Eur J Plant Pathol (2007) 118:93–104
DOI 10.1007/s10658-006-9097-1

REVIEW PAPER

Arguments for a low risk of establishment of Karnal bunt disease of wheat in Europe

David R. Jones

Received: 23 October 2006 / Accepted: 18 December 2006 / Published online: 12 April 2007

Abstract *Tilletia indica*, which causes Karnal bunt of wheat, has been perceived as a pathogen that has a high risk of establishment in the UK and other countries in Europe. This viewpoint is challenged with arguments for the risk of establishment being much lower. The present distribution of the pathogen and its likely widespread dissemination with consignments of germplasm and wheat seed from Mexico over a 20 year period without establishment outside the hot arid and semi-arid zones strongly suggests that *T. indica* is unsuited to environments other than those similar to where it is now found. A critical density of teliospores needs to remain viable and ungerminated between periods of wheat anthesis and then germinate during a narrow window of opportunity to ensure infection and establishment. Despite the results of outdoor teliospore longevity experiments, it is by no means certain that this can occur under European conditions. Generally cooler conditions and more frequent periods of rain in Europe are likely to trigger teliospore germination at times unsuitable for infection making establishment much more unlikely. A model based on year-round soil conditions, especially moisture content, is seen as appropriate to predict teliospore survival and germination potential. It is only after areas where teliospores can survive and germinate in sufficient numbers to guarantee regular infection cycles have been defined can another model, based on temperature, rainfall and/or humidity prior to anthesis, be applied to determine if environmental conditions are suitable for infection.

Keywords Karnal bunt · Pest risk analysis · Potential disease distribution · *Tilletia indica* · Teliospore · Wheat

Introduction

Karnal bunt is a disease of wheat caused by the fungus *Tilletia indica* (Carris, Castlebury, & Goates, 2006; Mitra, 1931). It usually has minimal impact on crop yield and quality, but its importance is that it is a disease of political significance and is used as a quarantine barrier to trade (Beattie & Biggerstaff, 1999). The disease needs to be placed in perspective by international co-operation between plant health authorities around the world, but this has not happened to date. As a result, the appearance of Karnal bunt in wheat crops or the discovery of teliospores of *T. indica* in grain shipments can result in a loss of export markets.

David Jones is a retired employee of the Central Science Laboratory (CSL). Statements and opinions expressed in this paper are those of the author and are not the views of the Central Science Laboratory.

D. R. Jones (✉)
Consultant in Plant Pathology and Pest Risk Analysis,
12 Charlotte Brontë Drive, Droitwich Spa,
Worcestershire WR9 7HU, UK
e-mail: bananadoctor@msn.com

Tilletia indica has been declared an A1 quarantine pest by the European and Mediterranean Plant Protection Organisation (EPPO) (Anon., 1997) and is designated an 1/A1 harmful organism whose introduction into, and spread within, all member states shall be banned by the European Commission (Anon., 2002b). These organisations recognise Karnal bunt as a serious exotic disease that threatens the wheat industries of Europe. Although symptoms of Karnal bunt have never been reported in Europe, wheat germplasm sent from Sweden to India in 1979 was contaminated with teliospores of *T. indica* (Lambat et al., 1983). As the origin of the germplasm was a Swedish plant breeding institute, it is possible that the contaminated seed could have been acquired from elsewhere and passed on to India without sowing (Diekmann, 1987).

Teliospores contaminating wheat seed or grain are believed responsible for long-distance dissemination in a manner similar to other bunt diseases of wheat (Wilcoxson & Saari, 1996). Teliospores in soil attached to machinery may also spread the disease, as can wind-blown inoculum (Bonde, Prescott, Matsumoto, & Paterson, 1987). Teliospores germinate on or near the soil surface to produce promycelia upon which primary sporidia develop. Primary sporidia give rise to secondary sporidia. This is aided by humidity, often provided by rainfall, under the wheat canopy (Goates & Jackson, 2006). Both primary and secondary sporidia are dispersed to the flowers by air currents or rain splash. Germ tubes from germinating sporidia infect the florets through stomata. Hyphae then grow to the base of the floret and invade the periderm of the nascent kernal (Goates, 1988).

Over a hundred primary sporidia may be produced by each teliospore and many more secondary sporidia. One experiment has shown secondary sporidia to be short-lived because of desiccation (Smilanick, Prescott, Hoffman, Secrest, & Weise, 1989) while another indicates that survival for longer periods is possible (Goates, 2005). The most vulnerable stage of wheat development is believed to be from spike emergence to the onset of anthesis, though infection can occur through to the soft dough stage. The window of opportunity for infection may be about 35 days (Goates & Jackson, 2006).

Tilletia indica is heterothallic and has four different bipolar mating types. Pathogenesis can only occur when individuals of two different mating types anastomose, which probably occurs within host tissue (Garrett & Bowden, 2002). Only a few spikelets are normally bunted within a wheat spike. *Tilletia indica* usually causes a partial bunt with teliospores replacing only a portion of the kernal. The kernal may rupture on harvest to release teliospores (Warham, 1986).

Research on Karnal bunt was recently funded as Project QLK5-1999-01554 under the EC Fifth Framework in order to define risks to European wheat crops. A pest risk analysis (PRA) submitted as Deliverable Reports DL 6.1 and 6.5 concluded that the pathogen had the potential of establishing in the UK and many other European countries (Sansford et al., 2006). This conclusion was reached after an analysis of weather data for a few weeks prior to wheat anthesis using a Humid Thermal Index (HTI) model. The model predicted that environmental conditions at the vulnerable period for infection were often ideal for disease initiation (Sansford et al., 2006). In addition, inoculation studies revealed that wheat cultivars grown in Europe were susceptible to *T. indica.* Furthermore, a proportion of teliospores buried in soil in different European countries was shown to retain viability for up to 3 years (Sansford et al., 2006).

The PRA proposed that the pathogen could survive as viable teliospores and still infect European wheat crops even if conditions immediately prior to anthesis over one or two consecutive seasons were unsuitable for teliospore germination and infection (Sansford et al., 2006). The establishment of *T. indica* in Europe was perceived as highly likely should it gain entry and the pathogen was considered a major threat to the continent's wheat industry (Sansford et al., 2006). A similar conclusion was reached in an earlier PRA prepared for the Department for Environment, Food and Rural Affairs in the UK (Sansford, 2004).

