

# Karnal bunt (*Tilletia indica*) in wheat

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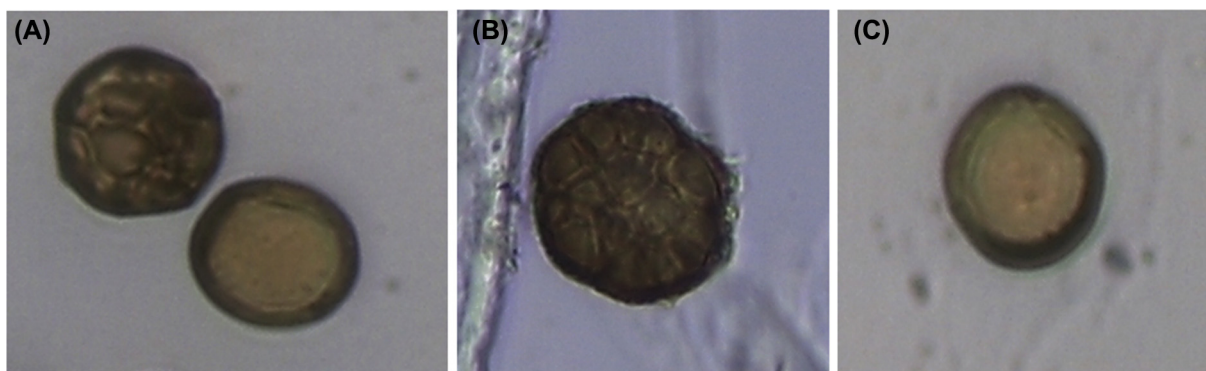
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Wheat is grown yearly on 215 million hectares, an area equivalent to that of Greenland, and it also occupies 30.3% of all land devoted to cereals. In the year 2017, the harvested surface area of wheat was 281.543.071 ha, and world production of wheat was 771.718.579 tons (CGIAR, 2018). Top four wheat-producing countries are China, India, the Russian Federation, and the United States, respectively. A worldwide average of wheat is 2.04 metric tons per hectare. Today, nearly US \$50 billion worth of wheat is traded each year globally and in the next decade. It is expected to pass rice as the world's most important food crop. Production of wheat must continue to increase annually by 2% to meet future demands imposed by population and prosperity growth (CGIAR, 2018; FAO, 2019).

Many factors affect the yield, quality, and the increase in wheat demand. One of the most important ones is plant disease arisen from infectious organisms and environmental factors such as drought, soil fertility, and quality. According to FAO estimates, diseases and pests in cereals have caused yearly 23 million ton loss, which is an amount that can feed 150 million people. Despite intensive crop protection practices, actual grain yield losses due to pathogens in global wheat production between the years of 2001 and 2003 were about 10%, while only 2% of losses were due to viruses (Oerke, 2006). The most important diseases affecting wheat are rusts, powdery mildew, loose smut, leaf blight, and bunt diseases.

Wheat bunt is known to be one of the most important fungal plant pathogens. It has been the focus of the publications in early plant pathology, such as M. Mathieu du Tillet (Tillet, 1755) who indicated that wheat bunt caused from the seed contamination by the greasy and blackish powder contained in infected seed. This contribution has later reflected in the generic name as "*Tilletia* Tul. and C. Tul.," published in 1847 (Tulasne and Tulasne, 1847).



**FIGURE 15.1** (A) *Tilletia caries* and *Tilletia foetida* spores. (B) *Tilletia caries* spore. (C) *Tilletia foetida* spore.

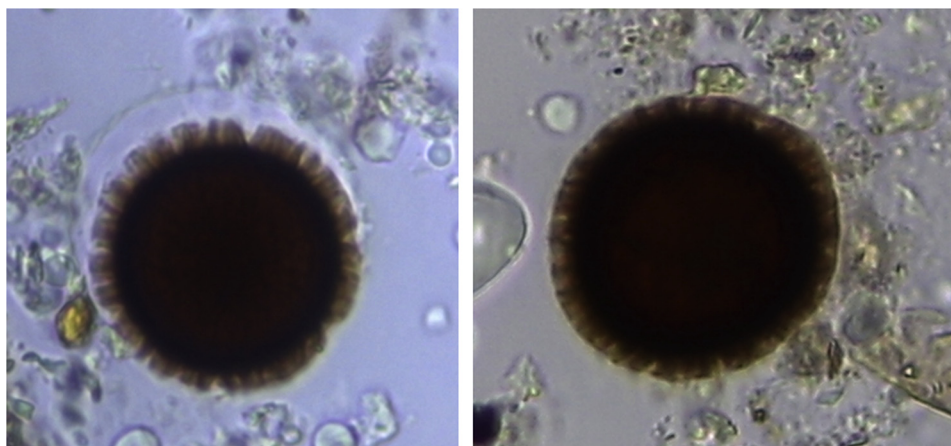
### 1. *Tilletia* species

The genus *Tilletia* is a grass disease fungus infecting cereal crops either locally or systemically. Smut fungi take place in the class of Ustilaginomycetes, subphylum Ustilaginomycotina, phylum Basidiomycota. As a result of the infection, symptoms of the plant appear blackened, because of this appearance; the name of the class is derived from the term “ustulatus” meaning burned (Carris, 2001a). The cereal-infecting *Tilletia* species forms teliospores in the ovaries of their hosts defined as bunt fungi. It is also considered to be the origin of the word “burned” (Duran and Fisher, 1961; Carris, 2001b).

Three important genera are causing *Tilletia* infection in the wheat. The most common one is common bunt, also known as stinking smut or covered smut, which causes disease both in spring and winter wheat. *Tilletia* infection has resulted from two very closely related fungi, *Tilletia caries* (DC.) Tul. [= *Tilletia tritici* (Bjerk.) Wint.] and *Tilletia laevis* Kühn. [= *Tilletia foetida* (Wallr.) Liro., *Tilletia foetons* (Berk & Curt)]. The two pathogens differ mostly in their spore wall structure. *T. laevis* has a smooth surface; *T. tritici* has a reticulated surface (Fig. 15.1). These fungi survive on the surface of the seed and in soil. The most important source of infection is contaminated seed, and pathogen infects wheat at the seedling stage (Mathre, 2000).

The other is dwarf bunt, caused by the fungus *Tilletia controversa* Kühn, which is a quarantine pest in many countries (Zhou et al., 2018). It occurs on autumn-planted wheat, and it has never reported on spring-planted wheat. The symptoms and life cycle of *T. tritici* and *T. controversa* are very similar, and the difference of teliospore structures can distinguish them. *T. controversa* teliospores cover with a conspicuous hyaline gelatinous sheath with a thickness of 1.5–5.5  $\mu\text{m}$  (Duran and Fischer, 1961).

Although its limited geographic distribution and lower yield impact, the third one, Karnal bunt (KB), is the most crucial smut fungi infecting wheat. It is also called with other scientific names such as Indian bunt of wheat, new bunt, partial bunt of wheat, and carie de Karnal (Fig. 15.2).



**FIGURE 15.2** *Tilletia indica* spores.

**TABLE 15.1** Differences of teliospore morphology of *Tilletia walkeri*, *Tilletia horrida*, *Tilletia ehrhartae*, and *Tilletia indica*.

