germling to the host surface (Kunoh *et al.*, 1991). The second is gaining access to water from the host to support development of the appressorial germ tube (Carver and Bushnell, 1983). The third is recognising the contact surface, leading to intracellular signaling results, eventually resulting in elongation of the appressorial germ tube (Carver *et al.*, 1995). The lower germination rate in the four resistant varieties, compared to E22072, implies that variation in host responses to the germinating mildew spore might be a component of partial resistance.

The next stages in development of the fungus are formation of the appressorium then the haustorium. The appressorial lobe, differentiated from the elongated appressorial germ tube, is a prerequisite to mildew infection, while successful formation of the appressorium then the haustorium indicates a successful infection (Johnson *et al.*, 1979; Wright and Heale, 1984; Carver, 1986). We speculate that the lower conidium germination rate and the low number of appressoria and haustoria formed on Chun1066 may imply that the same signals, between the fungus and the plant, and within the plant itself, are involved in the processes of appressorium and haustorium formation.

As well as variation in the number of haustoria formed, there was also variation in the size and morphology of haustoria. At 48 hpi, digitate processes could be seen only in the susceptible variety E22072, but not yet in the partially resistant varieties (data not shown). The haustorium size of oat mildew varies among oat genotypes with different levels of partial resistance (Carver and Carr, 1978); here there were significantly smaller haustoria in the resistant wheat varieties than in the susceptible variety (Table 5). The haustorium is the structure of *Erysiphe graminis* which extracts nutrients from the host cell (Hirata, 1967; Bracker 1968; Bushnell, 1971).

Table 6. Overall evaluation of the contributions of different components to the partial mildew resistance of four Chinese wheat varieties compared to a susceptible variety, E22072[§]

Variety	Germination rate of conidia[1]	Appressorium formation[2]	Haustorium formation[3]	Haustorium size[4]	Latent period[5]	Colony formation[6]	Sporulation capacity[7]	Disease severity in field[8]
Hx8541	2 b*	2 a	2 a	2 b	1 a	1 a	1 a	1 a
E28547	3 c	3 b	4 c	1 a	3 b	2 b	3 a	4 b
Chun1066	1 a	1 a	1 a	4 c	4 c	3 c	2 a	2 b
Ze88pin6	4 b	4 b	3 b	3 c	2 b	4 c	4 a	3 b
E22072	5 d	5 c	5 d	5 d	5 d	5 d	5 b	5 c

[§] Ranks of means from: [1] Fig. 1, [2] Table 3, [3] Table 4, [4] Table 5, [5] Table 2, [6] Fig. 2, [7] Fig. 3 and, [8] Fig. 4. Rank 1 indicates best performance, in terms of resistance, 5 the worst performance.

* Significant differences (p<0.05) are indicated by different letters.

Smaller haustoria may therefore be less effective in nutrient uptake, leading to slower development of the fungus.

The final and visible evidence of infection success is colonies on leaves. Reduction of infection is therefore one of the most important resistance components. This component is clearly expressed on the third leaf stage of Hx8541, E28547 and Chun1066. In Ze88pin6, this component was expressed by the fifth leaf stage (Figure 2).

Sporulation has been emphasized as an important component of partial resistance (Asher and Thomas, 1984; Knudsen, 1984) because reduction of sporulation reduces the rate of pathogen spread (Shaner 1973a). Here, there was little or no difference between the resistant varieties and the susceptible variety at the earlier sampling time, but significantly fewer spores were produced on resistant varieties later. This may imply that varieties with partial resistance have a shorter period of conidium production than susceptible varieties, which has been proposed as an important component of partial resistance not only in cereal diseases (Zadoks, 1972) but also in other crops (van der Zaag, 1959). A negative exponential relationship has been observed between the number of colonies and the cumulative number of conidia produced per colony (Rouse et al., 1984). Here, conidium production per colony did not show such a trend except on Ze88pin6, where conidiospore production decreased slightly with increasing colony number on third and fifth leaves. Sporulation capacity may be positively related to the size of colonies (Shaner, 1973b), and colony size may be inversely related to the number of colonies (Asher and Thomas, 1984). On any unit area of leaf, only when the colony number is sufficiently high will the colony size be limited. Colony numbers in this experiment may not have reached a density high enough to limit colony size. However, erroneous conclusions may be drawn in evaluating varieties' resistance by sporulation capacity per colony if the colony number is not taken into account.

Latent period is a major component contributing to partial resistance of potato to late blight (Umaerus, 1970), barley to powdery mildew (Asher and Thomas, 1984, Knudsen, 1984), and barley to leaf rust (Parlevliet and van Ommeren, 1975). Here, longer latent period was an important component of partial resistance of the four varieties. It was already expressed in Hx8451 in the primary leaf stage. As the plant grew, latent period became longer. This even occurred in the susceptible variety, E22072, but the difference in latent period between early formed and later formed leaves was much greater in the resistant varieties than in E22072 (Table 2). In third leaves of resistant varieties, the latent period was 30 to 40 hours longer than in primary leaves while it was less than 20 hours longer in E22072. Latent period is

also a component of adult plant resistance (Jones, 1978). The fact that longer latent period occurs in third and fifth leaves, not merely in adult plant leaves, indicates that the varieties studied here have resistance that is expressed well before the adult stage.

Partial resistance is characterised by a susceptible reaction in all growth stages, but the infection frequency, the latent period, the rate and the period of spore production may vary (Parlevliet, 1975). The four resistant varieties had a susceptible reaction (IT 4) with the isolates, as did the susceptible variety, but they expressed a wide range of traits, both before and after haustoria were formed, which contributed to partial resistance and resulted in low mildew severity in field trials. This indicates that the four resistant varieties studied here should have good levels of partial resistance and can be used in breeding wheat for durable resistance to mildew.

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General discussion

Wheat powdery mildew, caused by the obligate biotrophic fungus *Erysiphe graminis* f. sp. *tritici*, has been a major disease for the last 25 years in central China and has caused substantial yield losses. Research on this disease has only been conducted very recently because of shortages in research resources. Before rational control strategies can be developed, a fuller understanding of the pathosystem, such as details of the epidemic cycle, dynamics of the pathogen population, host resistance and other control practices, as well as the effectiveness of the control methods, must be obtained. This thesis aims at a fuller understanding of wheat powdery mildew in central China.

In the thesis, study of the epidemic cycle was focused on the sexual and asexual stage of the causal agent and their roles in transmitting the disease between wheat crops. In order to provide information on effective deployment of host resistance, the pathogen population dynamics and the pathway of movement of the pathogen population across regions in Hubei province have been investigated. The use of host resistance is the most economical and effective method of controlling disease. The specific resistance of the wheat cultivars grown along the Yangtze River valley and from some other parts of China had been studied under the assumption of gene-for-gene relationship. Resistance that follows a gene-for-gene interaction is readily overcome by changes in the pathogen population, so durable resistance is desirable. A substantial component of this thesis is aimed at assessing partial resistance, which has been proved durable in cereals to powdery mildew. Fungicide application is an important disease control method, so variation in the responses of pathogen populations to triadimefon, an important fungicide used to control powdery mildew in China was investigated, with the aim of providing information on the management of fungicide application. All these of aspects of the thesis have implications for the control of wheat powdery mildew in central China.

Epidemic zones and their implications for control of wheat mildew in central China

In temperate parts of the world, seasonal "bottlenecks" for cereal mildew are of limited consequence because the continuity of autumn- and spring–sown crops provides a "green bridge". (Wolfe and McDermott, 1994). However, in subtropical wheat growing regions, such as in central China, with monsoon climates, the seasonal "bottlenecks" are crucial to the understanding of wheat mildew epidemics.

The causal agent of wheat powdery mildew, *E. graminis* f. sp. *tritici*, is a biotrophic fungus, and there is a seasonal gap lasting several months between wheat crops in central China.