The arguments used in the two PRAs are persuasive, but all the available facts do not seem to have been considered in reaching the conclusion. Arguments in favour of *T. indica* being of low risk of establishment in Europe are presented here.

Seedborne fungal pathogens of wheat

Richardson (1990) reports 26 seedborne fungal pathogen species affecting wheat. Twenty-five of the 26 seedborne fungi are found in Europe, the only

exception being *T. indica*. Of the six seedborne bunts and smuts affecting wheat, *T. indica* is the only one not found in all six global regions defined as Africa, Asia, Australasia, Europe, North America and South America.

Wheat is a commodity that is commonly traded around the world. There is also an international trade in wheat seed and wheat germplasm is routinely exchanged between wheat breeders in different countries. The widespread distribution of most seedborne fungal pathogens and in particular the *Tilletia* spp. (Wilcoxson & Saari, 1996) reflects the ease with which they have moved internationally in the past. Why then has *T. indica* not spread, as have the other seedborne fungal pathogens of wheat? It is proposed that this is not through lack of opportunity, but the result of the unsuitability of most wheat-growing environments for establishment.

Present distribution of Karnal bunt and implications for potential disease spread

Karnal bunt affects wheat crops in the northern regions of Pakistan and India (Warham, 1986, 1992). As symptoms may have been seen in the Punjab in Pakistan in 1909 (Warham, 1986) and the pathogen first described in the Punjab in India in 1930 (Mitra, 1931), this region is likely to be the centre of origin of the disease. It is possible that it could have arisen further west with one of wheat's wild progenitors, but there is no evidence to support this hypothesis.

Karnal bunt is not common in the Indian subcontinent outside the northwest region. Disease incidence is very low in central and eastern India. Karnal bunt has never been reported in the south or northeast of the Indian subcontinent (Anon., 1996; Joshi, Singh, Srivastava, & Wilcoxson, 1983; Singh, 2005; Singh, Srivastava, & Joshi, 1985).

The disease has also been recorded in southern Nepal (Singh, Agarwal, Shrestha, Thrapa, & Dubin, 1989), Iraq (Mustafa, 1965) and southern Iran (Torarbi, Mardoukhi, & Jalaiani, 1996) in Asia. Wheat from Afghanistan (Locke & Watson, 1955), Lebanon, Syria and Turkey (Lambat et al., 1983), has been found contaminated with teliospores of *T. indica*, although the pathogen has never been reported in the field in these countries (Warham, 1986, 1992). Later wheat germplasm consignments from Lebanon, Syria and Turkey were free of teliospore contamination (Diekmann, 1987). The status of the disease in these countries is unclear. An international survey using a standardised protocol has been suggested as the only way of determining the exact distribution (Babadoost, 2000).

The disease has established in the New World in northwest Mexico (Warham, 1986) and has been reported in southern California, Arizona and Texas in the US (Rush et al., 2005). Da Luz, Mendes, Ferreira, and Urben (1993) reported finding *T. indica* in a seed lot produced in Rio Grande do Sul State, Brazil in 1989, but there have been no reports since and establishment has not been proven.

Karnal bunt also occurs in an area of northern Cape Province in South Africa (Crous et al., 2001).

All locations where Karnal bunt has established lie on or close to latitudes 30° North and 30° South (Fig. 1) and can be described as having arid or semi-arid climates with mild/cool winters and hot summers. In the main, they have been designated as dry environments ranging from 'desert' with little or no rain to 'arid steppe' or 'semi-desert' with a short rainy season. In the plains of the Ganges valley in northwest India, the climate is termed 'subtropical summer rain'. Karnal bunt has also been recorded in some fringing environments, but incidence is very low and outbreaks can be sporadic. The climates of these areas are termed 'subtropical rain' and 'tropical summer rain' (Pearce & Smith, 1990; Stefanski, Brusberg, Puterbaugh, Morris, & Motha, 1994).

A problem for the theory that Karnal bunt is likely to establish in Europe is that during the 76 years since *T. indica* was first described in India (Mitra, 1931), Karnal bunt has never established outside certain arid or semi-arid areas with mild/cool winters and hot summers. It has never been reported in the field in 'oceanic temperate', 'temperate continental' or 'subtropical winter rain' environments that prevail in Europe. Karnal bunt has not even been reported in southern parts of Mexico and India with different environments to areas in these countries where the disease is established, although there have been many opportunities for internal spread (Fuentes-Davila, 1996; Singh, 2005).

In the northwest of India, where incidence of Karnal bunt is relatively high, rain-fed wheat is sown in late October and irrigated wheat is sown in November. Harvesting generally starts in mid-May.

Fig. 1 World map showing regions where Karnal bunt may have established since the pathogen was first described in India in 1930. All areas where the disease now occurs (marked in white) lie in the hot arid and semi-arid zones along latitudes 30°N and 30°S. The disease has not been reported in Afghanistan, but grain from this country has been found contaminated with teliospores of *T. indica*. Southern Afghanistan has been included as an area where the disease is found on the map because it borders Pakistan and Iran where the disease is known to occur. Lebanon, Syria, Turkey and Sweden, where wheat germplasm contaminated with teliospores of *T. indica* has originated, but where disease symptoms have never been reported, have not been included. The areas in the US regulated for Karnal bunt are exaggerated. The disease is present in a few counties in the states of California, Arizona and Texas. A more precise indication of distribution can be found in Rush et al. (2005). Southern Brazil has also not been included, as there is only one record of contaminated grain in 1989 and the disease has never been reported in the field

Four to six irrigations are recommended with one coinciding with flowering (Anon., 2006). In Karnal-bunt infested areas in the state of Sonora in Mexico, Arizona in the US and northern Cape Province in South Africa, wheat is also irrigated. The influence of irrigation, as opposed to normal rainfall, on disease incidence is difficult to determine, though it would undoubtedly increase humidity and cool temperatures under the wheat canopy making conditions more suitable for infection (Smiley, 1997; Stansbury & Pretorius, 2001). This may be important in arid environments. Nevertheless, high Karnal bunt incidence is still correlated with periods of natural rainfall rather than irrigation by scientists in India (Damodaran, 2003).