	Mean spore size (μm)	Max spore size (μm)	Color and shape of spore	Spore ornamentation and median profile
<i>T. indica</i>	35–41	45–50+	Orange, generally dark reddish-brown to opaque black Globose to subglobose	Densely echinulate or finely cerebriform, truncate, sometimes curved and smoother appearance
<i>T. walkeri</i>	30–31	36–45	Pale yellow to dark reddish-brown, generally reddish-brown Globose	Coarse, cerebriform, and irregular with gaps between spines obvious in profile after bleaching
<i>T. horrida</i>	24–28	<36	Pale yellow—dark chestnut brown, generally dark or light chestnut-brown Globose to subglobose	Echinulate, sometimes cerebriform ridges. Generally curved and polygonal scales
<i>T. ehrhartae</i>	24–28	<28	Very dark olivaceous brown (mature spore). Opaque (melanization of the scales) Globose to subglobose	Cylindrical to slightly tapered spines and rarely cerebriform; broadly truncated to slightly rounded at apex

\**T. indica* and *T. walkeri* (Milbrath et al. (1998); Castlebury (1998); Castlebury and Carris (1999); Cunfer and Castlebury (1999)). \**Tilletia barclayana* and *T. horrida* (Duran and Fischer (1961); CMI description no. 75 (1965); Khanna and Payak (1968); Durán (1987); Aggarwal et al. (1990); Castlebury and Carris (1999); Castlebury (1998)). \**T. ehrhartae* (Pascoe et al. (2005)).

Modified from IPPC, 2016.

Three *Tilletia* species other than *T. caries*, *T. laevis*, and *T. controversa* can be confused with *Tilletia indica*, because of their morphological similarity. One of these species is *Tilletia walkeri* that infects *Lolium perenne* and *Lolium multiflorum*. *Tilletia horrida* is the other one *Tilletia* species that is a pathogen of *Oryza* spp., and the last one is *Tilletia ehrhartae* that is a pathogen of *Ehrharta calycina*. Briefly, these species infected Poaceae species. They also have been detected in harvested wheat (*Triticum aestivum*) or seed in Australia and America (Castlebury, 1998; Castlebury and Carris, 1999; Pascoe et al., 2005). Therefore, accuracy of identification of these species is essential.

*Tilletia barclayana* (smut of various Poaceae, e.g., *Panicum* and *Paspalum*), *Tilletia eragrostidis* (on *Eragrostis*), *Tilletia inolens* (on *Lachnagrostis filiformis*), *Tilletia rugispora* (on *Paspalum*), and *Tilletia boutelouae* (on *Bouteloua gracilis*) have similar morphological characteristics with *T. indica*. However, these species have been shown not to infect *T. aestivum* (EPPO, 2008). The morphological differences among *T. indica*, *T. walkeri*, *T. horrida*, and *T. ehrhartae* are the size, range, mean, ornamentation, and color of their teliospore (Table 15.1).

## 2. Morphology of *Tilletia indica*

Teliospore of *T. indica* is globose to subglobose. Generally, immature or rarely mature teliospores have a small hyphal fragment. The diameter of the teliospores usually ranges between 22 and 47 μm. This size of teliospores sometimes is larger up to 35–41 μm (Fig. 15.3). Colors of immature teliospores are rather different and change between pale orange-brown to dark, reddish-brown, whereas those mature teliospores can be black or opaque color. According to Carris et al. (2006), mature teliospore densely ornamented with sharply pointed to truncate spines, occasionally with curved tips, 1.4–5.0 (–7.0) μm high, which may appear as either individual spines or closely spaced, narrow ridges in surface view. A thin hyaline membrane covers the spines (Carris et al., 2006; CMI, 1983). Sterile cells of *T. indica* can be spherical, spheroidal, or tear-shaped, yellowish-brown, 10–28 × 48 μm, with or without

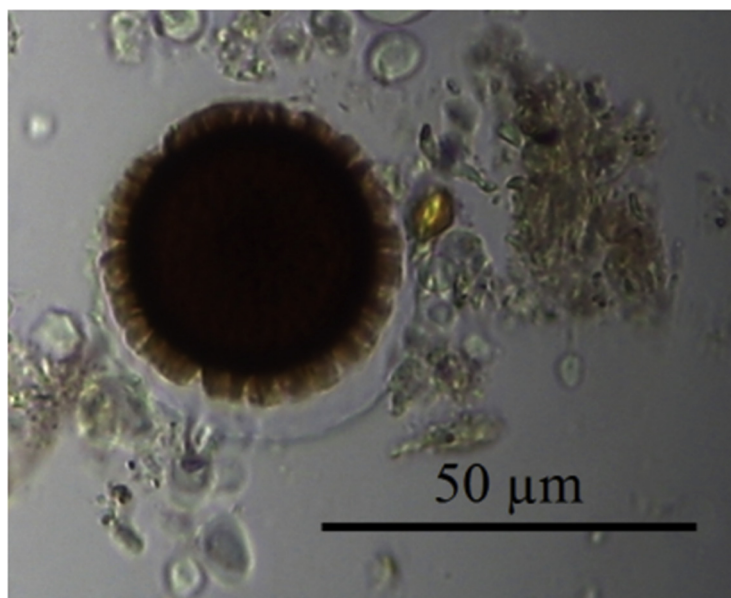


FIGURE 15.3 Teliospore of *Tilletia indica*.

an apiculus, with smooth walls up to 7  $\mu\text{m}$  thick and laminated. Sterile cells are probably uncommon in sieved washings (Carris et al., 2006; CMI, 1983).

### 3. Distribution of *Tilletia indica*

*T. indica* (synonym = *Neovossia indica*) causes KB disease in wheat. Although the first detection of KB was made from the region Faisalabad in Pakistan in 1909 (Anonymous, 1992), it was first formally recorded from a small city, Karnal, in the Indo-Gangetic plain, and it was identified by Mitra (1931). The pathogen is now a widespread problem in India (Delhi, Uttar Pradesh, Haryana, Punjab, Himachal Pradesh, Rajasthan, Madhya Pradesh, Jammu and Kashmir, West Bengal, and Gujarat). It has also become established in other parts of Asia including Pakistan (Punjab, Northwest Frontier Province), Afghanistan, Nepal, Iran, and Iraq. It was first confirmed outside Asia in 1972, in the state of Sonora in Mexico. In 1996, KB was detected in limited areas of Arizona, USA. KB disease has not been recognized in Turkey and the EPPO region (Crous et al., 2001; Fuentes-Davila, 1996; EPPO, 2007) (Fig. 15.4).

### 4. Hosts of *Tilletia indica*

The major host of the KB is *T. aestivum* whereas rare hosts are *Triticum durum*  $\times$  *Triticosecale* sp., *Secale cereale*. Although *Secale* spp. have been shown to have the potential to be a host (Sansford et al., 2008), *Aegilops geniculata*, *Bromus* spp. *Lolium* spp., *Triticum dicoccon*, and *Oloptum miliaceum* have been found to infect in *T. indica* in the greenhouse but has not been observed in the field (Inman et al., 2003).

### 5. Teliospores and life cycle of *Tilletia indica*

KB life cycle is based on information available of smut fungi even though biology and genetics of *T. indica* and cellular and molecular biology of KB disease are poorly understood. KB has three different morphological types of teliospore in the life cycle. The first morphological form is a nonpathogenic haploid phase. It grows like unicellular yeast (sporidial form). The primary sporidia or the macro (filiform) conidia are splash dispersed.





FIGURE 15.4 Distribution of *Tilletia indica* in the world.