The main aims of Chapter 2 were to determine how and where the fungus survives this gap. This was done through a thorough investigation of the sexual stage of *E. graminis* f. sp. *tritici* and its host in Hubei province where wheat growing can be considered in terms of three zones, and the effects of the zones on the epidemic cycle. It is clear that in the high mountainous region (so-called *highlands* in this thesis) of the northwestern part of the province, the fungus can survive the absence of wheat crops because of two reasons. The first is that the growing season of wheat crop in this zone is longer than in other zones, and the gap between wheat crops is short. Hence late season ejection of ascospores, the sexual propagules of the fungus, can coincide with seedlings emerging in early autumn and thus the disease is transmitted across seasons. The second is that volunteer plants can survive the summer in the highlands, so early released ascospores can infect hosts and produce conidia, the asexual propagules of the fungus, on the volunteers which provides initial inocula for wheat crop in autumn. In the intermediate altitudes, the seasonal gap between wheat crops is longer than that in the highlands and cleistothecia can only tide the fungus over the gap by releasing ascospores to infect volunteer plants on which conidia are produced, infecting autumn seedlings.

In the lowlands, neither the fungus nor wheat volunteers can oversummer. Cleistothecia in the lowlands are also capable of discharging viable ascospores, but the experimental results in Chapter 2 indicate that the duration of ascospore ejection is short because of the monsoon rains after wheat harvest which provide continuously wet conditions for cleistothecia and therefore shorten the duration of ascospore ejection. During liberation of ascospores, there are no volunteers to act as hosts, and even if there were, the temperature would be too high for the ascospores to infect them. When autumn seedlings emerge, there are no viable cleistothecia to release ascospores. So wheat powdery mildew in the lowlands of central China must be re-established each year from external sources.

Although the sexual stages of *E. graminis* f. sp. *tritici* in central China and *E. graminis* f. sp. *hordei* in the Middle East (Koltin and Kenneth, 1970) both transmit powdery mildew between seasons, there is a difference between the two situations. In central China, wheat volunteers are an important vehicles to bridge the seasonal gaps, especially in the intermediate altitudes, whereas in Israel for example, ascospores of *E. graminis* f. sp. *hordei* infect the barley crop directly when the rainy season starts.

This study has made several meaningful contributions to the control of wheat powdery mildew in central China. Sanitation of wheat straw bearing cleistothecia, some changes in methods of cultivating wheat, and more intensive cultivation of succeeding crops in the two epidemic zones in which ascospores are the means of transmitting the disease across seasons, may with time reduce the level of the disease. However, removal of wheat straw is impossible at present because farmers keep the straw as cooking fuel. Change in the cultivation of wheat, however, can achieve good results. Because of the limited duration of ascospore ejection, a delay in the time of sowing wheat has showed some benefit in reducing the initial level of mildew and delaying the disease epidemic (Yu *et al.*, *unpublished data*). This may be due to seedlings emerging later so avoiding the periods of ascospore discharge. Intensive cultivation of following crops to reduce the population of volunteer wheat is possible; for example, application of herbicides is a common cultural practice for many succeeding crops, such as cotton, potato and soybean, which are rotate with wheat in central China. At the time when volunteers are emerging in fields, application of herbicides to control weeds is now common practice. If attention is simultaneously paid to wheat volunteers, the control of volunteers may be achieved as a consequence of weed control of the succeeding crops. This, however, remains speculation as definitive work on this topic has not been done.

Although the "seasonal bottleneck" in temperate areas is of limited consequence, some attention has been given to effects of recombination of virulences during the sexual stage on the population structure of *E. graminis* f.sp. *hordei* (Welz and Kranz, 1987; Brown and Wolfe, 1990). The sexual stage was estimated roughly to contribute one quarter of the spores infecting a new crop of winter barley in autumn in the United Kingdom (Brown and Wolfe, 1990). The most important aspect is that sexual reproduction may be responsible for increasing of diversity of phenotypes by disrupting association between alleles and accelerating the rate of evolution of the pathogen populations. When a cultivar is introduced with two resistance genes that have not previously been used together, recombinant isolates carrying both matching virulences might be selected rapidly if the matching virulences were previously in negative gametic phase disequilibrium. Sexual reproduction of the pathogen may also increase the variation in fitness of progeny, compared with asexual reproduction (Williams, 1975). Variation in fitness may play a role in improving the adaptation of cereal mildew fungi to new control methods, such as new cultivars and fungicides (Wolfe *et al.*, 1983). The existence of very diverse virulence phenotypes in the population of *E. graminis* f. sp. *tritici* in central China (Chapter 4) may be partly explained by sexual recombination, but, the importance of the sexual stage with

respect to these aspects has not been studied in this thesis. Future investigations are required.

A differential set and connection of the populations of *Erysiphe graminis* f. sp. *tritici* in central China

A set of differential cultivars should be established in order to track virulence dynamics in pathogen populations. Two kinds of differential sets of host genotypes are in general use. One is a set of cultivars, landraces, breeding lines or other material, each of which has a different resistance specificity but also other differs from the other lines in its genetic "background", i. e. genes other than those controlling the specific resistance. The other type of set consists of near-isogenic lines, in which different resistance genes have been bred into a common genetic background (Brown, 1998). It is often assumed that near-isogenic lines are more suitable for identifying pathogen virulences, because each line should have a single, specific resistance gene. In fact, this is not always the case. Some near-isogenic lines have more than one resistance gene. Inevitably, near-isogenic sets do not necessarily include all recently discovered resistance genes (Brown, 1998).

Near-isogenic lines with a Chancellor background (Briggle, 1969) are often used to identify virulence genes in the populations of *E. graminis* f. sp. *tritici* in Europe and North America. Since resistance in Chinese wheat varieties may largely be controlled by genes which cannot be identified with pathogen virulence spectra defined by the Chancellor near-isogenic lines (Chapter 3), a set of varieties relevant for Chinese commercial varieties and breeding lines was established in this study. However, the main purpose of all pathogen surveys using a set of differential cultivars is often the early detection of new virulences that can overcome host resistance and the monitoring of changes in the virulence frequencies. The set of cultivars established in this study cannot fully meet this purpose as it cannot provide sufficient information on the risk to newly introduced resistances, which differ among breeding programmes at different institutions and where underlying genes are still unknown. The main purpose of the differential set is to track the relatedness of populations of *E. graminis* f. sp. *tritici* across different epidemic zones of central China. The set of cultivars, however, is relevant to the varieties grown and common breeding lines present in central China, and does reveal regional variations in the pathogen populations. It is thus an appropriate set although improvement is necessary and this remains an aim for future research.

In Chapter 4, results of the monitoring of pathogen populations showed that the

population structure in the lowlands of central China differs from year to year, which confirms the view that the disease in the lowlands is re-established each year. Disease in the highlands is a source of inoculum for disease in the lowlands, but there must be other inoculum sources affecting the epidemics in the lowlands. The structure of populations monitored at higher and intermediate altitudes over two years, including both spring and summer populations, does not differ significantly. This suggests that the populations at the higher and intermediate altitudes are the same population and that these two zones should be treated as one epidemic unit, although oversummering of the pathogen differs in detail between the two zones. The structure of the pathogen population in spring was different from that of the oversummering population in the highlands. This implies that the sexual stage of *E. graminis* f. sp. *tritici* plays a very important role not only in transmitting the disease between seasons, but also in determining the diversity of the pathogen population. In addition, it implies that, although the pathogen can oversummer in the highlands, the population in the highlands is not an isolated population; there must be external sources that affected evolution and structure of the population. Possible external sources contributing to the pathogen population in central China are discussed in Chapter 4. The involvement of external sources in epidemiology complicates the management of wheat powdery mildew in central China. Strategies covering larger areas than Hubei province should be considered.

Resistance of varieties and their use in controlling wheat mildew in central China

Although wheat powdery mildew has been a devastating disease for the last quarter of century in China, wheat disease research has focused on rusts, caused by *Puccinia* spp., in the northern parts of China, and wheat scab, caused by *Fusarium* spp., in central China, due to limited research resources. Consequently, information on cultivar resistance to powdery mildew and the evolution of mildew pathogen in China is limited. Results in Chapter 3 showed that mildew resistance in Chinese wheat varieties is largely controlled by unknown genes which may not be identified with the virulence spectra found on European and North American differential sets.

