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REVIEW PAPER

A reappraisal of the current status of *Tilletia indica* as an important quarantine pest for Europe

David R. Jones

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Abstract *Tilletia indica*, the fungus responsible for Karnal bunt disease of wheat, is currently recognised as a quarantine pest by both the EU and EPPO. The evidence that has been used to justify this status is reviewed and found to be in need of reappraisal. Yield losses caused by the pathogen are insignificant and disease levels are rarely high enough to cause serious quality problems. The sole reason for its designation as an important quarantine pest would seem to lie in the serious implications for trade should the pathogen be detected in an exporting country. Since there is strong evidence to suggest that *T. indica* may not even establish in Europe, it would seem logical to reclassify the pathogen as the cause of a minor disease that is likely to have little quarantine significance for Europe. However, since most wheat-growing countries have strict quarantine regulations aimed at preventing the introduction of *T. indica*, this reclassification is unlikely to occur unless plant health authorities around the world can agree on a new status of reduced importance for *T. indica* that suits its actual potential as a pest.

Keywords Economic impact · International trade · Karnal bunt · Quarantine pest · *Tilletia indica* · Wheat

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

Introduction

Tilletia indica (Mitra 1931; Carris et al. 2006) is a fungus that causes Karnal bunt disease of wheat in some countries in the arid/semi-arid zones in northern and southern latitudes (Jones 2007). Although the pathogen has never been found in climates that prevail in Europe despite many opportunities to spread internationally (Jones 2007), it is deemed to be a significant threat to European wheat crops (Sansford et al. 2006). As such, it is designated a 1/A1 harmful organism whose introduction into, and spread within, all EU member states is banned by the European Commission (Anon. 2002). In addition, *T. indica* has been declared an A1 quarantine pest by the European and Mediterranean Plant Protection Organisation (EPPO) based in Paris (Anon. 1997).

The long-distance dissemination of the *T. indica* is believed to be as a result of the dissemination of wheat seed or grain contaminated by teliospores in a manner similar to other bunt diseases of wheat (Wilcoxson and Saari 1996). Teliospores can also be wind-blown (Bonde et al. 1987) and transported in soil attached to machinery. Animals and birds have also been implicated in the movement of teliospores (Bonde et al. 1997). An analysis of Karnal bunt interception records in the USA has shown that wheat seed and grain with teliospores were found in personal baggage, trucks, automobiles, railroad cars and commercial cargo at land border crossings with Mexico from 1984 to 2000. It has been hypothesised that Karnal bunt may have been introduced into the southwest of the USA by this means (Marshall et al. 2003).

Teliospores germinate on or near the soil surface to produce promycelia upon which primary sporidia develop. Primary sporidia give rise to secondary sporidia. This process is aided by humidity provided by rainfall or irrigation (Goates and Jackson 2006). Both primary and secondary sporidia are dispersed to wheat flowers by air currents or rain-splash. 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). 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 and Jackson 2006).

It has been argued that, because a proportion of teliospores of *T. indica* have been found to remain viable in European soils for up to three years and environmental conditions in Europe prior to wheat anthesis can favour infection, there is a high risk of the pathogen establishing in Europe (Sansford et al. 2006). In addition, it has been proposed that the presence of *T. indica* on wheat in Europe will have a significant impact on grain quality, as well as on the trade of grain with other continents (Sansford et al. 2006).

An earlier review provided evidence for a low risk of establishment of *T. indica* in Europe (Jones 2007). This review assesses the evidence for *T. indica* causing a serious disease and thus deserving of its important quarantine pest status.

EU legislation

The origin of the EU legislation related to *T. indica* has been reported as a pest risk analysis (PRA) undertaken for the UK and the EU in 1996 (Sansford et al. 2006). This PRA was initiated because a significant new wheat trade pathway from the USA into Europe had established. The conclusions of this PRA were that the pathogen could establish and cause economic damage in the UK/EU. As a result, *T. indica* was added as a quarantine pest to the EC Plant Health Directive in 1997 (Anon. 2002; Sansford et al. 2006).