It germinates to give rise to interconvertible allantoid (infective entity) and filiform (reproductive) secondary sporidia. Being allantoid- and filiform-like sporidia, there are two types of secondary sporidia. Second is filamentous dikaryon. It occurs as a result of fusion of two compatible haploid cells. It colonizes and infects plant tissues and cells. Only allantoid sporidia can infect and cause the disease. Filiform sporidia increase the inoculum by division on host/soil surface (Dhaliwal and Singh, 1989a). When the wheat leaf begins to dry, an enormous amount of the secondary sporidia infecting wheat ear head is released (Dhaliwal and Singh, 1988, 1989b). This phase occurs due to sporogenous mycelium within host tissue (Kumar et al., 2000), and it is defined as teliospore.

While Mitra (1931) reported KB disease as a soilborne, now it is also known as an airborne and seedborne (CSL, 2004; Sharma et al., 2017). But it is not directly transmitted from the seed to the plant. The pathogen is airborne in the form of sporidia or teliospores. Primary sources of inoculum of diseases are soil or seeds (CSL, 2004; Sharma et al., 2017). Equipment, tool, or man moving from milling places, wheat straw, and farmyard manure move the spores of diseases (Sharma et al., 2017). Development of diseases is directly affected by environmental conditions. Suitable temperatures of germination of teliospores range from 15 to 25°C. While the disease progression also depends on moisture (water holding capacity) in the soil, the soil type would not have a significant effect on germination during the cropping season (Peterson et al., 2017). Period of these conditions is varied from country to country. For example, these conditions of North Indian plains progress from February to March (Dumalasová and Bartoš, 2009; Rush et al., 2005).

KB has a monocycle of its life cycle. The teliospores begin to germinate in the soil around the flowering period of wheat and form a promycelium carrying many sickle-shaped primary sporidia. Primary sporidia germinate to form mycelium on plant surfaces, and this stage is followed by the production of secondary sporidia resulting in the infection of wheat leaf due to dispersion effect of wind or rain splash. Secondary sporidia create germ tubes and grow toward stomatal openings of the glume, lemma, or palea where they enter the plant. The intercellular hyphae progress through the glume, lemma, palea, and possibly rachis and enter the ovary base from these tissues. The most favorable conditions for the infection of wheat ears during flowering periods are cloudy weather and increasing humidity after light rain showers (EPPO, 2008). These factors cause too rapid infection of the grains in the ear (Goates, 1988). At harvest, teliospores usually scatter locally from bunted grain and can stick to the surface of healthy grains as an external contaminant (CSL, 2004). Both bunted grains and teliospores in the soil are the primary sources of the infection.

## 6. Symptoms of *Tilletia indica*

Due to the initially small number of grains in the wheat head that is infected by *T. indica*, it is quite challenging to recognize these few grains. Initially, it is difficult to distinguish the appearance of the infected wheat head from healthy. For these reasons, there may be difficulties in detecting *T. indica* until after harvest. In the period after



**FIGURE 15.5** Grain samples contaminated with *Tilletia indica*.

the harvest, freshly infected grains contain an unwanted odor owing to the presence of triethylamine. Symptom indicates the presence of the disease. However, other criteria should be taken into consideration in the diagnosis of KB because it is a mutual feature with other smut diseases (common and dwarf bunt).

The symptoms of the disease depend on climate characteristics. The symptoms are most clearly during the flowering period of wheat in humid and warm weather. As a result of infection, the number of spikelets and the length of ears are reduced as well as an infected plant of height may be shorter than healthy wheat. Infected grains are generally empty. Inside the infested grains is filled with rectangular or oval sori dusty brown or black sports masses of 1–3 mm in diameter (Fig. 15.5).

Infection of grains starts from hilum and running along to suture. As a result, the endosperm remains intact, but the seed coat becomes partially or completely ruptured. When the infection is not severe, the symptom of the disease is only a black point just below the embryo toward the suture. When the infection is severe, spores are replaced by tissues throughout the suture and along with the adjacent endosperm. Infected grains are separated from their glumes, and such grains and both glumes and grains may fall to the ground. As a result of KB infection of whole or a part of the wheat, grains filled with a black powder pile consist of millions of teliospores. Grain quality is reduced, because of the color, smell, and taste of the products produced from such grains.

## 7. Climatic requirements of *Tilletia indica*

There have been many studies on the origin, life cycle, and epidemiology of *T. indica* (Smith et al., 1996; Bonde et al., 1997; Nagarajan et al., 1997; Stein et al., 2005; Mansoori, 2015). Moreover, the relationships between survival and growth of *T. indica* teliospore and surrounding environmental conditions have been densely investigated in situ and in vitro studies (Smilanick et al., 1989, 1985; Peterson et al., 2017; Kaur and Kaur, 2008). The growth, survival, and control of propagules of *T. indica* were investigated in several aspects in control conditions by Smilanick in 1989. The germination ability of *T. indica* secondary sporidia at 25°C was investigated at different relative humidity. Sporidial survival was found to decrease at the lowest relative humidity. The germination of teliospores was evaluated in two different locations. In both places, after 7 months of germination increased a little; however, after 22 months, the germination of teliospores, which were only buried in dry soil, was found to remain high. During the dry and hot summer months, when the maximum temperature exceeds 45°C, teliospores ensure the survival of the pathogen. Dormancy fresh teliospores may break their dormancy when exposed to 40–43°C in direct sunlight for 18 days or longer (Krishna and Singh, 1982). The disease of teliospores survives in the soil for several years (Bonde et al., 2004). *T. indica* teliospores can resist in harsh environments and sometimes can remain viable for

2–5 years in contaminated soils (Mathur and Cunfer, 1993). The spores germinating within 2 mm of the soil surface are the source of sporidia spreading to leaves (Smilanick et al., 1985). *T. indica* has three types of teliospore dormancy. The first of the dormancy is postharvest in that germination of long-term stored seeds with teliospores is abundant compared with the freshly harvested seeds (Bansal et al., 1983; Mitra, 1935; Rattan and Auja, 1990; Smilanick et al., 1985; Thinggaard and Leth, 2003). The long-term (second type) dormancy stimulates survival of teliospores in the field conditions. The third type of dormancy can be induced by cold temperatures (Carris et al., 2006) and has been recorded even 4–8 days of frozen conditions at 0°C (Sidhartha et al., 1995).

KB is a disease of arid or semiarid regions with warm summers and cold/mild winters (Jones, 2009). Microclimatic conditions in the plant canopy are more effective on the KB diseases than macroclimatic ones (Singh et al., 1996; Coakley, 1983). However, macroclimatic conditions produce the microclimate, and there is a limit to which the latter can facilitate disease development under microclimatic conditions unfavorable to diseases. The relationship between the average disease infection (%) and meteorological parameters has been demonstrated by the studies conducted during the most sensitive growth periods of wheat (Singh et al., 1996; Auja et al., 1989). After rainy days, the increase of relative humidity and the occurrence of cloudy weather provide the creation of favorable conditions for the development and spread of the disease. Such weather also helps to increase disease infection. During the ninth SMW, increased relative humidity after morning and evening rains is the most favorable conditions for the multiplication of secondary sporidia. Rainfall and rainy days during 10th, 11th, and 12th meteorological weeks helped in the multiplication of KB in wheat (Singh et al., 1996). A study showed that 35 days passed around wheat anthesis were critical for infection in the United States (Goates and Jackson, 1996).

