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Wheat Grain Discoloration in Argentina: Current Status

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ABSTRACT

Black point (BP) and smudge are discolorations of wheat and other cereal kernels and occur in all major crop-growing regions of the world. BP is defined as a distinct brown or black discoloration of the germ end and surrounding area. When the discoloration affects more than one-half of the kernel or extends into the crease it is interpreted as smudge. This disease is usually caused by fungi, though recent studies suggest it may be a result of abiotic stress conditions. Many of these fungi are saprophytic (non-pathogenic) and are commonly found growing on dead plant material, as the genus *Alternaria*, or are cereal pathogens such as *Bipolaris sorokiniana.* This discoloration reduces grain quality impairing flour, semolina and their products. Downgrading of discolored grains at the market can cause economic losses to producers. The development of BP is very dependent on weather, occurring in the field under conditions of high relative humidity or rainfall. BP can be partially controlled by reducing irrigation frequency after heading and by reducing nitrogen rates. Another alternative is the application of fungicide but its results are contradictory. Because BP can occur at damaging levels in some seasons despite modifications in cultural practices, the best option for control is to combine reduced input practices with BP-resistant cultivars. Current cultivars differ in the level of resistance or tolerance to the disease, although there are no completely resistant cultivars available. In Argentina, although this disease has been known since the 20th century, it has become important in the last 15 years. This review deals with the current status of this wheat grain disorder in Argentina.

Keywords: black point, kernel smudge, red smudge, wheat grain quality

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INTRODUCTION

Wheat (*Triticum* spp.) is a nutritious, convenient and economical source of food. It provides about 20% of the world's food calories and is a staple for nearly 40% of the world's population (Wiese 1987). In 2007, wheat was the most important crop in the cultivated area (214,207,581 ha) (FAO 2009). Only rice, corn, and perhaps potatoes are as important as wheat. Wheat grain provides important carbohydrates, proteins, vitamins, and minerals for growth and maintenance. It is due to its unique capacity for making doughs with its flour that it is consumed principally as bread.

In Argentina, the wheat cropping area comprises near 5.500.000 ha (5.77 million in 2008) (SAGPyA 2008) concentrated in 5 provinces of the temperate central-eastern zone. Since 1970, the wheat cultivated area has varied between 4 and 7 million ha, showing its importance as a main food in the diet composition in Argentina. Conversely, durum wheat (*T. durum* Desf.) has less importance in production and/or in cultivated areas. In the last 10 years this area varied between 50,000 and 90,000 ha. Thirty years ago the area cultivated with durum wheat reached 370,000 ha, although at present it represents only 50-70,000 ha.

Wheat plants at all growth stages and in all natural environments are subjected to various mechanical, physiologic and biological stresses that interfere with their normal growth and development. The main biological agents that cause wheat diseases are fungi, viruses, bacteria, and nematodes (Wiese 1987). These diseases are important limiting factors affecting wheat production and quality.

Among the diseases that affect grain quality, grain discoloration has been increasing over the last 15 years. It occurs on grain in all the major growing regions worldwide, not only in wheat growing areas, but also in barley (*Hordeum* spp. L.) and rye cropping areas (*Secale cereale* L.).

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Invited Review

Kernel discoloration of wheat can involve several types of symptoms as black point (BP), kernel smudge, red smudge and *Fusarium*-damaged kernels (Wang *et al*. 2002).

In this review several aspects of BP/kernel smudge discoloration are analyzed, with special reference to the Argentinean situation.

Background

Black grain discoloration is known in different countries by many names. It was first described as BP in the USA by Bolley (1913), a term which has later been used by many other authors (Coons 1918; Evans 1921; Drechsler 1923; Henry 1923; Dastur 1932; Brentzel 1944; Hanson and Christensen 1953). Other terms to describe this disease are "puntatura" in Italy (Curzi 1926; Peyronell 1926), "mouchetage" or "mouchetture" in Morocco, Algeria (Miège 1930) and in France (Rosella 1930; Ponchet 1966), black germ or black grain in the former USSR (Ziling 1932) and smudge or kernel smudge in Canada (Simmonds 1930; Greaney and Wallace 1943).