The incidence of disease declines significantly in India in the states of Bihar and West Bengal that lie southeast of the relatively high-incidence environments in the foothills of the Jammu region, lower Himachel Pradesh, Punjab, Haryana, Delhi and Uttar Pradesh (Joshi et al., 1983; Singh et al., 1985). It could be argued that this particular bunt pathogen, which may have evolved to infect wheat in the northwest of the Indian subcontinent, may not be suited to other climates, especially those with more rainfall. Other limiting factor in India may be higher than optimum temperatures for teliospore germination prior to anthesis and conditions in the mid and upper Himalayas where snowfall occurs every year (Singh, 2005). It cannot be purely by chance that *T. indica* has only established in certain arid and semi-arid environments with mild/cool winters and hot summers since it was first described in 1930.

Dissemination of Karnal bunt and implications for potential disease distribution

Karnal bunt was first found in the Yaqui and Mayo valleys of the northwestern state of Sonora in Mexico in 1969–1970 (Fuentes-Davila, 1996). It is likely to have been introduced as a contaminant of wheat seed

from India, though this has never been proven (Duran, 1972; Joshi et al., 1983). The pathogen was first reported in local experimental plots of the Centro Internacional de Mejoramiento de Maiz y Trigo (CIMMYT), an international institute dedicated to cereal improvement, in 1972 (Babadoost, 2000). Other evidence suggests that the disease was present in field nurseries producing seed as early as 1970 (Lambat et al., 1983). Teliospores of *T. indica* associated with seed germplasm lots from Mexico were reported in India (Lambat et al., 1983; Nath, Lambat, Mukewar, & Rani, 1981), China (Zhang, Lange, & Mathur, 1984), Syria (Diekmann, 1987) and Brazil (Mendes & Ferreira, 1994) over a period of 20 years. Work at the International Centre for Agricultural Research in the Dry Areas (ICARDA) has shown that 40.4% (136 of 337 samples) of wheat germplasm lots from Mexico in 1983 and 31.3% (71 of 227 samples) in 1985 were contaminated with *T. indica* teliospores (Diekmann, 1987).

CIMMYT records show that there were 1,778 individual shipments of wheat seed to Europe from 1973 to 1988 for wheat nursery trials where yield data was collected (T. Payne, CIMMYT, Mexico, pers. commun.). Results from these trials were received by CIMMYT from centres in 24 European countries, including England, France, Greece, Italy, Spain and Wales. This germplasm, a proportion of which would have undoubtedly been contaminated, was sown outdoors (T. Payne, CIMMYT, Mexico, pers. commun.). Positive findings of teliospores on CIMMYT seed led this organisation to develop a seed wash treatment against Karnal bunt in 1989 as a safeguard against further dissemination (Anon., 1990).

The evidence indicates that there was likely to have been a significant movement of CIMMYT wheat germplasm contaminated with teliospores out of Mexico from about 1970 to 1989. CIMMYT reports distributing 2,100 lines of wheat germplasm to various locations in 1988 alone (Anon., 1989).

In addition to small consignments of germplasm, 255,111 metric tonnes of wheat seed from the Yaqui and Mayo valleys in Mexico were exported between 1970 and 1982 without any phytosanitary precautions (Fuentes-Davila, 1996; G. Fuentes-Dávila, INIFAP-CIRNO, Mexico, pers. commun.). This was a time when Karnal bunt was present on wheat in the two valleys and most commercial bread wheat cultivars were susceptible to Karnal bunt. Shipments of seed for sowing to Algeria, China, Libya, Greece, Portugal, Spain, Brazil and other countries (Fuentes-Davila, 1996; G. Fuentes-Dávila, INIFAP-CIRNO, Mexico, pers. commun.) did not result in reports of Karnal bunt establishment.

Contaminated wheat germplasm was not just reported as intercepted from Mexico. Teliospores of *T. indica* were also found in 23.8% (5 of 21 samples) of germplasm lots from Pakistan in 1984 (Diekmann, 1987). The dissemination of pathogens with seed from plant genetic resource and breeding centres was unintentional, but not an uncommon event at this time (Jones, 1987).

Therefore, there is much evidence that teliospores of *T. indica* were being distributed internationally for many years as a contaminant of wheat germplasm and commercial seed lots sent to numerous countries. Most of this potentially contaminated material would have been sown in the field without much concern for quarantine. Why then did Karnal bunt not establish outside arid and semi-arid environments with mild/cool winters and hot summers? The answer must be that it is most likely unsuited to other environments because it has exacting climatic requirements (Babadoost, 2000). This is a convincing argument for re-evaluating the risk of establishment in Europe from high to low.

As it seems very likely that *T. indica* may only be capable of establishing in certain arid and semi-arid areas with mild/cool winters and hot summers, the arguments used for concluding otherwise would seem in need of closer scrutiny.

The Humid Thermal Index (HTI) model as a tool for determining potential disease distribution

The HTI, which is a disease forecasting model devised in the Punjab in India to predict Karnal bunt occurrence (Jhorar et al., 1992), was used in the EU-sponsored Karnal bunt programme as a tool to determine potential distribution within Europe (Sansford et al., 2006). The HTI model has also been the basis of attempts to predict potential distribution of Karnal bunt in Australia (Murray & Brennan, 1998; Stansbury & McKirdy, 2002) and South Africa (Stansbury & Pretorius, 2001). The model was developed when it was shown that there was a strong positive correlation between disease intensity and

both average daily temperature and relative humidity during the month leading up to anthesis of wheat crops (Jhorar et al., 1992; Mavi et al., 1992). The HTI has been used to show that environmental conditions prior to anthesis of European wheat crops would be expected to permit infection and this finding has been extrapolated to predict that Karnal bunt could establish on susceptible wheat cultivars in many parts of Europe (Baker et al., 2005; Sansford et al., 2006). However, the HTI may only be useful as a disease forecasting technique in areas where the disease occurs and not so useful at predicting potential disease distribution, as is discussed below.

Using long-term, average data, the HTI model predicts that a number of locations in Iran, Pakistan, India, Arizona and South Africa had unsuitable climates for Karnal bunt despite the disease being present. This result may be because local temperature and humidity levels are modified by irrigation (Stansbury & McKirdy, 2002).