Climate models have been developed to simulate the spread and development of the disease in the suitable meteorological condition in its country of origin, India (Jhorar et al., 1992; Mavi et al., 1992; Kaur et al., 2007). In many countries, some of these meteorological models have been used to predict the potential establishment and spread of KB, and many studies have been carried out to adapt these meteorological methods (Stansbury and McKirdy, 2002; Sansford, 1998; Murray and Brennan, 1998; Smiley, 1997; Holmes et al., 1996). Despite the high number of methods to predict the occurrence of *T. indica* such as Humid Thermal Index, the majority of these methods have limited to specific local conditions (DumalaSoVá and BarToš, 2009).

## 8. The thresholds of inoculum of *Tilletia indica*

Teliospores germinate on the topsoil and form promycelia, developing primary and secondary sporidia in turn. Secondary allantoid sporidia of *T. indica* released from mycelial colonies germinate and reproduce on the soil. The proliferations of sporidia on soil, leaves, and spikes indicate that the inoculum from soilborne teliospores can have an only starting role in KB epidemics (Jones, 2009). Running cycles of production sporidial in the ears ensure an adequate inoculum of *T. indica* to cause KB epidemics (Dhaliwal, 1989). Although the amount of teliospores is adequate during the postwintering period, this amount may sometimes be reduced due to the early germination of the present inoculum and cannot reach the necessary amount for the critical infection period. Factors that add to the dormancy of teliospores play a vital role in this issue. The evidence indicated that *T. indica*–contaminated seed samples (provided by CIMMYT Mexico where *T. indica* occurs) showed no formation of KB when sown in Europe. The teliospores cannot survive probably long enough on the soil surface conditions of Europe. As a result, for *T. indica*, a typical pathogen sample for the warm climatic region, there is much trap for the reasons for predicting pathogen risk from temperate zones. Even for areas where only the mean temperature rise is expected, the timing of warm and rainy periods with increasing temperature is vital for the location and spread of the disease.

## 9. Detection of *Tilletia indica*

The procedure of detection of teliospore of *T. indica* describes in the diagnostic scheme (Fig. 15.6). This diagnostic scheme contains the following steps:

1. Application of a size-selective sieving wash test, which is a quarantine method, is imported seeds or grain.
2. To detect morphological identification of teliospores, sieving wash test is used.
3. For the molecular confirmation, isolation and/or germination of teliospores are made.

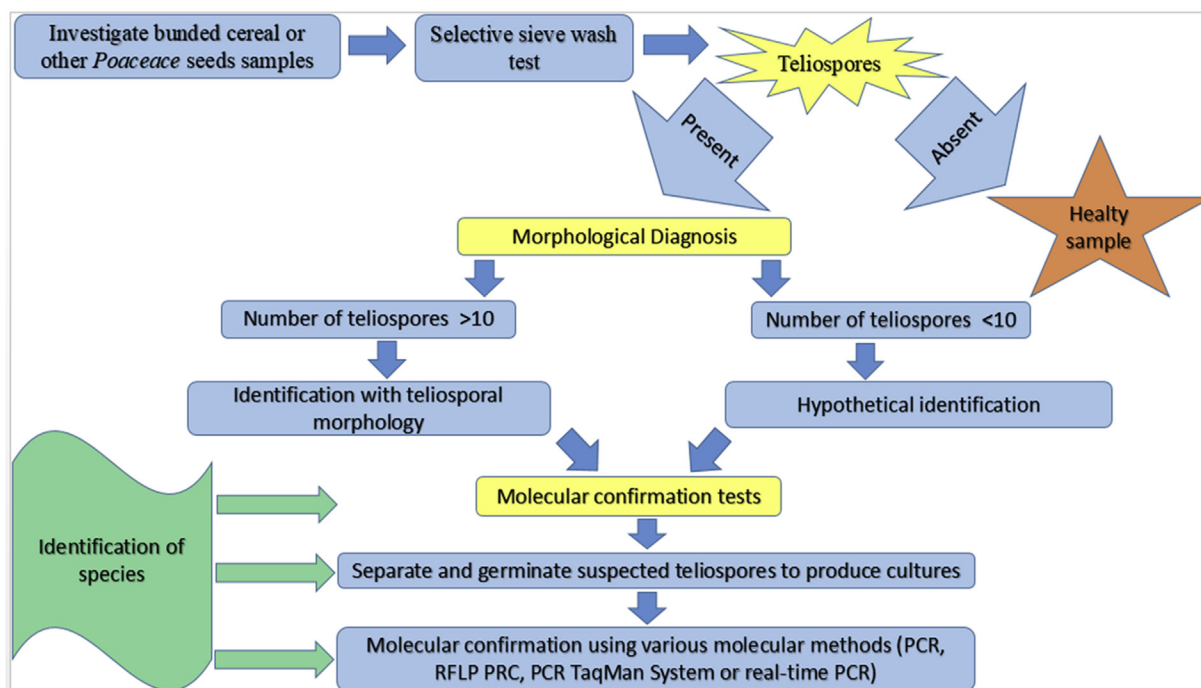


FIGURE 15.6 Diagnostic schemes for the detection of *Tilletia indica* in the grain samples (Anonymous, 2017).

EPPO published a quarantine method for detecting *T. indica* in *Triticum* spp. in 1991 (OEPP/EPPO, 1991). *T. indica* is A1 quarantine list for the European Union (EU). It means that EU is not present, it is forbidden to enter, and it is a potentially harmful organism. According to this protocol, field inspections should be carried out during the growing season between heading and harvest. The banded seed detected during these observations should be examined under microscopy for the proper characterization of teliospores of *T. indica*. For quarantine purposes, seeds infected should be tested by the washing test to check with the presence of the fungus as recommended by OEPP/EPPO (1991).

Direct visual observation for KB generally may not be adequate for quarantine purposes since low levels of infection may not be detected by only dry seed inspection (Agrawal et al., 1986), and even low seed infections can substantially contaminate healthy seed lots (Aujla et al., 1987). For these reasons, when a sample is detected contaminated with *T. indica*, the final decision needs to be confirmation tests. Therefore, to produce cultures for molecular confirmation tests, suspected spores are isolated, and it is germinated. Afterward, germinated individual teliospores remove to the culture for confirmation techniques that it is performed using advanced molecular methods based on PCR.

## 10. The social and economic impact

The yield loss of *T. indica* was found to be 0.3%–0.5% even in the highest epidemic years in India (Singh, 1994). Current yield losses are low in percentage. However, its financial impact is high in wheat production areas (Jones, 2007). The main economic losses due to *T. indica* are not related to the yield losses caused by the disease in the product.

Even though KB was determined first in the United States in 1996 and then in South Africa in 2000, it has long been known to occur in India, Afghanistan, Iraq, Mexico, and Pakistan. As a result of the concerns about its appearance in European countries, through agricultural trade activities, it has been included in the EC Plant Health Directive list of quarantine organisms, since 1997. Quarantine measures, losses in the export markets (seed and food) in the control, and treatment of infected cereals are mainly economic losses of the KB. After KB disease has been implemented to quarantine regulation in America, many countries in the world have created similar quarantine regulations (Bonde et al., 1997; Fuentes-Davila, 1998). The implementation of quarantine regulations harmed wheat grain trade and wheat research program. It is very costly to perform these regulations against KB disease. Strong expertise and a significant amount of funding are needed for these applications. This zero tolerance policy applied to the disease adversely affects grain trade. This negative situation increases wheat costs and affects consumers negatively.



Quarantine measure has also restricted the development of control measures to be taken against the KB disease and research on the development of resistant varieties. For example, in the United States, Washington State University studied intensively before the KB disease was quarantined in 1984. However, researches on KB have limited to the studies conducted in the containment facilities of America in Ft. Detrick, MD, since 1984, and the zero tolerance regulation has interfered with other researches on wheat by restricting access to germplasm and use of off-season nurseries in Mexico. Economic results are assessed by considering the disease levels of the countries affected by KB disease (Jones, 2007).