Symptoms and damage of BP/kernel smudge

Infected ears may look normal but there may be elliptical, brown to dark brown lesions on the inner side of the glumes (Mathur and Cunfer 1993). Diseased kernels are discolored and appear withered, black-pointed, or smudged (Wiese 1987) (**Fig. 1**). Discoloration generally occurs at the germ end of the caryopsis (BP) and in severe cases it extends along the crease and over the shoulders (kernel smudge) (Conner and Davidson 1988) causing the shrivelling of the whole seed. The dark brown discoloration at the embryo end is generally limited to the seed coat; the aleurone cells underneath are not damaged. However, the endosperm frequently turns slightly brownish-grey (Kietreiber 1971). The discoloration from the scutellum may also spread some distance in the mesocarp on the lower and upper sides of the grooves of the grain (Pasinetti 1931).

Black point is an insidious disease. It first appears when the grain begins to lose moisture. Symptoms are not readily observed until the plants are harvested and the grain is threshed from the head (Southwell *et al*. 1980b). Some authors agree that no important effect on seed germination is associated with the presence of BP (Dharam Vir *et al*. 1968; Rees 1984). Nevertheless, more recent studies showed that BP reduced the germination rate, number of embryonic roots, and coleoptile length (Toklu *et al*. 1999) and delayed seedling emergence and reduced seedling vigour (Özer 2005; Toklu *et al*. 2008).

The disease is a concern because it lowers the quality and interferes with the milling and baking properties of grain (Conner and Davidson 1988), impairing flour, semolina and their products (King *et al*. 1981; Dexter and Matsuo 1982; Lorenz 1986) and consequently limiting the crop market. Durum wheat is generally more susceptible to BP than bread wheat (*Triticum aestivum* L.) (Machacek and Greaney 1938; Greaney and Wallace 1943). Fernández *et al*. (2001) indicated that the high BP incidence in durum genotypes could be related to their white seed color. Semolina and pasta products manufactured from diseased grains often contain undesirable black specks and discolored particles (Harris and Sibbit 1942). These authors have reported on the effect of BP on semolina yield and spaghetti color, but no information has been published on the possible effects of smudge and BP on spaghetti cooking quality. Dexter and Matsuo (1982) observed that spaghetti color characteristics appeared to be slightly influenced by smudge and BP. The smudged samples seemed to have a slightly lower color intensity, as measured by purity, although in most cases differences in spaghetti yellow pigment and pigment loss were not significant. Some of the smudged samples were slightly duller (lower brightness) and slightly browner (longer dominant wavelength) than their respective controls. Spaghetti cooking quality was not related to smudge damage.

Fig. 1 Wheat grain discoloration.

In every case the elasticity and firmness of smudge-enriched cooked spaghetti were not significantly different from those of their respective controls.

Black specks in the semolina milled from these grains can be removed in the milling process, but this causes a reduction in semolina yield which may be as much as 20% (Bird 1975).

BP discoloration causes loss of grade. In the United States, blackened kernels are considered damaged, and only 2 and 4% are permitted in wheat graded as U.S. Nº1 and Nº 2, respectively, for all classes of wheat, except mixed wheat (USDA 1993). In some countries like Canada, BP-infected kernels with levels of 10 and 20% for Canadian Western Red Spring common wheat (CWRS) and 5 and 10% for Canada Western Amber Durum (CWAD) are allowed for grade Nºs.1 and 2, respectively. Downgrading thresholds for kernels are based on the incidence of affected kernels and the severity of infection (Canadian Grain Commission 2001). In Australia, 5 and 3% of BP and other stained grains are permitted for grade Nº 1 of Australian Prime hard wheat and Australian durum wheat, respectively (Australian Wheat Board 2000). In the United Kingdom, it has been estimated that 4% of wheat grain is downgraded or rejected in an average year due to the disease, and up to 15% in seasons when the disease is more severe (Culshaw *et al*. 1988; Ellis *et al*. 1996). In Argentina, though grain quality has been seriously affected by this discoloration in the last years, this disease is not included in the Official Standard of wheat grain commercialisation (Intagro 2010).