The HTI model fails in forecasting actual disease levels in the Punjab when wet and humid conditions favourable for teliospore germination come earlier than usual (Sharma & Nanda, 2003). As a consequence of early climatic conditions that suit teliospore germination, inoculum is depleted at a stage of crop development when the wheat is not susceptible to infection. Therefore, although conditions at anthesis are conducive to infection and a high disease level is forecast, most teliospores have already germinated and the disease level is actually low (Sharma & Nanda, 2003; I. Sharma, Punjab Agricultural University, Ludhiana, India in a personal communication to G. Murray, Wagga Wagga Agricultural Institute, NSW, Australia). This demonstrates that the HTI only works as an accurate forecasting model if inoculum is available at anthesis. If conditions before anthesis result in a depletion of inoculum, the model breaks down.

An inoculum supply at anthesis sufficient for infection would be far from certain in many environments where HTI has been used to predict potential distribution. A crucial factor is teliospore survival in the soil for the year or years between infection opportunities prior to wheat anthesis, which is not considered by the HTI model. Therefore, the use of the HTI model to determine potential distribution of Karnal bunt in Europe relies on the assumption that teliospores would survive between infection periods in numbers that would enable establishment. This limitation of the HTI model should be made clear in all discussions related to its use as a tool for predicting the potential distribution of Karnal bunt.

Teliospore survival and its role in determining potential disease distribution

The main argument that is used to justify the HTI model for determining the potential distribution of Karnal bunt in Europe rests on the assumption that viable teliospores of *T. indica* can survive for long periods in or on European soils and that there will always be some of these available to germinate just prior to anthesis in numbers sufficient to cause disease. Research undertaken for the EU-sponsored Karnal bunt programme indeed showed that a proportion of teliospores can survive outdoors in soil in Europe for at least 3 years and then germinate (Sansford et al., 2006). However, given the failure of Karnal bunt to establish in Europe and other countries outside the hot arid and semi-arid zones despite numerous opportunities to spread in the past, there must be other factors that also need to be considered.

Teliospore germination occurs between 2°C and 30°C, but the optimum temperature is 15–20°C. Freshly collected teliospores are affected by dormancy (Warham, 1986, 1992). A germination rate of 6.7% was reported when freshly harvested teliospores that had been stored dry for 1 week at 23–26°C were incubated on water agar at 15°C for 2 weeks (Smilanick, Hoffmann, & Royer, 1985). The percentage germination of teliospores steadily increased as storage time increased. After 5 months, 47.5% germinated and 55–60% germinated after 10–18 months. In research plots in Arizona, a few viable teliospores have been recovered from soil after 7 years (M. Bonde, USDA-Ft. Detrick, USA, pers. commun.). Germination is stimulated by light (Smilanick et al., 1985; Zhang et al., 1984). Optimum pH levels for germination are between 6.0 and 9.5 (Smilanick et al., 1985). Teliospore germination is arrested during freezing or desiccation, but resumes afterwards (Smilanick et al., 1985). Germination of teliospores stored dry for 1 year occurs after 1 week when the moisture levels are 10–45% g/g in sandy loam, sandy clay loam and clay loam soils and 15–

45% in silt clay soils. Optimum soil moisture levels for germination are 25% g/g (Sansford et al., 2006). Teliospores may have to be lying on or very close to the soil surface for the normal production and liberation of sporidia (Smilanick et al., 1985; Warham, 1986).

There is some evidence that teliospores begin breaking down in the soil as early as 3 months as a result of weathering of the outer sheath that may allow the entry of water (Bonde, Nester, Olsen, & Berner, 2004b). This could lead to their death and disintegration (Babadoost, Mathre, Johnston, & Bonde, 2004). A marked decrease in the percentage of extractable teliospores has been observed after 6 months (Bonde et al., 2004a). However, whether this is caused by spore breakdown, germination followed by breakdown, or the adherence of spores to soil particles during extraction has not been determined. Moisture is believed to be a significant contributing factor in decline and teliospores with strong sheaths may survive for longer periods (Babadoost et al., 2004).

Soil types may play a role in teliospore survival. In one experiment, average teliospore recovery from a number of soil types from two northern US states decreased on average by about 80% after 8 months and then remained fairly stable until 32 months (Babadoost et al., 2004). Recovery rates from 8 months onwards varied significantly between some soil types. The percentage germination of recovered teliospores was also significantly affected by some soil sources at the end of the 32-month experimental period (Babadoost et al., 2004). A decrease in teliospore recovery and percentage germination was also noted by Bonde et al. (2004a, b), who found significant differences in survival when teliospores were incubated in different soils from different regions of the US. Numbers of viable teliospores recovered from soils from Maryland and Kansas over time were much less than from soils from Arizona. However, the soil factors causing the differences could not be determined. The conclusion of the work was that in most soils under field conditions, the majority of teliospores rapidly lose viability with only a small percentage capable of long-term survival. It has been hypothesised that if numbers of viable teliospores in the soil were to drop below a certain threshold level required for establishment, the disease would disappear (Bonde et al., 2004b).

The density of teliospores of *T. indica* was negatively correlated with clay content in naturally infested soils in Texas (Stein, Maples, & Rush, 2005). It was speculated that because clay soils retained moisture for longer periods than other types of soil, this promoted more teliospore germination at times other than when infection can occur and thus viable spore numbers would decline over time.

Teliospores at or near the soil surface are very sensitive to environmental conditions and periods of high soil moisture and temperatures of around 5–20°C can induce germination (Sansford, 1998). If conditions conducive to germination are present other than at the vulnerable period for infection, teliospores undergo 'suicidal germination' and the teliospore population will decline without initiating disease. In contrast, if suboptimal conditions for germination prevail for most of the growing season, teliospores are more likely to survive longer with the potential to become an inoculum source for when the next crop develops a full canopy and a microclimate conducive for germination and infection (Bonde et al., 2004b).