Vast fields of wheat in the Mediterranean and European region are at a high risk of infection by *T. indica* (Sansford et al., 2008). Although there is no KB in the European Union countries, the socioeconomic impact of the disease in an outbreak situation has been made with a detailed pest risk analysis (PRA) (Brennan et al., 2004a, 2004b). The first PRA conducted in 1996 before 2004 was based on the available literature records. It stated that the loss in quality caused by *T. indica* would be more than the yield. It said that the potential damage, export control, and seed certification costs would be increased due to the quarantine effect of the pathogen. In 1996, Kehlenbeck et al. carried out a PRA for Germany in 1997, following a PRA in the United Kingdom. Considering the best scenario, only in terms of yield damage in this PRA, Germany estimated that there would be a 0.5% loss in wheat production and 5 million euros in economic damage.

In Australia, the semiarid region of growing wheat is also assumed to be at risk (Stansbury and McKirdy, 2002). It stated that the economic loss to occur when *T. indica* entered in Australia was 55 ADB dollars per ton. In this case, it is noted that the smallest share in financial loss will be caused by the loss of yield (Murray et al., 1996). In the detailed PRA of the United Kingdom carried out in 2004, the policies and regulations applied in the countries where the KB exists are examined. Outbreaks of KB and its components that may be associated with its formation are identified. These components are direct costs, reaction costs, and control costs (Brennan et al., 2004a, 2004b). It was estimated that reaction and control costs would constitute 99.5% of the total economic cost of the outbreak of KB in the United Kingdom. The values and their reasons are summarized below. The direct prices are the quality and yield losses (downgrading affected milling wheat to feed) in crops affected by KB. Reaction expenditures include the measures to be taken in the product infected with the disease, their costs, and the expenses for their management, for example, price and export effects, cost for the seed and the livestock industry, cost of quarantine and management diseases, machinery cleaning and facility cleaning, additional fungicide inputs as well as treatment of mill by-products (Sansford et al., 2008).

When a single outbreak occurs in an area of 50,000 ha, it is estimated that the total cost will be 454 million euros in 10 years due to the costs mentioned above and phytosanitary controls. If plant health official controls are less implemented and national spread, it is expected to cost 548 million euros. In such a case, if the disease spreads across the EU, then it is foreseen that the cost should be increased by 10 times for 10 years. As a result, the cost is estimated to be 4540 million euros. As stated, due to high control costs, it may be worthwhile in cases where controls prevent *T. indica* spreading nationally or spreading into the EU. Nevertheless, the least costless way is to keep the pathogen out through phytosanitary measures.

The value of damaged grain due to KB and the demand for products produced from infectious grain may vary depending on the color disorder and the content of triethylamine, the level of the disease, and the requirements of the market (Warham, 1986). The most important feature of this pathogen is that when more than 3% of grains are affected, the grain is no longer accepted for processing and is declared unfit for human consumption (Warham, 1986; Ullah et al., 2012). Disease incidence was 2%–28% (Mansoori, 2015). In India, experiments show that when the susceptible cultivars of wheat are cultivated in areas suitable for KB disease development, the level of infection is generally above 3% and affects the quality of the grains seriously (Gill et al., 1993; Sharma et al., 2004; Sansford et al., 2006). Studies on susceptible European wheat cultivars developed disease levels exceeding 3% (Riccioni et al., 2008). In countries such as India, wheat quality criteria are different from those of European countries. Quality assurance schemes in Europe, such as those in the United Kingdom, are zero tolerance of *T. indica*—affected or *T. indica*—contaminated grains. Such a situation in Europe will result in loss of income in the process from downgrading milling to animal feed. This will also affect product costs (Sansford et al., 2008).

## 11. Effect of climate change on *Tilletia indica*

Climate change has been the most significant threat of this century. It contributes to the death of approximately 400,000 people in a year, and the loss is more than 1.2 trillion dollars in the world (Anonymous, 2007). Plant diseases are directly affected by climate change and global agricultural productivity. As a result of effective management

strategy against diseases and pests, it has reached twice as much as food production; however, approximately 10%–16% of the global harvest is still threatened by pathogens (Chakraborty and Newton, 2011).

Due to climate change, it is valid on temperature, carbon dioxide, and humidity. It is thought that the temperature increase will lead to geographic expansion of pathogen and vector distributions in many cases. Thus, it is estimated that the pathogen will interact with more hosts and provide new opportunities for pathogen hybridization (Baker et al., 2000; Brasier et al., 1999).

Other characteristics such as the number of generations that the pathogen creates in the reproduction per time interval and the degree of participation affect the rate of evolution of a pathogen (Garrett et al., 2006). Temperature affects the rate of reproduction of many pathogens. Due to the increase in temperature, more time will be provided for pathogen development. Increased overwintering and oversummering rates will be contributed to pathogen evolution through existing large pathogen populations. Climate change may also affect sexually or asexual reproduction of pathogen populations. Changing temperatures in some cases contribute to the increase of sexual propagules and therefore may accelerate the evolutionary process of the pathogen population (Pfender and Vollmer, 1999). The crops, alternative hosts' biomass, and amount of inoculum of necrotrophic pathogens will be increased under the influence of climate change. This change will cause the pathogen to lose the advantage of a subsequent partially resistant variety that will reduce the density of inoculum (Melloyet et al., 2010).

Temperature changes will provide better opportunities for the overwintering of the sexual stages. This situation will accelerate the development of gene recombination and the development of more aggressive pathogen species (McElrone et al., 2005). Since soil is a highly complex ecosystem, different climate change parameters will be useful in various soil microorganisms and related biological processes. These changes depend on particular soil conditions. Therefore, the few generalizations of climate change interpretation can be made (Pritchard, 2011).

Due to the climate changes, many conditions such as warming, precipitation, and generation of polycyclic pathogens may also affect the geographic distribution of pathogens (Juroszek and Tiedemann, 2013). Short-life cycle pathogens adapt to climatic changes more rapidly due to high reproductive speeds and effective distribution mechanisms in the presence of host plants (Coakley et al., 1999). The survival of pathogens, the acceleration of the life cycle of vectors and fungi, and the increase in sporulation and infection are facilitated in winters with high night temperatures (Harvell et al., 2002).

In the past 30 years, analysis of weather data from different locations of Punjab, India, has determined that early warming came in February. Temperature changes affect the growth of crops and host–pathogen interaction. Hence, it is thought that the diseases such as yellow rust, KB, *Fusarium* head blight and root rust spread rapidly, if temperature and humidity increase in the absence of resistant wheat cultivars. As a result, this change is predicted to affect wheat production, which is an essential product of India, as it may cause changes in the profile of pathogens (Kaur et al., 2008).

Climate change has a vital role in geographic distribution (Mina and Sinha, 2008). Temperature changes can trigger the development of a pathogen in the dormant stage, inducing an epidemic. For example, an increase in temperature with adequate soil moisture may increase evapotranspiration, resulting in humid microclimate in crops. This situation may lead to the incidence of diseases favored under these conditions (McElrone et al., 2005). Diseases such as common bunt (*T. caries*) and KB may be important because of changes in soil moisture and temperature due to climate change in regions where the appropriate seed treatment is not applied (Oerke, 2006). Besides, the difference in the geographic distribution of *T. indica* due to climate change is stated in the studies of PRA in Europe (Baker et al., 2000; West et al., 2012).