Other symptoms of discoloration

Other discoloration symptom is the red smudge, which was previously referred to as pink smudge (Valder 1954). It is a reddish discoloration over most of the seed coat or in the crease of the seed caused by the fungus *Drechslera tritici-repentis* (Died.) Shoem.) (teleomorph = *Pyrenophora tritici-repentis* (Died.) Drechs.), which also causes "tan spot", a common leaf disease of wheat (Fernández *et al*. 1996). Kernel discoloration may cause downgrading of wheat (Canadian Grain Comission 1994). Natural red smudge infections reduce germination and seedling vigour (Fernández *et al*. 1996) and total emergence and rate of emergence in the field (Fernández *et al*. 1997). Kernel infection by *D. tritici-repentis* has also been reported to cause a reduction in 1000-kernel weight (Francl and Jordhal 1992). The reddish discoloration results from the secretion and accumulation of catenarin, an anthraquinone produced by this fungus as a secondary metabolite. Emodin, a direct precursor of catenarin, has been regarded as a dangerous mycotoxin so its detection in *D. tritici-repentis* infected grains is of great concern (Bouras and Strelkov 2008).

Scab of wheat, also called Fusarium head blight (FHB), is a destructive disease in the humid and semi-humid wheat-growing areas of the world (Schroeder and Christensen 1963). Many *Fusarium* species have been associated with wheat scab, however, *Fusarium graminearum* Schwabe (teleomorph = *Gibberella zeae* (Schwein.) Petch) is considered the principal pathogen responsible for head blight in many countries (Bai and Shaner 1994). Damage from head blight, involves shrunken and discoloured (pink or chalky white "tombstone") kernels, and causes reductions in yield and seed quality. This disease also reduces test weight and lowers market grade (Trigo-Stockli *et al*. 1998). Scab can cause additional loss for agriculture because of the potent mycotoxins produced by the fungus. The two most important mycotoxins are the estrogenic toxin zearalenone and the trichothecene deoxynivalenol (DON), a vomitoxin (Snijders 1990). Grain contaminated with *Fusarium* mycotoxins is unsuitable for both human and animal consumption because of adverse health effects of such toxins (Xu 2003).

Etiology

Although historically various fungi are associated with BP, there is no conclusive evidence that BP melanism is a direct result of fungal action (Mathur and Cunfer 1993). Many of these fungi are saprophytic (non-pathogenic) and are commonly found growing on dead plant material such as crop residues. For example, genus *Alternaria* and *Cladosporium* are commonly found on prematurely ripened wheat heads as "sooty molds", and can also cause BP or smudge (Wiese 1987).

Symptoms have been related to the presence of a pathogenic fungi complex on the grain, mainly dematiaceous, such as *Alternaria* spp., *Bipolaris* spp., *Curvularia* spp., *Drechslera* spp., *Exserohilum* spp. (Wiese 1987; Mathur and Cunfer 1993; Özer 2005) (**Fig. 2**). A weak mycoflora accompanies these pathogens always present in wheat grains (Sisterna and Lori 2005). These fungi can coparasitize seed but differ in aggressiveness. They are abundant spore producers and are widely distributed (Zillinsky 1983). They usually do not penetrate the seed and cause no harm to its quality. However, under certain conditions, they can go over from a saprophytic way of life to parasitic feeding type and, by penetrating inside the seed, they damage its quality and vigour (Lõiveke *et al.* 2004). The most common genera recorded are *Acremonium*, *Cladosporium*, *Epicoccum*, *Nigrospora*, *Phoma*, *Stemphylium*, *Torula*, *Trichothecium*, *Ulocladium* (Wiese 1987; Neergaard 1979; Warham *et al.* 1999) (**Fig. 3**). Also, other genera such as *Aspergillus* and *Penicillium* can cause discoloration of seed in storage resulting in germination failure, damaged embryos and production of mycotoxins (Malaker *et al*. 2008).