With respect to the resistance of wheat varieties, the most common conclusion drawn is that *Pm*8 has lost its effectiveness. This conclusion was drawn because Romanian varieties Lovrin10 and Lovrin13, possessing the 1BS-1RS wheat–rye translocation and known to carry *Pm*8, were introduced into China in the early 1970s for yellow rust resistance breeding (Zheng, 1993). This is a somewhat

speculative conclusion since no detailed research on the resistance background of Chinese wheat varieties has been conducted.

As shown in Chapter 3 frequencies of virulence matching *Pm*3b, *Pm*3c and *Pm*5 are much higher than that matching *Pm*8 in the pathogen population. This is also indirect evidence that the *Pm* genes listed above may be present in Chinese cultivars because a high frequency of virulences matching a resistance gene may have resulted from host selection for the pathogen population. Among the known resistance genes tested, *Pm*3d, *Pm*4b and *Pm*1+2+9 are valuable resources for mildew resistance in central China because frequencies of virulences matching these specific genes are very low. *Pm*2, *Pm*4a and *Pm*7 are also suitable for resistance breeding. Some Chinese varieties, such as Dian633, Ziza1028, Ningzi21 and Lin90-35, which may carry resistance factors differing from the known *Pm* genes, are also useful for breeding because they are effectively resistant against the major proportion of the pathogen population. The resistance genes in these cultivars may be easier than European or American cultivars to manipulate in breeding because the cultivars are closer to Chinese commercial varieties in agronomic characters. However, their resistance should be characterized and further studies on the genetics of their resistance to powdery mildew carried out.

Single specific resistance genes are easy to manipulate in breeding. However, a crucial aspect of cereal mildew resistance which involves the gene-for-gene relationship has been non-durability of cultivar resistance. "Break down" of resistance is a consequence of pathogen populations adapting more or less rapidly to the resistances deployed (Brown *et al.*, 1997). Thus a major goal of wheat breeding is durability of resistance. Johnson (1978) argued that the strongest test for durable resistance occurs when a cultivar is widely grown in an environment favourable to the disease and no single experimental method can be as strong as a long-term series of observations on varieties in agriculture.

A substantial component of this thesis (Chapter 6) has aimed at assessing partial resistance. Partial resistance has proved durable to powdery mildew of wheat (Shaner, 1973; Bennett, 1984) and other cereals (Jones and Davies 1985; Jones and Hayes, 1971) because adaptation by the pathogen has been limited (Chin and Wolfe, 1984; Newton, 1989). Mechanisms of partial resistance differ from those involved in gene-for-gene resistance, which are easy to recognize, (Brown *et al.*, 1997) and selection of partial resistance is best done on a large scale (Parlevliet and Van Ommeren, 1975). So selection for partial resistance is relatively difficult. Partial resistance is often recognized in field by epidemics in which there are relatively low levels of disease (Knudsen et al., 1986). However, the term "relatively low level of

disease" is somewhat ambiguous. When a large number of cultivars are involved, including some with race-specific resistances, and when frequencies of virulence matching those resistances are low, the cultivars will also have low level of disease. This makes the identification of partial resistance difficult. Because "breakdown" of resistance is caused by matching virulence, we inoculated a range of varieties with large number of isolates covering a wide spectrum of virulence, representing the pathogen population in central China. By regression of disease severity of cultivars on frequencies of virulences in the pathogen population, both disease severity of cultivars and frequencies of virulences in the pathogen population can be used to select partial resistance. This makes it possible to assess how the cultivars perform under a high pressure of virulence exerted by pathogen population, but avoids the problems with "relatively low level of disease". Estimation of the vertical distance from each cultivar data point to the fitted regression line (D) is a novel means of evaluating partial resistance and clarifies the term "relatively low level of disease". The biological meaning of D is the additional benefit of partial resistance, expressed as disease severity, compared with the expectation of the fitted line for a given frequency of matching virulence. A similar approach can also be applied to evaluating partial resistance by calculating the horizontal distance from a cultivar data point to the fitted regression line. If the evaluation is done in this way, the biological meaning of the horizontal distance is the additional amount of protection in terms of virulence frequency in the pathogen population compared with the expectation of the fitted line for a given disease severity. The vertical distance gives the benefit in terms of disease severity for a given virulence frequency, whereas the horizontal distance gives the benefit in terms of protection against virulence for a given level of disease severity. By this method, the "relative level" becomes an "absolute level". Wheat breeders can evaluate their materials without inoculation if the disease is present at sufficient levels. In this case, virulence frequencies should be estimated by sampling the local pathogen population.

Because partial resistance reduces epidemics, two parameters concerning epidemics, area under disease progress curve (AUDPC) and apparent infection rate (r), are often used to evaluate partial resistance. We used final disease severity, rather than AUDPC, to evaluate partial resistance of sixty Chinese wheat varieties. Both AUDPC and r require several disease assessments during epidemics, which is time-consuming and laborious. The two parameters also have some drawbacks in evaluating partial resistance when varieties also contain race-specific resistances. In the case that varieties with race-specific resistance face low frequencies of matching virulence in pathogen population, this will also lead to low AUDPC or r values. The

results in Chapter 6 show that final disease index is very well correlated with AUDPC, but that r is poorly correlated with both AUDPC and final disease index. So we recommend that final disease levels should be used to evaluate partial resistance instead of AUDPC as it is much easier for breeders to use.

The mechanisms underlying partial resistance differ from those for gene-for-gene resistance (Boyd *et al.*, 1995) and the nature of the signals for partial resistance are unknown (Brown *et al.*, 1997). Further evaluation of the components of partial resistance is often necessary to reveal important interactions between the pathogen and host. In order to confirm the field results, a thorough investigation of resistance from conidia germination to field expression was carried out on four Chinese varieties (Chapter 7). In the literature, it appears that no studies have been reported on conidium germination as a component of partial resistance to powdery mildew. Generally, later-formed leaves are more resistant to mildew pathogen than earlier-formed leaves. Conidium germination rate on later formed leaves was lower than on earlier formed ones. Also the rate of germination on resistant cultivars was lower than on susceptible cultivar. This may indicate that conidium germination as the first interaction between wheat variety and *E. graminis* f. sp. *tritici* may be first target for mechanism of partial resistance. All components investigated in the four varieties differed significantly from the susceptible control variety, with minor variation among resistant varieties. Differences in formation of appressoria and haustoria, latent period and sporulation capacity as resistance components were expressed in the third leaf stages in the four varieties, which indicates that the resistance they possess is different from adult plant resistance. Each of these components contribute to a low rate of disease development in field. This study confirmed that the four varieties have good levels of partial resistance and can be used as sources of durable resistance to wheat mildew in Chinese breeding programmes.

Pathogen population and deployment of resistance in central China

The deployment of host resistance in controlling wheat mildew is an important consequence of this work. As mentioned above, the wheat growing area in central China can be divided into three zones. There is a connection of the pathogen populations across the three zones. Accordingly, deployment of resistance separately in the three zones may be a way of managing the disease, especially in the lowlands where mildew is re-established each year. The same resistance used in the highlands should not be used in the lowlands. If the same resistances are used in both zones, virulent clones dispersed over long distances from the highlands will easily establish

disease in the lowlands.

Selection induced by two resistance genes present in the same variety is likely to generate positive gametic disequilibrium between the corresponding virulence genes. Selection induced by two resistance genes present in different varieties is likely to generate negative gametic disequilibrium between the corresponding virulence genes (Hovmoller and Ostergard, 1991). So, more resistance genes in separate varieties, not combined in the same variety, should be deployed in the highlands to generate negative gametic disequilibria between virulence genes. As a consequence, mildew selected in the highlands will not establish in the lowlands and *vice versa.*

Hitch-hicking is an important process in evolution of pathogen population. If two virulences matching two different resistance genes are associated in some clones in the highlands where only one of the two resistance genes is used, the virulence matching resistance gene which is not used in the highlands will be selected by hicth-hicking. Frequency of this unnecessary virulence will be high. If the resistance, not used in the highlands, used in the lowlands, the clones selected in the highlands will easily establish disease in the lowlands. So the process of hicth-hicking in evolution of the pathogen population should also be considered in deployment of host resistance.