As a result, a limited number of studies have been conducted on the impact of climate change on plant diseases in field conditions or on disease management under climate change. To overcome this lack of information and to have a broader perspective about the impact of climate change on plant diseases, (1) effectiveness of present physical–chemical and biological control methods, including disease-resistant varieties under the influence of climate change, should be carefully evaluated; (2) climate change scenarios should be included in all studies developing new tools and strategies; (3) disease risk analyses based on host–pathogen interactions should be implemented; and (4) the number of the studies questioning how slight differences in climate characteristics may affect host and pathogen adaptation should be increased.

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## 12. Conclusions

Although the economic loss is not very high, KB is considered to be a significant threat. KB of wheat has a different life cycle than the other smut (dwarf and common smut) diseases known in the world. The morphology and climate

requirements of teliospores play a significant role in the development of the disease. *T. indica* is soil and airborne pathogen that may remain viable in the soil for 2–5 years depending on surrounding environmental conditions.

Although *T. aestivum* is host to various *Tilletia* species such as *T. walkeri*, *T. ehrhartae*, *T. indica*, and *T. horrida*, *T. barclayana*, *T. eragrostidis*, *T. inolens*, and *T. boutelouae* are known not to infect *T. aestivum*. However, *T. walkeri*, *T. ehrhartae*, and *T. horrida* species have infected with *T. aestivum* that has similar morphological characteristics with *T. indica*. The size, range, mean, ornamentation, and color of their teliospores of *T. walkeri*, *T. ehrhartae*, and *T. horrida* are different from those of *T. indica*.

KB is usually evaluated morphologically, but sieve wash test is often used in case of doubt. PCR-based molecular techniques are preferred if doubt is still not resolved. Wheat yield losses due to KB are negligible, but the disease has economic importance due to the quarantine measures and their effective cost. The zero tolerance quarantine established to prevent the spread of the disease is a costly approach and may not be able to prevent the spread of the disease effectively. Although wheat yield losses caused by KB disease are regarded as less important, it is costly due to quarantine practices, and KB has a serious threat to international wheat industry in the world trade. Further studies are needed to determine the effects of climate change on the disease because our current data and information level would not be able to estimate actual yield loss/grain potential related to the changes in environmental conditions.

## References

- Aggarwal, R., Joshi, L.M., Singh, D.V., 1990. Morphological differences between teliospores of *Neovossia indica* and *N. horrida*. *Indian Phytopathology* 43, 439–442.
- Agrawal, K., Yadav, V., Singh, T., Singh, D., 1986. Occurrence and detection of Karnal bunt in wheat seed in Rajasthan. *Indian Journal of Mycology and Plant Pathology* 16, 290–291.
- Anonymous, 1992. Quarantine pests for Europe. In: Data Sheet for *Tilletia indica*, First ed. CAB International, UK, pp. 651–656.
- Anonymous, 2007. Intergovernmental Panel on Climate Change, Climate Change. The Fourth IPCC Assessment Report. Cambridge University Press, Cambridge, UK.
- Anonymous, 2017. Diagnostic protocol for *Tilletia indica*, the cause of karnal bunt. In: The International Diagnostic Protocol for *Tilletia indica* (ISPM-27 DP04) was Released March 2014. <https://www.ippc.int/en/publications/2457/>.
- Aujla, S.S., Indu, S., Sharma, I., 1987. New host records of *Neovossia indica*. *Indian Phytopathology* 40, 437.
- Aujla, S.S., Sharma, I., Singh, P., Singh, G., Dhaliwal, H.S., Gill, K.S., 1989. Propiconazole - a promising fungicide against Karnal bunt of wheat. *Pesticides* 23, 35–38.
- 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 nonindigenous pests under current and future climates. *Agriculture, Ecosystems and Environment* 82, 57–71.
- Bansal, R., Singh, D.V., Joshi, L.M., 1983. Germination of teliospore of Karnal bunt of wheat. *Seed Research* 11, 258–261.
- Bonde, M.R., Nester, S.E., Olsen, M.W., Berner, D.K., 2004. Survival of teliospores of *T. indica* in Arizona field soils. *Plant Disease* 88, 804–810.
- Bonde, M.R., Peterson, G.L., Schaad, N.W., Smilanick, J.L., 1997. Karnal bunt of wheat. *Plant Disease* 81, 1370–1377.
- Brasier, C.M., Cooke, D.E.L., Duncan, J.M., 1999. Origin of a new *Phytophthora* pathogen through interspecific hybridization. *Proceedings of the National Academy of Sciences of the United States of America* 96, 5878–5883.
- Brennan, J.P., Kelly, P.W., Thorne, F., 2004a. Report on Socio-Economic Costs of Karnal Bunt in the European Union. EU Karnal Bunt Risks Project. Deliverable Report 5-1. <http://karnalpublic.pestrisk.net/>.
- Brennan, J.P., Thorne, F.S., Kelly, P.W., Murray, G.M., 2004b. Defining the costs of an outbreak of Karnal bunt of wheat. In: Proceedings of the 48th Annual Conference of the Australian Agricultural and Resource Economics Society. AAARES, Melbourne, Australia. [http://www.aares.info/files/AAARES/rest2004/Brennan\\_et\\_al.pdf](http://www.aares.info/files/AAARES/rest2004/Brennan_et_al.pdf).
- Carris, L.M., Castlebury, L.A., Goates, B.J., 2006. Non systemic bunt fungi - *Tilletia indica* and *T. horrida*: a review of history, systematics, and biology. *Annual Review of Phytopathology* 44, 113–133.
- Carris, L.M., 2001a. Smut Fungi. See Ref. 76b, pp. 919–921.
- Carris, L.M., 2001b. Smut Diseases. See Ref. 76b, pp. 917–919.
- Castlebury, L.A., 1998. Morphological characterisation of *Tilletia indica* and similar fungi. In: Malik, V.S., Mathre, D.E. (Eds.), *Bunts and Smuts of Wheat: An International Symposium*. North American Plant Protection Organization, Ottawa, pp. 97–105, 445 + xv pp.
- Castlebury, L.A., Carris, L.M., 1999. *Tilletia walkeri*, a new species on *Lolium multiflorum* and *L. perenne*. *Mycologia* 91, 121–131.
- CGIAR, 2018. Consultative Group on International Agricultural Research, Wheat in the world. <https://wheat.org/wheat-in-the-world>.
- Chakraborty, S., Newton, A.C., 2011. Climate change, plant diseases and food security: an overview. *Plant Pathology* 60, 2–14.
- CMI, 1965. Description of Pathogenic Fungi and Bacteria, *Tilletia barclayana*. No. 75. CAB International, Wallingford (GB).
- CMI, 1983. Description of Pathogenic Fungi and Bacteria, *Tilletia indica*. No. 748. CAB International, Wallingford (GB).
- Coakley, S.M., 1983. Ambient meteorological factors light, temperature, and moisture. In: Kommedahl, T., Williams, P.H. (Eds.), *Challenging problems in plant health*. American Phytopathological Society, St Paul, USA, pp. 154–167.
- Coakley, S.M., Scherm, H., Chakraborty, S., 1999. Climate change and plant disease. *Annual Review of Phytopathology* 37, 399–426.
- 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.
- CSL, 2004. Pest Risk Analysis for *Tilletia indica*. <https://secure.fera.defra.gov.uk/phiw/riskRegister/downloadExternalPra.cfm?id=3923>.
- Cunfer, B.M., Castlebury, L.A., 1999. *Tilletia walkeri* on annual ryegrass in wheat fields in the Southern United States. *Plant Disease* 83, 685–689.