Among the dematiaceous complex, *Alternaria alternata* (Fr.) Keissler (=A. tenuis Nees.) and *Bipolaris sorokiniana* (Sacc.) Shoem. (=*Helminthosporium sativum* Pamm. King and Blake) (teleomorph *Cochliobolus sativus* (Ito & Kurib) Drechs. ex Dastur) (Brentzel 1944; Hanson and Christensen 1953; Kilpatrick 1968) have been isolated most frequently from grains and then considered the primary causal agents of the disease (**Fig. 4**). The often reported association between symptoms and the most common grain coloniser, *A. alternata*, is not strong and several studies have been unable to confirm any link. Black point is most commonly caused by this *Alternaria* species in several countries: the United Kingdom (Ellis *et al*. 1996), Italy (Languasco *et al.* 1993), Australia (Southwell *et al.* 1980a, 1980b; Rees *et al.* 1984; Klein 1987), Egypt (El-Helaly 1947), Turkey (Özer 2005; Toklu *et al.* 2008), India (Gill and Tyagi 1970; Adlakha and Joshi 1974; Rana and Gupta 1982), Chile (Madariaga and Mellado 1988; Mellado *et al.* 1990). On the other hand, in USA (Brentzel 1944; Hanson and Christensen 1953; Kilpatrick 1968; Statler *et al.* 1975) and Bangladesh (Hossain and Hossain 2001) *B. sorokiniana* was the main pathogen isolated. Some authors from Canada (Greaney and Machacek 1943; Greaney and Wallace 1943) found that *B. sorokiniana* was the predominating pathogen, while other researchers (Conner and Davidson 1988; Conner and Kuzyk 1988a; Fernández *et al.* 1994), determined that this disease was caused by *A. alternata*.

The relative importance of these two fungi in causing BP varies with year and location (Hanson and Christensen 1953). Several studies (Ziling 1932; Russell 1943; Kilpatrick 1968; Adlaka and Joshi 1974) reported different symptoms caused by the two fungi. Russell (1943) found that *B. sorokiniana* caused a black discoloration of the entire kernel tip, whereas *A. alternata* caused a brown discoloration around the embryo, but not extending to the ventral side of the grain. Nevertheless, Mathur and Cunfer (1993) stated that the visual distinction between effects of different fungal genera is rather difficult.

A. alternata (Johnston and Hagborg 1942) and *B. sorokiniana* (Adlaka and Joshi 1974) have also been associated with floret sterility, and infection by *B. sorokiniana* can result in seed shrivelling and rot, reducing seedling emergence and yield of the subsequent crop (Mathur and Cunfer 1993). In the case of *A. alternata* the seed setting is not affected (Adlaka and Joshi 1974; Rees *et al*. 1984) and this fungus evidently is less destructive than *B. sorokiniana* (Brentzel 1944).