Variation of responses to triadimefon and slowing down the development of fungicide resistance in central China

There has been a fall in the performance of triadimefon in controlling wheat powdery mildew in Hubei province. Variation in responses of *E. graminis* f. sp. *tritici* to triadimefon was very wide, covering a 370-fold of range of estimated ED50s. Variation in responses of *E. graminis* f. sp. *tritici* to triadimefon in central China showed the same dynamical trend from spring to September. In the spring population the variation in ED50s was smaller than for the September population. The smaller variation in the spring population was because of the decreasing proportion of sensitive isolates, which leads to the mean ED50 increasing. In 1996, the mean ED50 slightly decreased from February to April in the highlands although not significantly. Generally, in the time of disease epidemics, fungicide application gives selection for resistant isolates resulting in increase of the ED50. The decrease of mean ED50 in spring 1996 might be caused by immigration of mildew pathogen population into Hubei from areas where less triadimefon was applied. In the oversummering population in 1996, the proportion of more sensitive isolates

increased. In summer, migration of the pathogen population is surely limited. This increasing in proportion of more sensitive isolates may be due to a cost of fungicide resistance in terms of pathogen fitness. However, the mean ED50 did not decrease in 1997. Wheat powdery mildew was severe in 1997, which led to heavy application of triadimefon. A high mean ED50 in September 1997 may indicate that the level of fungicide resistance in the pathogen population may not reduce in the absence of the fungicide in summer if the fungicide was applied heavily in the late spring.

In order to slow down the development of resistance to triadimefon in *E. graminis* f. sp. *tritici* and prolong the life span of the fungicide, two strategies should be considered. In one, other fungicides with different modes of action should be introduced and applied alternately with triadimefon, which leads to relatively long periods without triadimefon being used in agriculture. In the other, triadimefon should not be heavily applied, especially in late spring, thereby slowing down the rate of resistance development.

No wild type, sensitive isolates were collected and maintained before the introduction of triadimefon into China. Hence the study described in Chapter 5 could not reveal the magnitude of the level of fungicide resistance in *E. graminis* f. sp. *tritici* in central China. This is very important in evaluating the effectiveness of a fungicide, slowing down the rate of resistance development, and predicting the life span. Wild type isolates are available in Europe and a further study should be carried out to reveal the fungicide resistance level in China using these isolates to provide baseline data, taking due caution to prevent escape of these isolates from laboratory.

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Summary

Wheat Powdery mildew, caused by the biotrophic fungus *Erysiphe graminis* f. sp. *tritici*, is now an important disease in China in terms of both severity and area of occurrence. This has been marked by two major phases of disease expansion from the Southwest Plateau to north China since the late 1970s, accompanying the changes in wheat cultivation methods, such as increasing densities of plant population, higher inputs of nitrogen fertilizer, shifts in cultivars from local landraces to dwarf and semi-dwarf yield-improved cultivars and expansion of the irrigated area in northern areas. Hubei province, located in central China, was in the region affected by the first phase of the expansion in the late 1970s and continues to be the most heavily attacked. The objectives of this study are to provide relevant knowledge about aspects of the disease epidemiology, pathogen population structure and evolution and host resistance for determining disease control strategies in Hubei province.

Wheat cultivation in Hubei province can be divided into three zones in terms of the epidemic cycle of the disease, characterised by the geographical and topographical features (Chapter 2). The northwest mountainous regions can be divided into two zones: over 800m altitude and between 500-800m. The remaining lowlands, is another zone. In all three zones, only autumn-sown wheat is grown. Therefore, there is a gap between crops. In the two zones at high altitudes, wheat volunteers can survive summer, but there is also a gap between the wheat crop and volunteers emerging from scattered seeds at harvest time. So conidia, the asexual spores of *E. graminis* f. sp. *tritici*, cannot survive summer in Hubei province. Above 800m altitudes, cleistothecia, sexual fruiting body of the fungus, can survive over summer by releasing ascospores infecting both volunteers and early emerging seedlings in autumn because the seasonal gap between crops is short. At 500-800m altitudes, the seasonal gap is longer and the sexual stage of *E. graminis* f. sp. *tritici* bridges the gap only by infecting volunteers. In the lowlands, however, there are no volunteers and cleistothecia of the fungus cannot oversummer because the weather is too hot and too wet. The disease is therefore re-established each year.

For monitoring the pathogen population, a differential set consisting of 9 cultivars plus a susceptible control cultivar was established in this

study (Chapter 3). Owing to lack of knowledge about the relatedness of known *Pm* genes to the local cultivars, the differential set was chosen from important commercial cultivars that revealed regional differences in the pathogen populations across the three zones.

Cleistothecia can tide the fungus over summer and establish disease in the highlands but not in the lowlands. Based on the differential set established in Chapter 3, a hypothesis that the mildew in the highlands provides initial inoculum for the lowlands was tested (Chapter 4). The pathogen population structure in the lowlands in spring 1996 was similar to that in the highlands. This is consistent with the hypothesis. In 1997, however, the pathogen populations were very different between the highlands and the lowlands, which indicates that the highland population is not the only initial source of mildew in the lowlands. Other external sources must also be involved in epidemics of the disease in the lowlands. The virulence matching the resistance of cultivar Mian8724 was at high frequency in spring populations and low frequency in late summer populations during a two-year monitoring of the pathogen population. Mian8724 and its related cultivars are widely grown in neighbouring regions in Sichuan province and Shaanxi province. This would surely have caused selection for the virulence. High frequency of V-Mian8724 in spring population in Hubei suggests that one possible external source may be the neighbouring area of Sichuan and Shaanxi.

Although the sexual stage tides the fungus over summer in the two zones in the highlands, ascospores infect both volunteers and autumn seedlings at over 800m altitudes whereas they only infect volunteers at 500-800m altitudes. However, the pathogen population structure was very similar in both spring and late summer in the two zones. The two zones, therefore, can be treated as a single epidemic unit.

Pathotypes were very diverse and no consistent associations between virulences were detected. This is consistent with sexual reproduction playing a very important role in the structure and evolution of the population of *E. graminis* f. sp. *tritici* in Hubei province.

Variation of responses in *E. graminis* f. sp. *tritici* populations to the triazole fungicide, triadimefon, the most important fungicide to control wheat mildew as well as wheat rust in Hubei province, was investigated (Chapter 5). There has been a reduced performance of triadimefon in controlling wheat powdery mildew in Hubei province. The variation of

responses among *E. graminis* f. sp. *tritici* isolates was very wide, covering a 370-fold range of estimated ED50s. Owing to lack of wild type standard isolates, any conclusion about the resistance level to the fungicide in the pathogen population is not easily drawn at present.

Twenty single-conidium isolates of *E. graminis* f. sp. *tritici* from central China were characterised for virulence to 14 known *Pm* genes. Specific resistance of Chinese wheat varieties grown along Yangtze River valley and some other regions was studied with reference of 14 known *Pm* genes (Chapter 3). *Pm1*, *Pm3b*, *Pm3c*, *Pm5* and *Pm8* were postulated to be present in 17 of 37 varieties tested. But these *Pm* genes were associated, in every case, with other, unknown resistance factors that differed from one variety to another. Resistance in 19 varieties cannot be identified with the isolates. These results indicate that the resistance of many Chinese wheat varieties is largely controlled by unknown resistance genes that differ from those in the European and North American differential sets. Frequencies of virulences matching the 14 known Pm genes in the pathogen populations ranged from 0% to 80% in Hubei. Pm3d, Pm4b and Pm1+2+9 had frequencies of matching virulence less than 1%. These genes are therefore of value in resistance breeding to mildew in central China. Local varieties Dian633, Ziza1028, Ningzi21, Mian8855 and Lin90-35 had frequencies of matching virulence in the *E. graminis* f. sp. *tritici* population less than 4%. The resistance of these varieties can be useful in resistance breeding programme.