- Dhaliwal, H.S., 1989. Multiplication of secondary sporidia of *Tilletia indica* on soil and wheat leaves and spikes and occurrence of Karnal bunt. Canadian Journal of Botany 67, 2387–2390.
- Dhaliwal, H.S., Singh, D.V., 1989a. Up-to-date life cycle of *Neovossia indica*. Current Science 57, 675–677.
- Dhaliwal, H.S., Singh, D.V., 1989b. Production and interrelationship of two types of secondary sporidia of *Neovossia indica*. Current Science 58, 614–618.
- Dhaliwal, H.S., Singh, D.V., 1988. Inter-relationship of two types of secondary sporidia of *Neovossia indica*. Indian Phytopathology 41, 276.
- DumalaSoVá, V., BarToš, P., 2009. Will climatic changes enhance the risk of *Tilletia indica* in Europe? Plant Protection Science 45 (Special Issue), S38–S40.
- Duran, R., Fischer, G.W., 1961. The Genus *Tilletia*. Washington State University Press, Pullman, WA, USA, p. 138.
- Durán, R., 1987. Ustilaginales of Mexico: Taxonomy, symptomatology, spore germination, and basidial cytology. Washington State University, Seattle, 331 + xvi pp.
- EPPO, 2007. Diagnostic protocols for regulated pests. PM 7/29(2). *Tilletia indica*. OEPP/EPPO Bulletin 37, 503–520.
- EPPO, 2008. Diagnostics PM 7/29 (3) *Tilletia indica*. OEPP/EPPO Bulletin 48 (1), 7–31.
- FAO, 2019. Food and Agriculture Organization of the United Nations, Food Outlook Biannual Report On Global Food Markets. <http://www.fao.org/3/ca4526en/ca4526en.pdf>. (Accessed 3 December 2019).
- Fuentes-Davila, G., 1996. Karnal bunt. In: Wilcoxson, R.D., Saari, E.E. (Eds.), Bunt and Smut Diseases of Wheat: Concepts and Methods of Disease Management. International Maize and Wheat Improvement Center (CIMMYT), Mexico, DF, pp. 26–32.
- Fuentes-Davila, G., 1998. Karnal bunt of wheat. In: Malik, V.S., Mathre, D.E. (Eds.), Bunts and Smuts of Wheat, an International Symposium. North American Plant Protection Organization, Ottawa, Canada, pp. 69–81.
- Garrett, K.A., Dendy, S.P., Frank, E.E., Rouse, M.N., Travers, S.E., 2006. Climate change effects on plant disease: genomes to ecosystems. Annual Review of Phytopathology 44, 489–509.
- Gill, K.S., Sharma, I., Aujla, S.S., 1993. Karnal Bunt and Wheat Production. Punjab Agricultural University, Ludhiana, India, p. 153.
- Goates, B.J., Jackson, E.W., 1996. Susceptibility of wheat to *Tilletia indica* during stages of spike development. Phytopathology 96, 962–966.
- Goates, B.J., 1988. Histology of infection of wheat by *Tilletia indica*, the Karnal bunt pathogen. Phytopathology 78, 1434–1441.
- Harvell, H.C., Mitchell, C.E., Ward, J.R., Altizer, S., Dobson, A.P., Ostfeld, R.S., Samuel, M.D., 2002. Climate warming and disease risks for terrestrial and marine biota. Science 296, 2158–2162.
- Holmes, G.J., Jackson, L.F., Perring, T.M., 1996. Imperial Valley conditions limit Karnal bunt in wheat. California Agriculture 51 (3), 29–32.
- Inman, A.J., Hughes, K.J.D., Bowyer, R., 2003. Protocol for extracting teliospores from untreated seed or grain by size-selective sieving. In: EU Recommended Protocol for the Diagnosis of a Quarantine Organism: *Tilletia indica*, pp. 21–26.
- IPPC, 2016. International Plant Protection Convention, Diagnostic protocols for regulated pests *Tilletia indica* Mitra. [https://www.ippc.int/static/media/files/publication/en/2016/01/DP\\_04\\_2014\\_En\\_2015-12-22\\_PostCPM10\\_InkAmReformatted.pdf](https://www.ippc.int/static/media/files/publication/en/2016/01/DP_04_2014_En_2015-12-22_PostCPM10_InkAmReformatted.pdf). (Accessed 2 December 2019).
- Jhorar, O.P., Mavi, 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., 2007. A reappraisal of the current status of *Tilletia indica* as an important quarantine pest for Europe. European Journal of Plant Pathology 118, 105–113.
- Jones, D.R., 2009. Towards a more reasoned assessment of the threat to wheat crops from *Tilletia indica*, the cause of Karnal bunt disease. European Journal of Plant Pathology 123, 247–259.
- Juroszek, P., Tiedemann, A.V., 2013. Climate change and potential future risks through wheat diseases: a review. European Journal of Plant Pathology 136, 21–33.
- Kaur, G., Kaur, S., Handal, S.S., 2007. Weather based empirical model to predict infective sporidial stage of *Tilletia indica* during wheat crop season. Indian Phytopathology 60 (2), 173–179.
- Kaur, S., Dhaliwal, L., Kaur, P., 2008. Impact of climate change on wheat disease scenario in Punjab. Journal of Research 45 (3&4), 161–170.
- Khanna, A., Payak, M.M., 1968. Teliospore morphology of some smut fungi. II. Light microscopy. Mycologia 60, 655–662.
- Krishna, A., Singh, R.A., 1982. Evaluation of fungicides for the control of Karnal bunt of wheat. Pesticides 16, 7.
- Kumar, A., Singh, U.S., Singh, A., Malik, V.S., Garg, G.K., 2000. Molecular signalling in pathogenicity and host recognition in smut fungi taking Karnal bunt as a model system. Indian Journal of Experimental Biology 38, 525–539.
- Mansoori, B., 2015. Biology and epidemiology of *Tilletia indica* inducing Karnal bunt (partial bunt) of wheat (*Triticum aestivum*) in arid regions. Indian Phytopathology 68 (1), 39–41.
- Mathre, D.E., 2000. Stinking smut of wheat. The Plant Health Instructor. <https://doi.org/10.1094/PHI-I-2000-1030-01Updated-2005>.
- Mathur, S.B., Cunfer, B.M., 1993. Karnal bunt. In: Mathur, S.B., Cunfer, B.M. (Eds.), Seed-borne Diseases and Seed Health Testing of Wheat. Jordbrugsforlaget, Frederiksberg, Denmark, pp. 31–43.
- Mavi, H.S., Jhorar, O.P., Sharma, I., Singh, G., Mahi, G.S., Mathauda, S.S., Aujla, S.S., 1992. Forecasting Karnal bunt disease of wheat - a meteorological method. Cereal Research Communications 20, 67–74.
- Mcelrone, A.J., Reid, C.D., Hoyer, K.A., Hart, E., Jackson, R.B., 2005. Elevated CO<sub>2</sub> reduces disease incidence and severity of a red maple fungal pathogen via changes in host physiology and leaf chemistry. Global Change Biology 11, 1828–1836.
- Melloy, P., Hollaway, G., Luck, J., Norton, R., Aitken, E., Chakraborty, S., 2010. Production and fitness of *Fusarium pseudograminearum* inoculum at elevated carbon dioxide in FACE. Global Change Biology 16, 3363–3373.
- Milbrath, G.M., Pakdel, R., Hilburn, D., 1998. Karnal bunt spores in ryegrass (*Lolium* spp.). In: Malik, V.S., Mathre, D.E. (Eds.), Bunts and Smuts of Wheat: An International Symposium. North American Plant Protection Organization, Ottawa, pp. 113–116, pp. 445 + xv.
- Mina, U., Sinha, P., 2008. Effects of climate change on plant pathogens. Environmental News Network 14 (4), 6–10.
- Mitra, M., 1935. Stinking smut (bunt) of wheat with special reference to *Tilletia indica* Mitra. Indian Journal of Agricultural Science 1, 51–74.
- Mitra, M., 1931. A new bunt of wheat in India. Annals of Applied Biology 18, 178–179.
- Murray, G., Brennan, J., Hare, R., 1996. Karnal bunt of wheat: getting closer to Australia? Agricultural Science 9 (6), 45–48.
- 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.