In Europe, North America and Australia studies have shown that wheat grain samples are infected with other *Alternaria* spp., being also associated to BP symptoms (Webley and Jackson 1998). Information available on the *Alternaria infectoria* species-group is limited as the taxa it comprises have often been misidentified as other small-spored *Alternaria* species (e.g. *A. alternata*, *A. tenuissima*), due to the use of insufficient methods for identification (Andersen and Thrane 1996) (**Fig. 5**). The *A. infectoria* species-group comprises nine known species and an unknown number of distinct taxa yet to be described (Andersen *et al* 2002). Discrimination between species of *Alternaria*, especially *A. alternata* and *A. tenuissima* on the one hand and *A. infectoria* on the other, is very important for assessing the potential for contamination by mycotoxins. *A. infectoria* has diminished capacity for mycotoxin production compared to members of the *A. alternata* group (Andersen and Thrane 1996; Webley *et al*. 1997). There is considerable variability between grain samples regarding the frequency of isolation of *A. alternata* versus *A. infectoria* (Bruce *et al.* 1984; Webley *et al.* 1997); reports of the incidence of *Alternaria* in grains and feeds have frequently failed to distinguish between these two species (Webley *et al*. 1997). The literature on mycotoxins in cereal grains is extensive (Frisvad 1995), but there is little information available on *Alternaria* mycotoxins (Dugan and Lupien 2002).

Another species, *A. triticina* responsible for the leaf blight, causes significant yield losses in wheat on the Indian subcontinent, from where it originates and has spread throughout the world (Prasada and Prabhu 1962; Prabhu and Prasada 1966) (**Fig. 6**). It develops on plants approaching maturity, causes premature death of the uppermost leaves and heads. Mexican wheats introduced in India showed moderate susceptibility (Kulshrestha and Rao 1976). Alternaria leaf blight is likely to develop near irrigation ditches, in low areas, or wherever humidity and soil moisture are high. Bhowmik (1969) found this fungus and *A. alternata*, infecting grains and causing BP and observed an incidence of 13 to 22% in samples collected from areas receiving rains during the last part of the crop season. In his study he tried to determine the relative role of the two *Alternaria* spp. in causing natural infection. *A. triticina* is a quarantine pathogen in many countries, so it would be important to investigate the incidence of this disease in new wheat areas (Perelló and Sisterna 2006).

Fusarium spp. has also been reported to be associated with BP or kernel smudge in wheat (Hanson and Christensen 1953; Maloy and Specht 1988; Conner and Kuzyk 1988a). Kilpatrick (1968) isolated *Fusarium oxysporum* and *Fusarium* spp. at low frequency from BP kernels of wheat. However, the ability of *Fusarium* spp. to cause BP in wheat has never been clearly established until Conner *et al*. (1996) demonstrated that it can be caused by a species of *Fusarium*: *F. proliferatum* (Matsushima) Niremberg, a major cause of maize ear rot and fumonisin contamination. The BP symptoms produced by this species in wheat were similar to those produced by *A. alternata*, as the discoloration