Partial resistance in 60 Chinese wheat varieties was assessed in field trials under inoculation with wide range of virulences of *E. graminis* f. sp. *tritici* isolates (Chapter 6). Disease severity in inoculated and uninoculated plots was significantly correlated, which indicates that the inocula used in assessing partial resistance represented the local pathogen population. Final disease severity was regressed against virulence frequency matching each variety and a novel method, measuring the vertical distance (*D*) from the data point for each variety to the expected fitted line, was applied for assessing partial resistance. Five varieties Hx8541, E28547, Chun1066 Ze88pin6 and Lin5064 consistently expressed relatively low disease severities despite high frequencies of matching virulence and had high D values. These results indicate that the five varieties have good levels of partial resistance and are therefore valuable partial resistance sources for breeding for durable

resistance to powdery mildew.

Components of partial resistance in four varieties with good levels of partial resistance were analysed (Chapter 7). All components investigated contributed to the resistance of the varieties. Latent period, number of colonies formed and sporulation capacity are the most important and common components. Germination rate of conidia signals the first interaction between host and the parasite and can be considered a component of partial resistance. Low formation of appressoria and haustoria leads to low infection frequency. Small haustorium size results in less effective nutrient uptake by the fungus leading to slower development of the fungus.

Based on the results and insights of this study, management of wheat powdery mildew in central China by host resistance through breeding and deployment of resistance, cultural practices for wheat and succeeding crops, and application of fungicides are proposed (Chapter 8). Implementation of the proposed practices for management of wheat powdery mildew, and further researches on some aspects to improve understanding of epidemiology in central China are needed to achieve effective control of the disease.

Samenvatting

Meeldauw van tarwe wordt veroorzaakt door de biotrofe schimmel *Erysiphe graminis* f.sp. *tritici*, en is heden een belangrijke ziekte in China. Zowel de mate van aantasting als de verspreiding van de ziekte wordt gemarkeerd door twee belangrijke fasen in de verbouw van tarwe. Uitbreiding van de tarweteelt van het Zuidelijk plateau naar Noord China ging niet alleen gepaard met een hogere plantdichtheid en stikstofbemesting en het vervangen van landrassen door kortstrotarwerassen met een hoger opbrengstpotentieel, maar ook met een toename van de geïrrigeerde tarweteelt in het noorden. Eind zeventiger jaren werd de provincie Hubei, die deel uitmaakt van centraal China, tijdens de expansie van de tarweteelt naar het Noorden getroffen door meeldauw en is nog steeds één van de zwaarst getroffen gebieden. Deze studie beoogt inzicht te geven in relevante aspecten van de epidemiologie, de populatiestructuur en - ontwikkeling van tarwemeeldauw in relatie tot waardplant resistentie ten behoeve van de ontwikkeling van gewasbeschermingsstrategieën in Hubei.

De tarweteelt in Hubei kan op grond van de epidemiologie van de schimmel in drie zones worden ingedeeld die worden gekarakteriseerd door geografische en topografische kenmerken (Hoofdstuk 2). Het Noordwestelijk gebergte kan in twee zones worden ingedeeld: gebieden die gelegen zijn tussen 500 en 800 m en gebieden boven 800m. De laaglanden vormen een derde zone. In deze drie gebieden wordt tarwe in de herfst gezaaid. Hierdoor ontstaat een belangrijke onderbreking van de meeldauw cyclus. In de twee hoger gelegen gebieden kunnen opslagplanten overleven tijdens de zomer, maar de deze planten verschijnen geruime tijd na de oogst. Hierdoor kunnen conidiën, de ongeslachtelijke meeldauw sporen, de zomer in Hubei niet overleven. In gebieden boven 800m kunnen cleistotheciën, de geslachtelijke vruchtlichamen van de schimmel, de zomer wel overleven door ascosporen te vormen die in de herfst opslagplanten en opkomende tarwezaailingen kunnen infecteren omdat de tijd tussen oogst en zaai beperkt is. In gebieden die gelegen zijn tussen 500 en 800 meter is de tijd tussen oogst en inzaai langer. De geslachtelijke sporen van *E. graminis* f.sp. *tritici* kunnen hier alleen de opslagplanten infecteren. In de laaglanden zijn echter geen opslagplanten en worden geen cleistotheciën van de schimmel gevormd door de te warme en te vochtige weersomstandigheden waardoor de schimmel niet kan overzomeren. De ziekte vestigt zich daarom ieder jaar opnieuw.

Tijdens deze studie werd een set differentiërende rassen samengesteld die bestaat uit negen rassen en een vatbaar controle ras om de pathogeenpopulatie

te volgen (Hoofdstuk 3). In verband met onvoldoende informatie over de aanwezigheid van bekende *Pm* genen in lokale rassen werd de differentiërende set samengesteld uit belangrijke commerciële rassen waarmee regionale verschillen in de samenstelling van de pathogeenpopulatie over de drie zones konden worden vastgesteld.

Cleistotheciën kunnen de schimmel helpen te overzomeren in de hooglanden maar niet in de laaglanden. De differentiërende set tarwerassen die in hoofdstuk drie werd beschreven vormde de basis om de hypothese te onderzoeken dat meeldauw in de hooglanden initieel inoculum vormt voor de laaglanden (Hoofdstuk 4). De structuur van de pathogeenpopulatie in de laaglanden in de lente van 1996 was vertoonde overeenkomst met de populatie in de hooglanden, hetgeen overeenkomt met de hypothese. Echter, in 1997 vertoonden de populaties van de hoog- en laaglanden grote verschillen, wat er op wijst dat de populatie in de hooglanden niet de enige inoculumbron voor de laaglanden is. Andere externe bronnen moeten een daarom een rol spelen in de epidemiologie van meeldauw in de laaglanden. De virulentie voor het ras Mian8724 vertoonde een hoge frequentie in lentepopulaties maar een lage frequentie in nazomerpopulaties gedurende de tweejarige monitoring van de pathogeenpopulatie. Mian8724 en daaraan gerelateerde tarwerassen worden op grote schaal verbouwd in belendende regio's van de provincies Sichuan en Shaanxi. Dit heeft zeker een rol gespeeld in de selectie van virulentie voor dit ras. De hoge virulentiefrequentie voor het ras V-Mian8724 in lentepopulaties van Hubei suggereert dat deze gebieden een externe inoculumbron vormen voor deze provincie.

Hoewel de geslachtelijke cyclus de schimmel helpt te overzomeren in de twee hooglandzones, infecteren ascosporen opslagplanten en herfszaailingen in gebieden boven de 800m, maar zij infecteren alleen opslagplanten op 500-800m hoogte. Desondanks vertoonde de populatiestructuur tijdens de lente en de nazomer een grote overeenkomst. Deze twee gebieden kunnen daarom als twee afzonderlijke epidemiologische units worden beschouwd.

De gekarakteriseerde fysio's waren zeer divers zonder enige consistente associaties tussen virulentiefactoren. Dit stemt overeen met het feit dat de geslachtelijke voortplanting een zeer belangrijke rol speelt in de ontwikkeling van de populatiestructuur van *E. graminis* f.sp. *tritici* in de Hubei provincie.

Triademefon, een triazool fungicide, is het belangrijkste fungicide ten behoeve van de bestrijding van meeldauw en roestziekten in Hubei. De variatie in gevoeligheid van *E. graminis* f.sp. *tritici* voor dit fungicide werd onderzocht (Hoofdstuk 5). Triademefon vertoont een verminderde werkzaamheid bij de

meeldauwbestrijding in Hubei. De geschatte ED50 van meeldauw isolaten voor triademefon vertoonde een zeer groot spreiding en bestreek een reeks tot aan een 370-voudige gevoeligheid. Door het ontbreken van wildtype isolaten is het echter niet mogelijk iets te vermelden over het resistentieniveau tegen dit fungicide in de pathogeenpopulatie.