A long, dry season, as occurs in hot arid and semi-arid environments, may be an important suboptimal condition that prevents 'suicidal germination'. The dark-coloured, thick-walled teliospores of *T. indica* tolerate harsh, dry summer conditions during the post-harvest period (Singh, 2005). Dormancy and a long dry period ensures that large numbers of teliospores remain ungerminated until water stimulates germination during the next growing season. Seasonal rains and/or irrigation water are needed for wheat production in dry regions and, because of sizeable inoculum reserves in areas where the disease is prevalent, some teliospores are almost certain to germinate just prior to anthesis and sporidia would be available to infect wheat crops. However, the situation is different in environments where rain is more evenly distributed throughout the year, such as in many parts of Europe. Moisture would be available much more often and also for longer periods because of lower evaporation rates than in warmer regions. Consequently, conditions would be suitable for teliospore germination more often. Persistent moisture may also stimulate microbial antagonism and degradation that may affect viability. Interactions between moisture and low winter temperatures could further reduce the longevity of teliospores. Therefore, although climatic conditions prior to anthesis of

wheat crops may predict infection is possible, as has been found in many parts of Europe, there may be no great reservoir of inoculum capable of sustaining the disease because of a gradual depletion of teliospore reserves. Even if infection did occur under favourable circumstances, low teliospore population levels would result in reduced disease incidence and increase the possibility of pathogen extinction (Bonde et al., 2004b; Garrett & Bowden, 2002).

Tilletia indica is likely to have been introduced into Mexico on wheat seed and seed transmission is obviously an important pathway for spread if the new environment is ideal for the pathogen. A bunted seed lying on the soil surface may result in a local concentration of teliospores that could initiate an infection and cause disease development. However, following the handling and mixing of seed, one would normally expect a distribution of relatively low levels of teliospores in any accidental entry into a country. The pathogen may not establish if the climate is not ideal and/or when numbers of teliospores in any one location are insufficient to reach a threshold necessary to maintain a population in that particular environment. Nevertheless, in some situations, it may be possible for infection to occur under exceptionally favourable and perhaps unusual circumstances in areas outside ideal environments for the pathogen. However, these unusual circumstances would need to re-occur regularly for disease establishment. This may not happen in environments other than certain hot arid and semi-arid environments and the disease would not become established. The one reported occurrence in southern Brazil may be an example of this type of infection. The sporadic and unpredictable occurrence of Karnal bunt in only a few counties in Texas (G.L. Peterson, USDA-Ft Detrick, USA, pers. commun.; Stein et al., 2005) suggests that this may be a marginal environment for establishment. As the threshold numbers of teliospores in a given area required to initiate and maintain the disease in different environments is not known, the reason for the non-establishment of *T. indica* outside certain hot arid and semi-arid zones is still the subject of speculation. However, it would appear that the mere presence of teliospores in or on the soil does not guarantee infection and establishment (Stein et al., 2005).

Numbers of teliospores recovered from soils gradually decreased in outdoor longevity experiments undertaken in Europe over 3 years, (Sansford et al., 2006). A mean of a third or less of teliospores recovered intact from the soil germinated immediately while the remainder did not. A long unbroken dormancy or natural loss of viability could explain why some teliospores did not germinate. However, what prevented those that did germinate from germinating in the soil, which would have been moist for a proportion of the time, before extraction? It is possible that old viable teliospores remaining under the soil surface may stay ungerminated until they lose viability and degrade. Not enough is known about teliospore physiology and critical numbers required to initiate disease for the results of longevity experiments to be used to predict establishment with much accuracy.

The climate throughout the year is important and not just environmental conditions prior to anthesis

Smiley (1997) came to the conclusion that wheat in certain regions in the US Pacific Northwest were at risk from Karnal bunt because temperature and suitable rainfall events causing persistent high humidity levels near anthesis favoured teliospore germination and infection. His model was modified and used to predict potential distribution in South Africa (Stansbury & Pretorius, 2001) and Australia (Stansbury & McKirdy, 2002). Results were similar to predictions based on the HTI model. An analysis of the risks to North America using a model based on planting dates, temperature factors and rainfall patterns concluded that the majority of production areas were not at high risk (Anon., 2001). However, the model failed to identify coastal areas of Sonora and Sinaloa states in Mexico, where Karnal bunt has established, as high-risk zones. It was thought that temperatures in these coastal areas were being overestimated and did not consider the cooling effect of coastal winds on crops. In these analyses, the presence of an inoculum source capable of initiating infection was assumed.

Prediction models based on climatic conditions prior to or at anthesis assume that teliospores will survive in sufficient numbers between anthesis and then germinate to ensure infection and establishment, and this may not be the case. As teliospores of *T. indica* are exposed to the environment for all of their existence as viable propagules, the factors that control their long-term survival and then their

capacity to germinate in situ also determines in part the potential distribution of Karnal bunt. Consequently, a distribution prediction model that also reflects the environmental parameters that either maintains or destroys the capability of teliospores to survive between growing seasons and then allows them to germinate would also seem of great importance.

Diekmann (1993) recognised three temperature-related parameters as sufficient for discriminating environments where Karnal bunt had established and those where it had not. They were (1) the difference between mean daily maximum and minimum temperature in the month of planting, (2) the mean daily maximum temperature in the month of flowering and (3) the mean daily minimum temperature in the coldest month of the year. In an analysis of the risk to some locations using this model, establishment was considered possible in parts of Ethiopia and Australia. The risk index was low for locations within Germany, Morocco and Tunisia. Neither soil moisture nor rainfall was selected as an essential parameter. The criticism levelled at this model, which is based on temperature parameters that exist in areas that already have the disease, is that it would not be able to predict new areas with different conditions that may be suitable for establishment (Sansford et al., 2006). However, any prediction system based on climatic parameters where a pathogen presently occurs is open to this argument.

The use of a tool, such as CLIMEX (Sutherst & Maywald, 1985), which takes into account climatic conditions throughout the year, including soil moisture content, seems more attractive for determining potential distribution based on teliospore survival. However, CLIMEX has been reported to identify coastal areas worldwide as being at risk and to fail in identifying several regions where Karnal bunt is now endemic (Royer, 1990). Climatic mapping has a role to play in predicting potential geographical distribution of pests, but there are limitations (Baker et al., 2000).

Another approach may be to devise a distribution prediction model that assesses the number of suitable teliospore germination periods throughout the year other than just prior to anthesis in any given environment. The more germination periods, which would be related to prevailing temperatures and frequency of rainfall, the less chance of teliospores surviving ungerminated until anthesis (G. Murray, Wagga Wagga Agricultural Institute, Australia, pers. commun.).