- Nagarajan, S., Aujla, S.S., Nanda, G.S., Sharma, I., Goel, L.B., Kumar, J., Singh, D.V., 1997. Karnal bunt (*Tilletia indica*) of wheat — a review. Review of Plant Pathology 76, 1207–1214.
- OEPP/EPPO, 1991. Quarantine procedure No. 37. *Tilletia indica*. Inspection and test methods for wheat seeds. Bulletin OEPP/EPPO Bulletin 21, 265–266.
- Oerke, E.C., 2006. Crop losses to pests. The Journal of Agricultural Science 144, 31–43.
- Pascoe, I.G., Priest, M.J., Shivas, R.G., Cunnington, J.H., 2005. Ustilospores of *Tilletia ehrhartae*, a smut of *Ehrharta calycina*, are common contaminants of Australian wheat grain, and a potential source of confusion with *Tilletia indica*, the cause of Karnal bunt of wheat. Plant Pathology 54, 161–168.
- Peterson, G.L., Berner, D.K., Phillips, J.G., 2017. Observations of the germination behavior of *Tilletia indica* teliospores on the soil surface under varying simulated environmental conditions. American Journal of Plant Sciences 8, 2878–2897.
- Pfender, W.F., Vollmer, S.S., 1999. Freezing temperature effect on survival of *Puccinia graminis* sub sp. *graminicola* in *Festuca arundinacea* and *Lolium perenne*. Plant Disease 83, 1058–1062.
- Pritchard, S.G., 2011. Soil organisms and global climate change. Plant Pathology 60, 82–99.
- Rattan, G.S., Aujla, S.S., 1990. Survival of Karnal bunt (*Neovossia indica*) teliospores in different types of soil at different depths. Indian Journal of Agricultural Science 60, 616–618.
- Riccioni, L., Inman, A., Magnus, H.A., Valvassori, M., Porta-Puglia, A., Conca, G., Di Giambattista, G., Hughes, K., Coates, M., Bowyer, R., Barnes, A., Sansford, C.E., Razzaghian, J., Prince, A., Peterson, G.L., 2008. Susceptibility of European bread and durum wheat cultivars to *Tilletia indica*. Plant Pathology 57, 612–622.
- Rush, C.M., Stein, J.M., Bowden, R.L., Riemenschneider, R., Boratynski, T., 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. Karnal bunt (*Tilletia indica*): detection of *Tilletia indica* Mitra in the US: potential risk to the UK and the EU. In: Malik, V.S., Mathre, D.E. (Eds.), Bunts and Smuts of Wheat: An International Symposium. North Carolina, 17–20 August 1997. NAPPO, Ottawa, pp. 273–302.
- Sansford, C., Baker, R., Brennan, J., et al., 2006. Pest Risk Analysis for *Tilletia indica* for the European Union. EU Karnal Bunt Risks Project. Deliverable Report 6-1 and 6-5. <http://karnalpublic.pestrisk.net/>.
- Sansford, C.E., Baker, R.H.Z., Brennan, J.P., Ewert, F., Gioli, B., Inman, A., Kinsella, A., Magnus, H.A., Miglietta, F., Murray, G.M., Porta-Puglia, A., Porter, J.R., Rafoss, T., Riccioni, L., Thorne, F., 2008. The new pest risk analysis for *T. indica*, the cause of Karnal bunt of wheat, continues to support the quarantine status of the pathogen in Europe. Plant Pathology 57, 603–611.
- Sharma, A., Sharma, P., Dixit, A., Tyagi, R., 2017. Karnal bunt of wheat in India and its management: a review. Plant Pathology and Quarantine 7 (2), 165–173.
- Sharma, I., Nanda, G.S., Singh, H., Sharma, R.C., 2004. Status of Karnal bunt disease of wheat in Punjab (1994–2004). Indian Phytopathology 57, 435–439.
- Sidhartha, V.S., Singh, D.V., Srivastava, K.D., Aggarwal, R., 1995. Some epidemiological aspects of Karnal bunt of wheat. Indian Phytopathology 48, 419–426.
- Singh, A., 1994. Epidemiology and Management of Karnal Bunt of Wheat. Research Bulletin No. 127. G.B. Plant University of Agriculture and Technology, Pantnagar, India.
- Singh, D., Singh, R., Rao, V.U.M., Karwasra, Beniwal, M.S., 1996. Relation between weather parameters and Karnal bunt (*Neovossia indica*) in wheat (*Triticum aestivum*). Indian Journal Agriculture Science 66 (9), 522–525.
- 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.
- Smiley, R.W., 1997. Risk assessment for Karnal bunt occurrence in the Pacific Northwest. Plant Disease 81, 689–692.
- Smith, O.P., Peterson, G.L., Beck, R.J., Schaad, N.W., Bonde, M.R., 1996. Development of a PCR-based method for identification of *Tilletia indica*, causal agent of Karnal bunt of wheat. Phytopathology 86, 115–122.
- 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.
- Stein, J.M., Maples, H.W., Rush, C.M., 2005. Epidemiology of *Tilletia indica* teliospores in regulated wheat fields in Texas. Plant diseases 89 (8), 828–833.
- Thinggaard, K., Leth, V., 2003. Use of the fluorochrome vital dye acridine orange to determine viability and germination of *Tilletia indica* teliospores in soil. Seed Science and Technology 31, 329–340.
- Tillet, M., 1755. Dissertation on the Cause of the Corruption and Smutting of the Kernels of Wheat in the Head and the Means of Preventing These Untoward Circumstances. Bordeaux, p. 150.
- Tulasne, L.R., Tulasne, C., 1847. Mémoire sur les Ustilaginées comparées aux Uredinées. Annales des Sciences Naturelles 3 (7), 12–127.
- Ullah, H.M.Z., Haque, M.I., Rauf, C.A., Akhtar, L.H., Munir, M., 2012. Comparative virulence in isolates of *T. indica* and host resistance against Karnal bunt of wheat. Journal of Animal and Plant Sciences 22, 467–472.
- Warham, E.J., 1986. Karnal bunt disease of wheat: a literature review. Tropical Pest Management 32, 229–242.
- West, J.S., Townsend, J.A., Stevens, M., Fitt, B.D.L., 2012. Comparative biology of different plant pathogens to estimate effects of climate change on crop diseases in Europe. European Journal of Plant Pathology 133, 315–331.
- Zhou, Y., Duan, X., Jia, W., 2018. Risk assessment of *Tilletia controversa* establishment in China. Czech Journal of Genetics and Plant Breeding 42 (Special Issue), 84.