De virulentie van 20 mono-conidium isolaten van *E. graminis* f.sp. *tritici* uit centraal China werd gekarakteriseerd voor 14 bekende *Pm* genen. De aanwezigheid van deze genen is onderzocht in Chinese tarwerassen met een specifieke resistentie tegen meeldauw die in het stroomgebied van de rivier de Yangtze en enige andere gebieden worden geteeld (Hoofdstuk 3). De aanwezigheid van *Pm*1, *Pm*3b, *Pm*3c, *Pm*5 en *Pm*8 werd gepostuleerd in 17 van de 37 onderzochte rassen. Deze genen waren echter altijd geassocieerd met onbekende resistentiefactoren in elk van deze rassen. Negentien rassen vertoonden geen resistentie tegen de gebruikte meeldauw isolaten. Deze resultaten geven aan dat de resistentie van vele Chinese tarwerassen grotendeels wordt bepaald door onbekende genen die niet aanwezig zijn in de Europese en Noord-Amerikaanse differentiërende rassenset. De virulentiefrequenties voor de 14 bekende *Pm* genen liepen uiteen van 0-80% in de pathogeenpopulatie van Hubei. De virulentiefrequenties voor *Pm*3d, *Pm*4b en *Pm*1+2+9 waren lager dan 1%, terwijl de frequentie voor de onbekende meeldauwresistentie in de lokale tarwerassen Dian633, Ziza1028, Ningzi21, Mian8855 en Lin90-35 kleiner was dan 4%. Deze genen(combinaties) en rassen zijn daarom waardevol voor resistentieveredelingsprogramma's, in het bijzonder ten behoeve van centraal China.

De partiële resistentie van 60 Chinese tarwerassen werd in het veld beoordeeld door deze te inoculeren met een breed spectrum van virulente meeldauwisolaten (Hoofdstuk 6). De mate van aantasting in geïnoculeerde en niet geïnoculeerde veldjes vertoonde een significante correlatie. Dit is een aanwijzing het gebruikte inoculum een voldoende representatief was voor de lokale pathogeenpopulatie. Er werd een regressieanalyse uitgevoerd van de uiteindelijke mate van aantasting tegen de virulentiefrequentie voor ieder ras waarbij een nieuwe methode werd gehanteerd die de verticale afstand van ieder datapunt tot de regressielijn (D) gebruikt als schatter voor de partiële resistentie in dat ras. Vijf rassen, Hx8541, E28547, Chun1066 Ze88pin6 en Lin5064, hadden een hoge D waarde en vertoonden een consistente relatief lage mate van aantasting, ondanks de hoge virulentiefrequentie voor de resistentie in deze rassen. Deze resultaten geven aanleiding tot het veronderstellen van goede partiële resistentieniveau's in deze rassen waardoor zij waardevolle bronnen

zijn voor veredelingsprogramma's die gericht zijn op duurzame resistentie tegen meeldauw.

In vier rassen met een goed niveau van partiële resistentie werd een componentenanalyse uitgevoerd (Hoofdstuk 7). Alle componenten die werden onderzocht droegen bij aan het niveau van resistentie. De latente periode, het aantal gevormde kolonies en de sporulatiecapaciteit zijn de belangrijkste en gemakkelijkst te bepalen componenten. Het kiemingspercentage van de conidiën is een resultaat van de eerste interactie tussen de waardplant en het pathogeen en kan worden beschouwd als een partiële resistentie component. Lage aantallen appressoria en haustoria leiden tot een lage infectiefrequentie. Kleine haustoria beperken, door een minder efficiënte voedselopname, de ontwikkeling van de schimmel.

De resultaten van en inzichten door deze studie vormen de basis voor een voorstel om het management van tarwemeeldauw in China te verbeteren door resistentieveredeling en -diversificering, cultuurmaatregelen voor tarwe en opvolgende gewassen en het gebruik fungiciden (Hoofdstuk 8). De implementatie van deze maatregelen en een voortzetting van het onderzoek naar de epidemiologie van meeldauw in centraal China zijn gewenst om een effectieve beheersing van deze ziekte te bewerkstelligen.

后记

这个后记包括三个方面，有些是写在前言的话，有些是补记，另外是研究工作的中文小结。因为论文必须用英文发表，我还是不得不在前言的基础上再用母语 – 中文补写一些。

这本论文与其说记下了在湖北小麦白粉病的研究方面所作的一些工作，不如说记下的是一条艰辛而曲折的小路。一九九二年的此时，我背着沉重的行李，离开家人，离开家乡，来到世界著名的农业研究中心之一的荷兰瓦赫宁根，开始徒步这条小路。后来这条小路从荷兰沿伸到瑞士，丹麦，荷兰，英国，最后落脚荷兰，整个形成一道圆圈，更准确地说是一道怪圈。我走完这道怪圈历经了八年时间，几次意欲放弃，但有许许多多人从正面和反面支持，特别有了反面的支持，使我欲止不能。最后由于有了很多人的帮助，我才沿着这条小路走到了预定的目的地。如果没有许许多多人的支持，帮助和理解，发表在此论文中的工作是不可能完成的，这道怪圈也不可能成为今天的一个句号。

首先，我真诚地感激我的两位导师，荷兰瓦赫宁根大学，英国伦墩大学崴学院教授迈克·杰格博士和英国约易利斯中心詹姆斯·布朗博士。我不会忘记五年前从小贝尔特海峡吹到丹麦若斯基德海边冬天的海风。我从瑞士苏黎世湖畔去到若斯基德，你们从荷兰和英伦去到那儿，我们简短的讨论我的研究计划，在你们的启迪下，我豁然觉得来自那小贝尔特海峡的风不是冬天的大风，而是春天的微风，正在吹绿中国中部广袤大地上的麦苗。迈克，没有您飞越欧亚的指导和鼓励，我的信心不会十足。在崴的日子里，尽管您刚落坐于你新的座椅，你的新桌旁堆满要您及时处理的事情，但您仍然花费大量的时间关注我的论文。詹姆斯，没有您频传而富有意味的电子邮件和图形示范，我对"种群"这个自然和社会兼有的概念的理解会十分肤浅。我深深的感谢黛安娜，她包揽所有的家务，使你能在晚上和周末修改我的论文。在伦墩大学崴学院和约易利斯中心的日子是令人难忘的。冬天英伦天气之恶劣令人沮丧，论文修改之枯燥令人乏味，但您的学识，对科学的执着，对我的论文的投入深深地打动着我。

我十分感谢荷兰农业部研究院植物保护研究所赫特·克马博士及其夫人海娜和她们的孩子们。赫特，作为朋友和同事，是你最初于一九九二年邀请我作为访问学者来到欧洲，来到荷兰，来到瓦赫宁根。这一邀请给我铺垫了一条道路，并且带来了一篇你我之间悠长而有趣的故事，这篇故事记下了我人生的一段历程，也在你的人生篇章中写下了重要的一笔。我不可能忘记我们为小麦叶枯病病理组织学所付出的辛劳，不可能忘记我们每每看到了菌丝在细胞间一点细微变化而不同所引起的欢欣鼓舞，这一辛劳使我们成为叶枯病经典文献的作者。我不可能忘记我们每个周末晚上那种海阔天空的漫谈。你们和你们的孩子们欣赏着古老中国的菜肴，我了解着曾经比英伦民族更强大的荷兰民族的文化。海娜的热情不象西方女子那么狂放，倒象东方女性体贴入微。海娜，谢谢你的那些丰盛的晚餐。