A method using soil moisture levels, duration of high soil moisture levels, desiccation periods and temperature parameters may not take all the factors that affect a teliospore's capacity to remain viable and then germinate into consideration, but it would be a starting point for the development of a more precise system utilising additional and perhaps more pertinent parameters as they become apparent through experimentation. Only when such a model predicts with accuracy that teliospores could survive in sufficient numbers in a particular environment and then germinate to initiate infection would the use of models similar to the HTI become appropriate for predicting the likelihood of infection.

Potential for further spread of Karnal bunt

Predictions of the possible potential distribution of Karnal bunt appear speculative in the absence of a definitive model based on biometeorological data for determining whether teliospores of *T. indica* can survive in numbers sufficient to establish disease in a particular environment. However, the arguments presented here for Europe being at low risk have merit. A similar argument can be made for a low likelihood of Karnal bunt establishing in northern North America. As well as climatic unsuitability, several thousand metric tonnes of commercial wheat seed from Karnal bunt-infested areas of northwestern Mexico were exported to the USA in 1970, 1975 and 1980 (Fuentes-Davila, 1996; G. Fuentes-Dávila, INIFAP-CIRNO, Mexico, 2006, pers. commun.) without any reported disease outbreaks. In addition, much CIMMYT germplasm was distributed to many locations in the USA and Canada at a time when contamination by teliospores of *T. indica* was a strong possibility (Babadoost, 2000; T. Payne, CIMMYT, Mexico, pers. commun.) with no cases of Karnal bunt being recorded. Since then, Karnal bunt has only established in the USA in areas where the environment is similar to that of infested areas in Mexico.

CIMMYT has described 17 major world wheat growing environments based on conditions during the coolest, warmest or wettest consecutive 3 months of the year and annual means or totals (Anon., 2002a).

The CIMMYT map showing these environments indicates that most areas where Karnal bunt has established have the same classification. Here, spring-habit wheat is sown in low rainfall regions in the autumn or winter with conditions in the cropping season ranging from temperate to late heat stress, which especially affects late-sown crops. The wheat is usually irrigated. If this growing environment is one in which teliospores can survive in sufficient numbers to ensure disease establishment, wheat in the Nile valley in Egypt may be vulnerable if local environmental conditions prior to anthesis indicate temperature and humidity levels are suitable for infection.

Wheat in Australia is grown in a different CIMMYT wheat-growing environment (Anon., 2002a). However, conditions are dry and the country has hot summers and mild/cool winters, which would seem to suit the long-term survival of teliospores. As conditions in some parts of Australia prior to anthesis have been shown to be conducive to infection using the HTI model (Murray & Brennan, 1998; Stansbury & McKirdy, 2002), it is possible that Karnal bunt could establish.

Conclusions

There has not been a significant increase in the incidence and distribution of Karnal bunt in recent years despite being classified as an emerging disease (Anderson et al., 2004). Karnal bunt has spread very little in the US in the past 10 years since gaining a foothold (Carris et al., 2006). The rate of establishment of *T. indica* in new wheat-growing regions over the last 76 years has been slow for a seedborne pathogen of a major cereal. This is most likely because Karnal bunt has a strict environmental niche.

Arguments outlined in this article strongly suggest that the risk of Karnal bunt establishing in Europe is likely to be low. This conclusion challenges recent pest risk analyses that have concluded that it is highly likely that the disease could establish in Europe.

The sole use of models for determining infection using biometeorological data prior to wheat anthesis would not seem appropriate. Conditions just prior to anthesis are important, but cannot be the only consideration, as is shown by the present limited distribution of Karnal bunt despite many opportunities to spread. Further research on the survivability of teliospores in environments with more frequent rains and with longer periods of high soil moisture than occurs in environments where it is now established may shed some light on why the disease is not found in most wheat-growing areas of the world.

A model for determining teliospore survival rates would reveal environments where viable teliospores were likely to be present in sufficient numbers to initiate infection if conditions were favourable. If sufficient numbers of teliospores to initiate infection could survive, then a biometeorological model for determining if conditions immediately prior anthesis were suitable for infection could be applied.

Acknowledgements The author gratefully acknowledges Guillermo Fuentes-Dávila for providing information on commercial wheat seed exports from Karnal bunt-infested areas of northwestern Mexico and Tom Payne for data on the distribution of wheat germplasm from CIMMYT.

References

Anderson, P. K., Cunningham, A. A., Patel, N. G., Morales, F. J., Epstein, P. R., & Daszak, P. (2004). Emerging infectious diseases of plants: Pathogen pollution, climate change and agrotechnology drivers. *TRENDS in Ecology and Evolution, 19*, 535–544.

Anon. (1989). Genetic resources – germplasm bank. CIMMYT 1988 Annual report delivering diversity (p. 37). El Batán, Texcoco, México.

Anon. (1990). A simple treatment for Karnal bunt. In: CIMMYT 1989 Annual Report (International Maize and Wheat Improvement Centre), Beyond Subsistence: New Options for Asian Farmers (p. 54), Centro Internacional de Mejoramiento de Maiz y Trigo, El Batán, Texcoco, México.

Anon. (1996). *Tilleta indica*. *Distribution maps of plant diseases* (No. 173, Edition 5). Egham, Surrey, UK: International Mycological Institute.

Anon. (1997). *Tilletia indica*. In: Smith, I. M., McNamara, D. G., Scott, P. R., & Holderness, M. (Eds.), *Quarantine Pests for Europe* (2nd ed., pp. 938–943). Wallingford, UK: CAB International.

Anon. (2001). An Epidemiological Approach to Assessing the Risk of Establishment of Karnal Bunt, *Tilletia indica* Mitra, in North America. NAPPO Pest Risk Assessment Panel [http://www.nappo.org/Publications/KB-Aug25–01.pdf].

Anon. (2002a). A Map of the World for Wheat Breeding. CIMMYT Annual Report 2001–2002 (pp. 62–64). Centro Internacional de Mejoramiento de Maiz y Trigo, El Batán, Texcoco, México.

Anon. (2002b). Unofficial Consolidation of the European Community Plant Health Directive 2000/29/EC as last amended by Commission Directive 2002/28/EC (of 19

March 2002). Commission of the European Communities, Directorate-General for Agriculture, Brussels, Belgium.

Anon. (2006). Field crops. Krishiworld – the pulse of Indian Agriculture [http://www.krishiworld.com/html/field_-crops1.html].