A quarantine pest has been defined as 'a pest of potential economic importance to the area endangered thereby and not yet present there, or present but not widely distributed and being officially controlled' (Anon. 2006a). Therefore, in effect, *T. indica* is considered by the EU authorities as capable of establishing in the EU and of causing economic damage. EPPO also consider this to be the case. Measures aimed at preventing entry are in place in EU member states and in many EPPO countries.

EU quarantine requirements to help prevent the introduction of *T. indica* are currently applied to seed and grain of *Triticum*, *Secale* and x *Tritosecale* from Afghanistan, India, Iran, Iraq, Mexico, Nepal, Pakistan, South Africa and the USA where Karnal bunt is either known or strongly suspected to occur. *Secale* (rye) is being considered for deletion from the legislation, as it is no longer considered to be a natural host of *T. indica* (Sansford et al. 2006).

The requirements are that seed imported into the EU must originate in an area where *T. indica* is known not to occur and imported grain must originate either in an area where *T. indica* is known not to occur or where no symptoms of *T. indica* were observed on wheat at the place of production during the last complete cycle of vegetation. In addition, representative samples of the grain must have been taken both at the time of harvest and before shipment and found free of the pathogen in tests (Anon. 2002).

These requirements place a burden on an exporting country where Karnal bunt is known to occur to undertake tests on exported grain and also conduct surveys to define production areas where *T. indica* is not found. There is an obligation under international agreement on exporting countries to prove that production areas are free from Karnal bunt (Anon. 2006b). The consequences are that wheat-producing countries without Karnal bunt have a distinct advantage over those that have the disease. There would appear little incentive for those countries considered free of the problem to undertake the detailed surveys that are necessary to find the pathogen if it occurs at a low level.

When imposing restrictions on wheat imports because of Karnal bunt, countries usually provide evidence that they either do not have the disease or the disease is confined to certain areas and is being controlled. A statement that the disease has never been seen in the field by inspectors from national plant protection organisations is evidence. Surveys for Karnal bunt symptoms and teliospores of *T. indica* in grain specifically undertaken by the UK and the Netherlands have proved negative (Sansford et al. 2006). Surveys conducted in the USA indicate that the disease is absent outside known infested areas (Rush et al. 2005).

Despite EU quarantine precautions, five consignments of wheat grain from India during the period 2003–2006 were found with teliospores of *T. indica* when tested on arrival in the UK (Sansford et al. 2006). The plant health authorities in India would have been alerted to these incidents of non-compliance with EU regulations as is required by international agreement (Anon. 2001). Teliospores of *T. indica* have never been detected in grain arriving in the EU from the USA

Economic damage caused by Karnal bunt

In addition to the perceived high establishment potential of Karnal bunt in Europe, it has also been the perceived economic consequences of introduction that led to *T. indica* being declared a quarantine pest in the EU (Sansford et al. 2006). The pathogen has been described as normally causing minor yield losses, but serious quality losses for wheat crops. The loss of export markets and other economic repercussions should *T. indica* be found associated with wheat in a EU country was also a factor that led to the PRA conclusion that the pathogen causes serious economic damage and is a significant quarantine pest (Sansford et al. 2006). The available evidence is reappraised below.

Yield losses

The greatest global incidence of Karnal bunt may occur in the northwest of the Indian subcontinent. For many years after its discovery in experimental wheat plots at Karnal in Haryana state in India in 1930, only traces of the disease were found in the plains region. However, the disease was common in Delhi, Punjab, Haryana and western Uttar Pradesh by 1969–1970. In the crop season of 1974–1975, the disease was referred to as ‘severe’ in many places in north India, but particularly in the Himalayan foothills and the Tarai region of Uttar Pradesh, Punjab and Himachal Pradesh. This upsurge in incidence was linked to the large-scale cultivation of Mexican-bred wheat cultivars, which were deemed to be more susceptible to the disease than native types, in infested areas. By 1983, the disease was present in northern India from its northwestern borders to Bihar and West Bengal States, but incidence was highest in the Punjab, Haryana, lower Himachal Pradesh, the Jammu region of Jammu and Kashmir, Delhi and Uttar Pradesh (Joshi et al. 1983; Wareham 1986; Wareham 1992). Since 1983, there have been no reports of further spread in India despite there being no restrictions on the movement of seed. Occurrence of the disease in eastern India is erratic and seldom exceeds trace levels to very low levels of infection (Singh 2005; Singh et al. 1996). Eastern India would seem marginal for disease development. Southern and northeastern regions of the Indian subcontinent would appear unsuitable for Karnal bunt (Jones 2007). The planting of wheat cultivars less susceptible to Karnal bunt has reduced damage levels in recent years (Singh 2005).