多谢艾尔丝在我在荷兰植保所工作期间以及后来编辑论文时所给予的帮助。阿丽和科芮所给予的帮助令人难忘。

赫特，你和可尔博士把我引见给世界权威植病学家扎多克斯教授和育种学家帕立威利艾特教授，我十分荣幸曾经得到扎多克斯教授的赏识。由于国内的种种原因，我没有能在

小麦黄斑病上做任何工作，没有在他们的指导下完成博士学位学习，但我从和他们的谈话及他们的著作中获得了知识。没有在他们的指导下完成博士学位也带给我另一个契机，使我在阿尔卑斯山脚下，苏黎世湖畔认识了世界白粉病权威马丁·沃尔夫教授和艾克哈德·林伯特博士。

艾克哈德，谢谢你邀请我去到美丽的山国—瑞士。在苏黎世的日子是一段美好的记忆。瑞士那美不胜收的风光令人留连忘返，天之高，云之淡，水之蓝，树之绿是世界独一无二的。那一城必靠一山，必伴一湖的景观是人与自然的最完美的结合。英特拉肯地区的山顶覆盖的白雪使其终年银装素裹，而山脚下的湖面却始终万尾鱼跃。山国的景观不是仙境，胜似仙境。最为有趣的是三种语言交融一起组成这个小小山国的官方语言而使其更具国际化的氛围。我欣赏那儿的风光，欣赏那儿的风情，但我最欣赏的是你们的科研水平。你安排我在作为世界禾谷白粉病研究中心的瑞士联邦理工大学的学习是我博士学位学习的第一阶段，你的指导为我的学习打下了坚实的基础，我在那儿学到了真正的种群研究的方法和手段，马丁和你是我最初进入这个领域的导师。克里斯蒂娜，我的衣服脏了，你为我洗衣，我的肚子饿了，你为我做饭，你问寒问暖的那种关照使我感到宾至如归。你们安排了我的学习费用又让我住在你们家却决不收取分文的慷慨会令西方人感到尴尬，但令一个漂泊他国的东方人感到的却是一种友情。

马丁，您是禾谷类白粉病的世界权威，您有英国人的伸士风度，但您不失作为一个普通的科学导师的本份，热爱学生永远是您的风范。是您给我博士入学考试。我永远记得当我问到我的考试成绩时您的幽默，您说您不是中国龙。是您把我推荐给迈克，送我去丹麦，使我能走过从荷兰到荷兰这样一道圆圈。是您帮我修改开题报告，为我完成学习奠定了坚实的基础。我荣幸我能和您曾经相识，我荣幸我曾经是您的学生并得到过您的教诲。

如果没有朋友的鼎力相助，这本论文更不可能完成。我万分感谢我的同学，学友，朋友，农业部周开忠司长，中国农科院研究员，科技管理局局长叶志华博士，中国农科院研究员，植保所曹雅忠所长在项目安排上所给予的支持。开忠，在我最困窘的时候，是你和志华商量着拉我一把，才让我得以动弹。志华，我不会忘记你对我的所有帮助，我懂得你的帮助对我意味着什么。雅忠所长，我们已是两个五年计划科技攻关的挚友了。我对该项目负责人倪汉祥老师表示深深的谢意。这本论文凝聚着你们的帮助和支持及友情。如果说这本论文有其躯体，所有来自你们的帮助才是它的灵魂。

同时，我还要感谢我们合作多年的伙伴，中国农科院植保所白粉病组的专家们，农业部农技推广中心邵振润处长，刘万才农艺师。十分感谢你们的帮助，你们的帮助使我懂得了白粉病的很多层面，这些层面的理解帮助了我学习任务的完成。

此时，我对我自己所里面的同事们怀着深深的谢意。特别要感谢王少南所长。王所长，在我两度远涉欧洲，放下家里的管理工作时，您不辞劳苦地接下本该我承担的担子，当我在国内科研任务压力太大时，也是您接下本该我承担的担子。没有您这种无私的支持，这本论文将很难完成。谷守礼先生在我出国期间也承担了该我完成的大量任务，对你们的支持深表感谢。我要感谢我的老师甘启芳先生这么多年来对我的鼓励，关怀和教诲。

我很难找到恰当的语言来描述我的两位学生，助手加同事杨小军和杨立军对这项工作的贡献，他们辛劳的工作够成了这本论文的骨架。小军，郭西北的山，尽管没有阿尔卑斯

山那样雄奇，鄂西北的川，尽管也没有莱茵河和泰吾士河那样罗漫，但是，那是家乡的山川，你的工作让外人知道了我们家乡的山川。我想为了寻找白粉病原流行的蛛丝马迹，如果现在还让你冒着酷暑和严寒再徒步鄂西北的山山水水，你还会打起背包，带上你的采集盒再走遍鄂西北的麦田和稻场，你还会在农民家里借着月光计划第二天你的去向。因为你懂得你在为家乡的山添绿，为家乡的水添绣。立军，科研是有趣和枯燥的结合，它正在陶冶着你我。谢谢你种了那么多的麦苗，剪了那么多的叶片，调查了那么多的数据。两只"小羊"，感谢你们在实验室度过了那么多的周末。没有你们的辛苦就没有这本论文。我们是这本论文的共同作者。

我在这里还要对湖北省农科院院长，教授，刘定富博士表示深深的感谢。刘院长，你首先树立的是榜样，然后是支持与理解，这些成为我完成此项工作的重要动力。陈廷济院长给了我很多道义上的支持，给了我很多克服困难的勇气和力量。其他院领导也给予了专业上和其他方面的诸多支持。没有您们的鼓励、指导、支持，也不会有此论文。

我很高兴有机会感谢胡伟华先生，他对这项工作始终如一的支持也凝聚在此论文中。同时，对省人事厅技干处的领导对此项工作所给予的支持表示感谢。

借此机会，我衷心地感谢我高中的老师梅保林先生，感谢您在我世界观形成过程中对我的教诲，助我成长。感谢我儿时的朋友们涂光良，王洪胜及夫人熊小桃，李植，涂范荣，熊建国，鲁天生，是这些儿时的朋友们对生活和事业深层的理解和执着的追求的榜样作用鼓励我上进，是我们持之以恒的友情使我有力量奋斗。

远离家乡是痛苦的。在我为博士学位作早期准备的时候，胡志红，梁俊奕，张守健，郑小鹰，杨伟才夫妇，张筱平及夫人纪勤，曾令文夫妇这些同胞在瓦赫宁根给了我精神上巨大的支持，我们也结下了深厚的友情。在苏黎世的日子里，如果没有清华大学教授周志伟博士及夫人彭凌以及谢志鹏博士的友情，瑞士的风景再美，生活将显得乏味。特别要感谢黄如国博士对我的计划初稿的修改和从加拿大发回国内的一些传真指导以及他夫人在生活上的关照。再返瓦赫宁根的日子里，又得到了许多朋友的关照。老朋友纷纷离去，新朋友纷至沓来。这儿我不提那么多新朋友们的大名，他们在我心中。然而，有两位新朋友，我无法不提及。马耀明及贾立在我再返瓦市时给予的照顾是令人难忘的，短暂的相处使我们成为了朋友。在英国约易利斯中心期间，漆小泉博士及夫人王英坤，周发松博士及夫人给予了很多关照，这些关照极大地缓解了我修改论文的紧张。

在这个谢忱的最后，我深深地谢谢我的家人和亲戚。我父母使我受教育，教我热爱生活，教我做人的道理，这些是我学习的基础，也是我永远的前进动力。感谢我的哥嫂和孩子们的支持。感谢我妻子的姐姐，姐夫，弟弟，弟媳和她们的孩子们，在我多次奔波他国的时候，你们给了我家庭许多的关照。特别要感谢我的妻子王久珍在我的博士学习的整个过程中付出的巨大艰辛。我深深地感谢你，久珍。感谢你在我远涉他乡之时，你承担着既当爹又做妈的双重重担，料理家庭；感谢你在我情绪低落的时候，给我鼓励，使我恢复前进的力量；感谢你在我困倦的冬夜，给我一杯浓茶，感谢你在我饥渴的夏午，给我一碗甜汤。没有你的相濡以沫的理解，鼓励，合作与支持，我绝对无法坚持这个比别人时间更长，道路更艰辛的学习过程。我十分感谢我的女儿喻理。喻理，感谢你当我不能照顾你时，你对我的理解，感谢你认真学习，使我得以宽心。