Babadoost, M. (2000). Comments on the zero tolerance quarantine of Karnal bunt of wheat. *Plant Disease, 84*, 711–712.

Babadoost, M., Mathre, E., Johnston, R. H., & Bonde, M. R. (2004). Survival of teliospores of *Tilletia indica* in soil. *Plant Disease, 88*, 56–62.

Baker, R. H. A., Sansford, C. E., Gioli, B., Migletta, F., Porter, J. R., & Ewert, F. (2005). Combining a disease model with a crop phenology model to assess and map pest risk: Karnal bunt disease (*Tilletia indica*) of wheat in Europe. In: *Plant Protection and Plant Health in Europe: Introduction and Spread of Invasive Species, Symposium Proceedings* No. 81 (pp. 89–94) British Crop Protection Council, Alton, Hampshire, UK.

Baker, R. H. A., Sansford, C. E., Jarvis, C. H., Cannon, R. J. C., MacLeod, A., & Walters, K. F. A. (2000). The role of climatic mapping in predicting the potential geographical distribution of non-indigenous pests under current and future climates. *Agriculture, Ecosystems and Environment, 82*, 57–71.

Beattie, B. R., & Biggerstaff, D. R. (1999). Karnal bunt, a wimp of a disease...but an irresistible political opportunity. *Choices*, Second Quarter 1999, pp. 4–8.

Bonde, M. R., Berner, D. K., Nester, S. E., Petersen, G. L., Olsen, M. W., Cunfer, B. M., & Sim, T. (2004a). Survival of *Tilletia indica* teliospores in different soils. *Plant Disease, 88*, 316–324.

Bonde, M. R., Nester, S. E., Olsen, M. W., & Berner, D. K. (2004b). Survival of teliospores of *Tilletia indica* in Arizona field soils. *Plant Disease, 88*, 804–810.

Bonde, M. R., Prescott, J. M., Matsumoto, T. T., & Peterson, G. L. (1987). Possible dissemination of teliospores of *Tilletia indica* by the practice of burning stubble. *Phytopathology, 77*, 639.

Carris, L. M., Castlebury, L. A., & Goates, B. J. (2006). Nonsystemic bunt fungi – *Tilletia indica* and *T. horridae*: A review of history, systematics and biology. *Annual Review of Phytopathology, 44*, 113–133.

Crous, P. W., Van Jaarsveld, A. B., Castlebury, L. A., Carris, L. M., Frederick, R. D., & Pretorius, Z. A. (2001). Karnal bunt of wheat newly reported from the African continent. *Plant Disease, 85*, 561.

Da Luz, W. C., Mendes, M. A. S., Ferreira, M. A. S. V., & Urben, A. F. (1993). *Tilletia indica* on wheat in the south of the state of Rio Grande do Sul., Brazil and measures for eradication. *Fitopatologica Brasileira, 18*(supplement), 329.

Damodaran, H. (2003). Karnal bunt threat to wheat on untimely rains – crop scientists keeping fingers crossed. The Hindu Business Line, Wednesday Feb 19, 2003. [http://www.thehindubusinessline.com/2003/02/19stories/htm].

Diekmann, M. (1987). Monitoring the presence of Karnal bunt (*Tilletia indica*) in germplasm exchange at ICARDA. *Phytopathologia Mediterranea, 26*, 59–60.

Diekmann, M. (1993). *Epidemiology and geophytopathology of selected seed-borne diseases*. Aleppo, Syria: International Center for Agricultural Research in the Dry Areas.

Duran, R. (1972). Aspects of teliospore germination in North American smut fungi. II. *Canadian Journal of Botany, 50*, 2569–2573.

Fuentes-Davila, G. (1996). Karnal bunt in Mexico. Transcripts of the American Phytopathological Society Karnal Bunt Symposium, June 14 – August 16, 1996. [http://www.aspnet.org/online/karnal/kbspaper/index.htm].

Garret, K. A., & Bowden, R. L. (2002). An allee effect reduces the invasive potential of *Tilletia indica*. *Phytopathology, 92*, 1152–1159.

Goates, B. J. (1988). Histology of infection of wheat by *Tilletia indica*, the Karnal bunt pathogen. *Phytopathology, 78*, 1434–1441.

Goates, B. (2005). Durability of secondary sporidia of floret infecting *Tilletia* species: Implication for epidemiology. *Phytopathology, 95*, 961.

Goates, B. J., & Jackson, E. W. (2006). Susceptibility of wheat to *Tilletia indica* during stages of spike development. *Phytopathology, 96*, 962–966.

Jhorar, O. P., Marvi, H. S., Sharma, I., Mahi, G. S., Mathauda, S. S., & Singh, G. (1992). A biometeorological model for forecasting Karnal bunt disease of wheat. *Plant Disease Research, 7*, 204–209.

Jones, D. R. (1987). Seedborne diseases and the international transfer of plant genetic resources: An Australian perspective. *Seed Science and Technology, 15*, 765–776.

Joshi, L. M., Singh, D. V., Srivastava, K. D., & Wilcoxson, R. D. (1983). Karnal bunt: A minor disease that is now a threat to wheat. *The Botanical Review, 49*, 309–329.

Lambat, A. K., Nath, R., Mukewar, P. M., Majumdar, A., Rani, I., Kaur, P., Varshney, J. L., Agarwal, P. C., Khetarpal, R. K., & Dev, U. (1983). International spread of Karnal bunt of wheat. *Phytopathologia Mediterranea, 22*, 213–214.

Locke, C. M., & Watson, A. J. (1955). Foreign plant diseases intercepted in quarantine inspection. *Plant Disease Reporter, 39*, 518.

Mavi, H. S., Jhorar, O. P., Sharma, I., Singh, G., Mahi, G. S., Mathauda, S. S., & Aujila, S. S. (1992). Forecasting Karnal bunt disease of wheat – a meteorological method. *Cereal Research Communications, 20*, 67–74.

Mendes, M. A. S., & Ferreira, M. A. S. V. (1994). Pathogenic fungi detected in vegetable germplasm introduced into Brazil from 1990 to 1992. *Fitopatologica Brasileira, 19*, 449–454.

Mitra, M. (1931). A new bunt of wheat in India. *Annals of Applied Biology, 18*, 178–179.