Karnal bunt usually affects only a few spikelets within a wheat spike. In addition, the pathogen usually causes a partial bunt with teliospores replacing only a portion of the kernal. Yield losses in the Punjab and Jammu regions of India were estimated at 0.2% during 1969–1970 (Munjal 1975; Wareham 1986). Even during the worst years of ‘epidemic’, the damage to wheat crops was reported as only 0.2–0.5% of total production in infested areas (Joshi et al. 1983). Losses of 0.3–0.5% have been suggested during the most severe years between 1982 and 1989 particularly in Uttar Pradesh (Singh 1994, 2005). Higher losses of 5–20% have been reported in experimental plots and at specific locations (e.g. McRae 1934 reported by Joshi et al. 1983; Shukla et al. 1982 reported by Wareham 1986), but these severe infection levels would seem to be isolated and exceptional (Nagarajan et al. 1997). Many reports of high losses emanate from the mid- to late-1970s (Wareham 1986) when susceptible wheat cultivars were grown.

Yield losses in northwestern Mexico have been estimated at 0.12% per year assuming a 25% loss of weight in infected grains (Brennan and Wareham 1990). Disease incidence appears to be so low in the USA that impact on yield is non-existent (Rush et al. 2005). In Texas, outbreaks are sporadic and unpredictable (G. Peterson, USDA-Ft Detrick, USA, pers. comm.), which indicates the environment may be marginal for establishment (Jones 2007). Estimates of yield losses in other countries are not readily available if they exist.

The conclusion reached by most plant pathologists is that the low incidence and low severity of the disease results in low yield losses (Beattie and Biggerstaff 1999; Bonde et al. 1987; Murray and Brennan 1998; Sansford et al. 2006). Yield losses alone would seem insufficient for *T. indica* to warrant quarantine pest status in the EU.

Quality losses

Kernels with only traces of infection do not suffer any significant deterioration in germination, but badly affected kernals show considerable loss in viability and any germination is abnormal (Joshi et al. 1983). Plants grown from infected seed suffer yield reductions (Jatav et al. 2003 reported in Sansford et al. 2006).

High percentages of infected grain fed to rats, chicks, monkeys and goats caused no adverse effects in toxicological studies. Although no alkaloids or mycotoxins have been found in infected grain, secondary invaders, such as *Aspergillus flavus*, did produce aflatoxins (Bhat et al. 1980, 1983 and Bedi et al. 1981 reported in Wareham 1986).

Bunted wheat grains have a 20–25% lower lysine content than healthy grains indicating a loss of protein quality, but nutrient composition only differs slightly in other respects. Kernal weight may also be reduced by 34–50% (Bhat et al. 1980 and Singh, 1980 reported in Joshi et al. 1983). Infected seed also gives off a 'fishy' odour caused by the volatile trimethylamine. This odour is reported lost after a year in storage (Joshi et al. 1983).

The quality of flour can be affected in India as a result of Karnal bunt infection of grain. 'Chapatties' made from flour derived from 10% infected grain were dark in colour and inedible. Flour made from grain with an infection level of 1% was said to only slightly affect the palatability of 'chapatties'. About a 3% infection level resulted in 'chapatties' being unpalatable because of a disagreeable odour (Mehdi et al. 1973; Joshi et al. 1983). However, washing 5% infected grain before milling was reported to result in flour that produced edible 'chapatties'. Even flour made from 10% infected grain could be used if the grain was steeped after washing (Sekhon et al. 1981; Wareham 1986).