　　这本论文的完成凝聚了许许多多人的心血．这些心血的付出可能会给小麦白粉病的防治带来一些益处。

　　此论文用八个章节写了湖北麦区小麦白粉病发生流行的病原菌种群生物学，小麦品种抗病性及其该病害防治的有关问题。

　　禾谷白粉病发源于中东野生禾谷杂草上，随着人类将这些草本植物驯化为作物作为人类的食物来源后，它也就成为我们获得好的谷物收成的一大障碍。小麦白粉病一直是世界上冷凉潮湿地区小麦的重要病害，该病在我国二十世纪七十年代以前仅仅在西南地区和山东半岛沿海地带造成经济损失而具有经济重要性。随着小麦种植水平的提高，特别是矮杆和半矮杆品种的推广，抗病性的单一化，种植密度的加大，氮肥投入量的急剧增加以及北方麦区灌溉面积的扩大，该病在我国从南向北几度扩迁，在我国几乎有凌驾于锈病之上成为第一大小麦病害的趋势。这本论文的第一章讨论了小麦白粉病在我国流行的方方面面，在湖北麦区的重要性，主要提出了湖北麦区小麦白粉病流行过程中一些急待解决的问题和一些问题的成因。这些分析够成了此论文的研究方向和研究目的。

　　小麦白粉病菌在湖北麦区每年在小麦生长的后期发生一次有性世代。因为湖北麦区只有冬麦种植，白粉病菌的专性寄生性使其不可能离开寄主而生存。从上季收获到再种小麦这段时间白粉病原的踪迹何在？有性世代在完成从上季到下季的侵染过程中起什么作用？这些不仅关系流行的问题还关系到种群变异的问题在第二章作了研究和分析。

　　自布瑞革勒建立一套抗小麦白粉病的近等基因系以来，至目前为止在小麦染色体的 23 个位点上已定位 28 个抗白粉病基因（包括复等位基因），其中 *Pm10, Pm11, Pm14, Pm15* 只抗僵麦白粉病而不抗小麦白粉病。国际上这些抗病的单基因材料常作为鉴别寄主来研究病原种群的进化与变异以及预测抗性基因的风险，国内也不乏这种研究。这种研究在我国的重要性只能用于哪些以知的抗病基因有效和风险。但是，并不能很好地监测病原种群的时空变化，因为我国小麦品种的遗传背景只知部分，含有哪些以知的抗性基因只是臆测。例如，许多专家说 *Pm8* 在我国完全丧失了抗性（病原中相应毒性基因固定）。因为我国在抗锈病育种中引进了罗马尼亚品种洛夫林系，洛夫林系含有从黑麦转来的 1B/1R 易位系上的 *Pm8* 基因，我国又很多种植品种与洛夫林有血缘关系，另外加上她们所用的捕捉菌原的品种本身含有 *Pm8* 基因，得出病原中对 *Pm8* 有毒基因以经固定的结论不足为奇。其实不然，*Pm3* 的某些等位基因所受到的病原种群的压力绝对比 *Pm8* 更大。因此，要了解某地区病原种群的时空变化，必须根据病原和寄主协同进化的规律来研究。第三章主要报道了建立一套鉴别菌株并对湖北及周边麦区 37 个主栽品种和育种材料根据伏洛的基因对基因学说进行抗病基因的推断，在这 37 个品种（系）中，*Pm1, Pm3b, 3c, Pm5,* 和 *Pm8* 可能存于 17 个品种中，但是，这些品种多带有其他抗性因子。因此，简单地用已知基因来鉴别监测病原群有所弊端。为此，我们筛选出了一套对白粉病菌种群能够有效监测并更接近生产的品种材料。

　　毒性基因的突变，重组，相互搭载，寄主筛选和以群体或个体方式的迁移是病原种群变异的主要来源。所有这些变异方式在白粉病菌的变异中均能找到例证，所有这些也都是以种群遗传学为主要内容的种群生物学的研究对象。然而种群的远距离传播是病原种群变异最快捷的方式。湖北麦区是一个地貌复杂，环境各异，品种多样的麦区。因而，白粉病

在区内不同的地方表现出不同的流行规律。但在病菌种群生物学概念上是否有着相互联系呢？湖北小麦白粉病菌种群是一个独立的种群还是有外来种群加速了它的变异呢？论文的第四章以鄂西北山区不同海拔以及江汉平原为背景地区，研究了该麦区白粉病菌种群迁移这一与防治关系极大的重要问题，部分地回答了上述有关种群生物学的疑问。

化学防治是病害治理的有效方法之一。长期单一，大量使用化学药剂防治一种病害所带来的恶果必是病菌在其筛选压力下产生巨大的抗药性。群体抗药性产生的途径与种群变异的所有过程有关，也可能本身存在抗性个体而被筛选成为群体的主体部分。无论是什么原因，抗药性的产生将造成两大恶果，一是使人们加大该药剂的使用，造成环境的严重污染和食品中农药残毒增加，二是病原群体对药剂抗性达到一定程度，化学药剂完全丧失效用，人们就完全失去了一种防治的手段。粉锈宁是我国广泛用于防治小麦白粉病和三种锈病的最主要的药剂。白粉病菌种群抗药性水平多高？能否继续长期使用？怎样能使其长期使用？这是迫在眉睫必须回答的问题。第五章报道了两年中一千多采自不同地方的白粉病菌株的群体对粉锈宁的反应变异水平。与此同时，对抗药性首次在国内作为监测标记物用来研究菌原迁移问题的手段也作了报道。

品种的专化性抗性是很容易被病原克服的，持久抗性在病害防治中有着巨大作用，也是持续农业的一个主题。持久抗性的定义是某一品种在病害易于发生的环境下大面积长期种植而不失抗病性，这个定义包涵着"易发病的环境"，"大面积"，"长期"和"抗病性"四大概念。没有很好的研究和试验方法能够用于持久抗性的筛选，至少目前没有。但有证据证实部分抗病性具有持久性(并非所有的部分抗性)。论文的第六章对 60 个小麦品种进行了部分抗性的田间筛选，其中几个品种表现出较好的部分抗性特点，它们可以作为持久抗性育种的很好抗源。

第七章针对在田间筛选出的几个具有部分抗性的品种进行了抗性组份的分析，并用病原与寄主相互作用及病理组织学的方法确定了部分抗性的存在。

第八章最后总结和讨论了湖北麦区小麦白粉病流行的全貌，品种抗病性的利用，粉锈宁作为有效药剂用于防治中应注意的问题。本章还对整体防治管理体系的建立及其运作进行了讨论。

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喻大昭

二零零零年四月于荷兰瓦赫宁根

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Curriculum vitae

Yu Dazhao was born on January 14, 1956 in Wuhan, Hubei province, China. After completed high school study in 1972, he was dispatched to countryside in Hubei to receive so-called re-education from farmers during Cultural Revolution period of China. He entered Huazhong (Central China) Agricutural University, majored in plant protection, in 1978 and completed his Bsc study in 1982. In 1982, He commenced his scientific career when he was employed by the Plant Protection Institute of Hubei Academy of Agricultural Sciences (HAAS), Wuhan, China. He participated in National Project of research on integrated control of wheat head blight caused by *Fusarium* spp. and wheat powdery mildew from 1982 to 1990. In 1988, he studied rice disease control in Kobe University, Japan, for one year. From May 1992 to May 1993, he worked in IPO-DLO, the Netherlands, on *Septoria tritici*, as a visiting fellow, which initiated his PhD programme in Wageningen University. In May 1995, he studied in ETH, Zurich, Switzerland, as the first stage of his PhD study.

He was appointed as the head of Phytopathology Section of Plant Protection Institute, HAAS in 1988, the head of Plant Protection Institute and senior researcher in 1994. He intends to continue his research on fungal disease of wheat.