Murray, G. M., & Brennan, J. P. (1998). The risk to Australia from *Tilletia indica*, the cause of Karnal bunt of wheat. *Australasian Plant Pathology, 27*, 212–225.

Mustafa, F. H. (1965). A list of common plant diseases in Iraq. *Bulletin of the Ministry of Agriculture, Iraq, 111*, 1–16.

Nath, R., Lambat, A. K., Mukewar, P. M., & Rani, I. (1981). Interceptions of pathogenic fungi on imported seed and planting material. *Indian Phytopathology, 34*, 282–286.

Pearce, E. A., & Smith, C. G. (1990). *The world weather guide* (2nd ed.). London, UK: Hutchinson.

Richardson, M. J. (1990). *An annotated list of seed-borne diseases* (4th ed.). Zurich, Switzerland: International Seed Testing Association.

Royer, M. H. (1990). Pros and cons of using modelling versus non-modelling approaches to predict Karnal bunt outbreaks. *Proceedings of the Seventh Biennial Workshop on Smut Fungi* (p. 24). June 4th–7th, 1990, Frederick, Maryland, USA.

Rush, C. M., Riemenschneider, R., Stein, J. M., Boratynski, T., Bowden, R. L., & Royer, M. H. (2005). Status of Karnal bunt of wheat in the United States 1996 to 2004. *Plant Disease, 89*, 212–223.

Sansford, C. (1998). An assessment of the significance of the initial detection of *Tilletia indica* Mitra in the USA in early 1996 and the potential risk to the United Kingdom (and the European Union). In: Malik, W. S., & Mathre, D. E. (Eds.), *Bunts and smuts of wheat: An international symposium* (pp. 273–302). Ottawa, Canada: North American Plant Protection Organization.

Sansford, C. (2004). UK pest risk analysis for *Tilletia indica.* Prepared by the Central Science Laboratory for Plant Health Division, Department for Environment, Food and Rural Affairs, UK. [www.defra.gov.uk/planth/pra/tilletia.pdf].

Sansford, C., Baker, R., Brennan, J., Ewert, F., Gioli, B., Inman, A., Kelly, P., Kinsella, A., Leth, V., Magnus, H., Miglietta, F., Murray, G., Peterson, G., Porta-Puglia, A., Porter, J., Rafoss, T., Riccioni, L., Thornes, F., & Valvassoni, M. (2006). Deliverable Report, D. L., 6.1. Report on the Risk of Entry, Establishment and Economic loss for *Tilletia indica* in the European Union. EC Fifth Framework Project QLKS-1999-01554 Risks Associated with *Tilletia indica*, the Newly Listed, E. U., Quarantine Pathogen, the Cause of Karnal Bunt of Wheat. [http://karnalpublic.pestrisk.net/].

Sharma, I., & Nanda, G. S. (2003). Effect of prolonged period of fog/rain from sowing of wheat to pre-boot stage on Karnal bunt development. *Annals of Plant Protection Services, 11*, 93–95.

Singh, D. V. (2005). Karnal bunt of wheat: A global perspective. *Indian Phytopathology, 58*, 1–9.

Singh, D. V., Srivastava, K. D., & Joshi, L. M. (1985). Present status of Karnal bunt of wheat in relation to its distribution and varietal susceptibility. *Indian Phytopathology, 38*, 507–515.

Singh, D. V., Agarwal, R., Shrestha, J. K., Thrapa, B. R., & Dubin, J. J. (1989). First report of *Tilletia indica* on wheat in Nepal. *Plant Disease, 73*, 273.

Smiley, R. W. (1997). Risk assessment for Karnal bunt occurrence in the Pacific Northwest. *Plant Disease, 81*, 689–692.

Smilanick, J. L., Hoffmann, J. A., & Royer, M. H. (1985). Effect of temperature, pH, light and desiccation on teliospore germination of *Tilletia indica*. *Phytopathology, 75*, 1428–1431.

Smilanick, J. L., Prescott, J. M., Hoffman, J. A., Secrest, L. R., & Weise, K. (1989). Environmental effects on survival and growth of secondary sporidia and teliospores of *Tilletia indica*. *Crop Protection, 8*, 86–90.

Stansbury, C. D., & McKirdy, S. J. (2002). Forecasting climate suitability for Karnal bunt of wheat: A comparison of two meteorological methods. *Australasian Plant Pathology, 31*, 81–92.

Stansbury, C. D., & Pretorius, Z. A. (2001). Modelling the potential distribution of Karnal bunt of wheat in South Africa. *South African Journal of Plant and Soil, 18*, 159–168.

Stefanski, R., Brusberg, M., Puterbaugh, T., Morris, B., & Motha, R. (1994). Major World Crop Areas and Climatic Profiles (Revised). Agricultural Handbook No. 664, National Technical Information Service, US Department of Commerce, United States Department of Agriculture, Washington, DC, USA.

Stein, J. M., Maples, H. W., & Rush, C. M. (2005). Epidemiology of *Tilletia indica* teliospores in regulated wheat fields in Texas. *Plant Disease, 89*, 828–833.

Sutherst, R. W., & Maywald, G. F. (1985). A computerised system for matching climates in ecology. *Agriculture, Ecosystems and Environment, 13*, 281–299.

Torarbi, M., Mardoukhi, V., & Jalaiani, N. (1996). First report on the occurrence of partial bunt on wheat in the southern parts of Iran. *Seed and Plant, 12*, 8–9.

Warham, E. J. (1986). Karnal bunt disease of wheat: A literature review. *Tropical Pest Management, 32*, 229–242.

Warham, E. J. (1992). Karnal bunt of wheat. In: Singh, U. S., Mukhopadhyay, A. N., Kumar, J., & Chaube, H. S. (Eds.), *Plant diseases of international importance* (Vol. 1, pp. 1–24). New Jersey, USA: Prentice Hall.

Wilcoxson, R. D., & Saari, E. E. (Eds.). (1996). Bunt and smut diseases of wheat: Concepts and methods of disease management. Centro Internacional de Mejoramiento de Maiz y Trigo, El Batán, Texcoco, México.

Zhang, Z., Lange, L., & Mathur, S. B. (1984). Teliospore survival and plant quarantine significance of *Tilletia indica* (causal agent of Karnal bunt) particularly in relation to China. *EPPO Bulletin, 14*, 119–128.