Consignments containing large levels of bunted grain have been downgraded to animal feed in India. Singh (1988) reported that this amounted to a 0.96% loss in epidemic years. Quality losses in grain from infested areas of Mexico have been estimated at 0.72% of the value of the average crop in infested areas (Brennan et al. 1992). In the USA, incidence in most infested fields is below 0.1% and effects on quality are minimal (Rush et al. 2005).

Wareham (1986) noted that grain is not accepted for industrial processing and is declared unfit for human consumption when more than 3% of grains are diseased. Growers in Mexico were reported to receive a 1% price discount for each percent of infected grain up to 3%. Above 3%, consignments were accepted as feed grain at a discount of 20% from the price of food wheat. However, no estimates of the numbers of wheat consignments being rejected as food grain were given (Brennan et al. 1992).

Cereal grain cleaning machines, such as air blowers or gravity selectors, are available that should be able to remove many bunted kernals from healthy grain on the basis of a difference in weight and density. Optical sorters, that have been used to detect and remove kernels infected with *T. indica* from seed lots (Dowell et al. 2002), could be adapted for commercial consignments of grain. In addition to cleaning, infected lots can be mixed with non-infected lots diminishing the effects of bunted grains on the quality of flour.

To summarise, grain infected by *T. indica* only becomes a major quality issue if levels of 3% or more are reached. Very susceptible Mexican-bred wheat cultivars are no longer grown in northwest India and, as a consequence, levels of Karnal bunt have declined significantly. In fields with the disease, incidences now only rarely exceed 1% (S.K.Kaur, Punjab Agricultural University, India, pers. comm.). During the 2005–2006 season, the range of disease incidences in wheat fields in northwest India was reported as 0.003–0.550% (R. Gogoi, Indian Agricultural Research Institute, India, pers. comm.).

Bunted kernal problems are not unlike other grain quality defects, which are handled routinely by quality standards, grading, price discounts and blending. Quality defects have never inhibited grain markets (Beattie and Biggerstaff 1999).

At usual low levels of infection, the effect of *T. indica* on the quality of wheat grain does not seem to be an issue that can be used to justify an important quarantine pest status for *T. indica*. No serious economic repercussions of the disease because of effects on yield or quality are evident in countries where Karnal bunt occurs (Singh 2005).

Marketing implications

As Karnal bunt has only minor effects on yield and manageable effects on grain quality in areas where it now occurs, why has it become such an important global quarantine issue? Its present status does not seem justified on grounds of pathologic impact. In addition, *T. indica* would seem to have only limited potential for further global spread (Jones 2007). Arguments in favour of *T. indica* being retained as an important quarantine pest for Europe and elsewhere seem to rest not on its direct impact, but on the marketing implications resulting from its introduction to a wheat-growing country.

Rush et al. (2005) lists 71 countries other than the USA with specific regulations on the importation of wheat from countries with *T. indica*, either through national regulations or through being a member of a regional plant protection organisation. Once *T. indica* has been identified on wheat in the field or in grain/seed stocks in a wheat-exporting country, the export of wheat to those countries with specific regulations would be severely restricted. If wheat consignments were allowed entry to countries without the disease, they would most likely need to be tested to guarantee pathogen-freedom, as is required by the EU today.

An economic analysis in 2002 showed that if the US Department of Agriculture (USDA) ended the issuance of Karnal bunt phytosanitary certificates for export shipments of wheat, as is now required by many overseas buyers, exports to some countries would be jeopardised. It was thought that this would only be partially offset by increased domestic use of grain as animal feed. The result would be that prices would remain low for domestic markets and there would be reduced wheat production (Vocke et al. 2002). The USA continues to regulate Karnal bunt probably as a result of this analysis. Although wheat farmers in regulated areas where the disease is found are disadvantaged, growers in most other parts of the USA are not and the industry overall remains strong. It also allows USA quarantine requirements as regards Karnal bunt to be maintained. The zero-tolerance policy of the USA towards Karnal bunt prevents wheat being imported from regions of countries with the disease.

Costs, should Karnal bunt become established in some countries currently free of the disease, have been estimated by economic analysis. Total costs associated with an outbreak in the UK were estimated to be €453.7M over 10 years if 50,000 ha were affected and €15.6M if 1,000 ha were affected (Sansford et al. 2006). If Karnal bunt was introduced into Western Australia, it has been calculated that the economic impact in the long term could range from 8 to 24% of the total value of wheat production in that state depending on the rate of spread (Stansbury et al. 2002).

In addition to deleterious effects on the sale of grain and seed for domestic and export markets because of quarantine concerns, costs associated with surveys to define outbreak sites, sampling and testing for the pathogen in the field and grain shipments, measures aimed at eradicating or containing the disease, such as the disinfestations of contaminated grain silos and trucks, and costs to governments on implementing research programmes, have also been identified. Reaction and control management costs have been estimated to account for over 99.5% of the total economic effect should an outbreak occur in the UK (Sansford et al. 2006). However, all these indirect costs are artificial in that they are a result of the current undeserved international status of the pathogen as a significant quarantine pest.

Origin of quarantine restrictions aimed at preventing the introduction of *T. indica*

The reason for *T. indica* becoming an important quarantine consideration is believed to date back to 1981. This was the year when the USA first prohibited the introduction of wheat grain from countries where Karnal bunt was known to occur.

In 1982, kernels infected with *T. indica* were intercepted on grain imported from Mexico. After confirmation of the presence of the disease in Sonora state in Mexico, the USA prohibited the importation of seed, grain, straw and dried plants of wheat, durum and triticale from Mexico. In 1983, Mexico was permanently added to the official US list of countries with Karnal bunt. After the USA implemented a zero-tolerance level for *T. indica*, many other countries subsequently established quarantine regulations for Karnal bunt. (Babadoost 2000).

Despite import prohibitions, *T. indica* was found in the USA in Arizona in 1996 (Bonde et al. 1997) and later reported from California and Texas (Rush et al. 2005). Because of reports of *T. indica* in three states, the USA today finds itself the subject of trade restrictions from those countries that followed its lead in implementing Karnal bunt quarantine regulations. In addition, with the knowledge that Karnal bunt was present in the USA, 11 countries that had never had requirements for the disease asked for an additional declaration from the USA that grain shipments came from areas where the disease was not known to occur (Rush et al. 2005).

Why did the USA introduce regulations to prevent the introduction of a minor disease like Karnal bunt in 1981? At the time, it was perfectly legitimate and thought appropriate to introduce quarantine regulations to prevent the entry of any disease that was deemed to be a threat to a major agricultural industry. It was also considered that pathogens may become more aggressive in a new environment. The main quarantine philosophy was 'when in doubt, keep it out'. These were the years before assessments of risk based on scientific evidence were required by the World Trade Organization (WTO) to justify import restrictions (Anon. 2006b). Although most plant pathologists would agree that it would be beneficial if all plant pathogens, whether major or minor, could be prevented from spreading between countries, the political reality of the current situation is that trade cannot be restricted for unimportant issues.

Obligations of WTO member nations under the SPS Agreement

Karnal bunt is viewed by some as a 'political' disease of wheat in that, although a minor problem, it provides an opportunity to gain economic advantage in world and regional markets. In this way it is seen to fall into the same category as import tariffs and quotas, and rules and regulations to restrict the production or consumption of competing goods and services (Beattie and Biggerstaff 1999). However, if true, this is against the principles of the Sanitary and Phytosanitary (SPS) Agreement that countries who are Members of the WTO are obliged to follow. Article 2, Paragraph 3 of the SPS Agreement states that phytosanitary measures shall not be applied in a manner that would constitute a disguised restriction on international trade (Anon. 2006b).

Enshrined in Article 5 of the SPS Agreement is the need for quarantine measures of Members to be justified by PRA developed following international guidelines (Anon. 2004). The PRA should take all relevant scientific data into account. The potential damage in terms of loss of production in the event of the entry, establishment or spread of a pest should also be considered in the PRA. If a pest is found to be of quarantine concern, measures adopted should only be as restrictive to trade as they need to be to prevent introduction of the pest. Members are advised that they should, when determining the appropriate level of phytosanitary protection, take into account the objective of minimising negative trade effects (Anon. 2006b).

In cases where relevant scientific evidence is insufficient for risk to be determined, a Member may provisionally adopt phytosanitary measures on the basis of available pertinent information. This includes advice from relevant international organizations as well as phytosanitary measures applied by other Members. In such circumstances, Members shall seek to obtain the additional information necessary for a more objective assessment of risk and review the phytosanitary measure accordingly within a reasonable period of time (Anon. 2006b). *Tilletia indica* would not appear to fall into this category, as there is much evidence to support the contention, as outlined in this review, that it is a minor pathogen that causes very little direct economic damage to wheat. However, there may still be some additional evidence required to confirm that *T. indica* is restricted in its distribution to those environments similar to where it exists today (Jones 2007).

Discussion

It would seem that under the SPS Agreement and given the information provided in this review and the review by Jones (2007), countries and trading blocks with restrictions related to Karnal bunt need to justify their quarantine concerns about *T. indica* from a more realistic viewpoint.

Many scientists involved with Karnal bunt now believe that *T. indica* is a minor pathogen that does not warrant its status in the USA as a zero-tolerance quarantine organism (Babadoost 2000; Beattie and Biggerstaff 1999; Rush et al. 2005). The American Phytopathological Society is also on record as suggesting that *T. indica* is a minor plant pest of no consequence (Babadoost 2000). As is shown in this review, its only claim to an important quarantine pest status would seem its unjustified detrimental effects on trade.

An unbiased PRA developed today on purely pathological and direct economic damage evidence would come to the conclusion that *T. indica* does not qualify for important quarantine pest status. However, it is the threat of trade restrictions resulting in deleterious indirect economic impacts associated with control management that still keeps this minor pathogen, which seems confined to specific wheat-growing areas in arid/semi-arid regions either side of the 30° latitudes (Jones 2007), as a quarantine pest.

Some national quarantine regulations aimed at preventing the introduction of *T. indica* could be viewed as too restrictive given the minor importance of the disease with a likely limited potential for establishment outside its current area of distribution. The restrictions imposed on imported grain for human consumption, which is a commodity of much lower risk status than seed, as it is processed in flour mills and not sown, would now seem particularly inappropriate and in need of revision.

The USA recently reviewed its quarantine regulations in the light of an international requirement that measures taken to prevent the introduction of a quarantine pest should be no more stringent than measures taken to prevent its spread within national boundaries (Anon. 2005). This change did not affect its zero-tolerance policy towards *T. indica*, but did enable wheat to be imported from regions of Mexico known to be free of the pathogen. Continued zero-tolerance policies applied to wheat grain imports as regards Karnal bunt would also seem inappropriate since the disease is unimportant and unlikely to establish outside known infested areas in the south west of the country (Jones 2007).

A reappraisal of the quarantine significance of *T. indica* by world plant health authorities is urgently needed. Unilateral action by any one country or trading block is unlikely to happen. A multilateral agreement needs to be reached that will put the risks posed by the pathogen into perspective. The removal of the important quarantine pest status of *T. indica* by common consent, although desirable and appropriate based on the scientific evidence of its potential to cause real economic damage, would be perhaps an unrealistic initial goal. However, a general lowering of its quarantine significance to allow grain to be imported more easily into countries with environments where *T. indica* is unlikely to establish would reduce problems associated with trade.

More research aimed at determining key parameters for a model that will predict where teliospores of *T. indica* will survive between periods of wheat anthesis in sufficient numbers to allow Karnal bunt to establish (Jones 2007) may be needed before any agreement can be reached. However, until an agreement is reached, Karnal bunt will continue to be a contentious issue for wheat-trading nations.

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