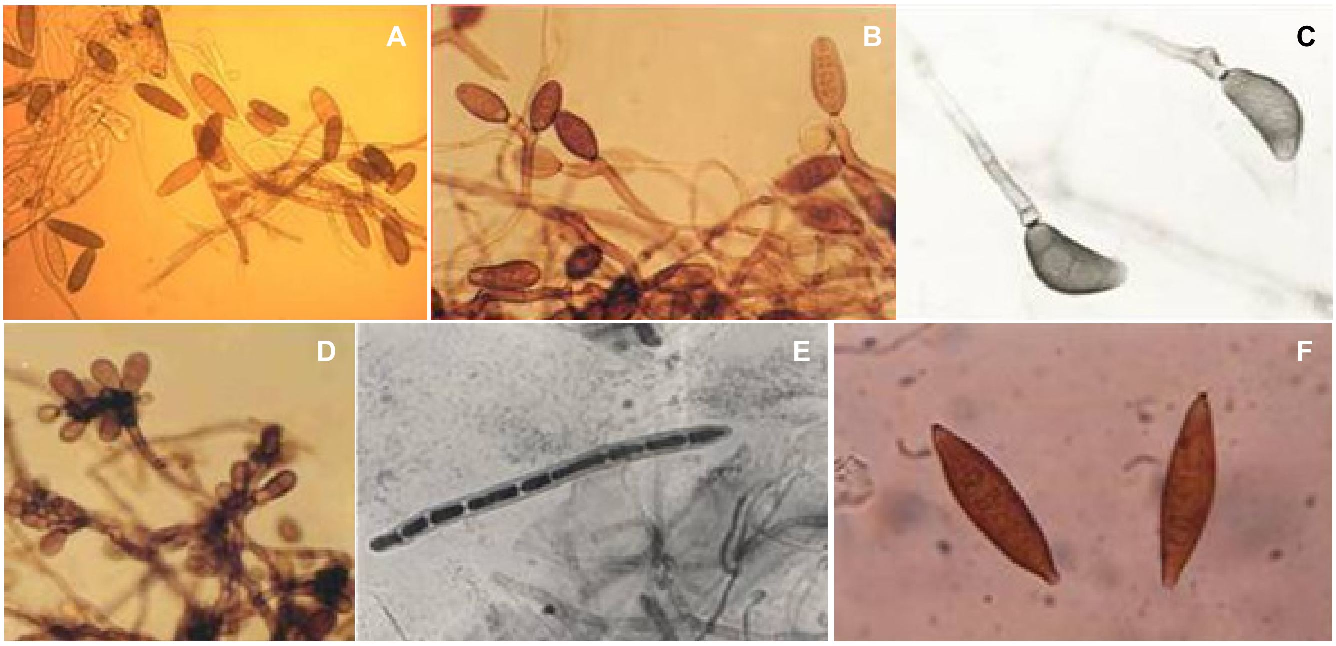

Fig. 2 Fungi complex. (**A**) *Bipolaris cynodontis*; (**B**) *B. nodulosa*; (**C**) *B. papendorfii* (**D**). *Marielliottia biseptata* (=*Drechslera biseptata*) (reprinted with kind permission from Boletín de la Sociedad Argentina de Botánica, ©2006. El género Marielliottia (Hifomicetes, Ascomycota): Nuevo taxón asociado a la Micoflora del Grano de Trigo en Argentina, Sisterna and Minhot **41 (3-4)**, 177-182); (**E**) *Drechslera tritci-repentis* (courtesy Dr. Virginia Moreno); (**F**) *Exserohilum* spp.

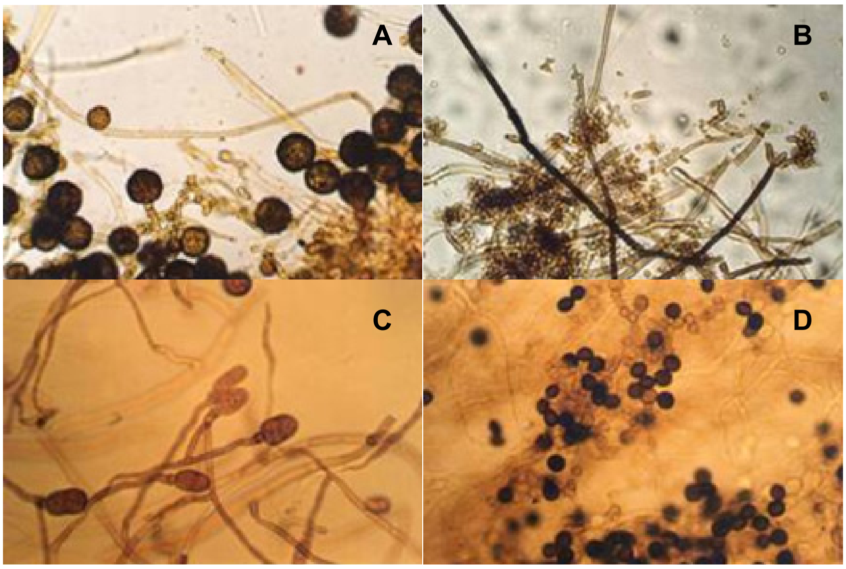

Fig. 3 Microscope images of weak mycoflora. (**A**) *Epicoccum* spp.; (**B**) *Cladosporium* spp.; (**C**) *Stemphylium* spp.; (**D**) *Nigrospora* spp.

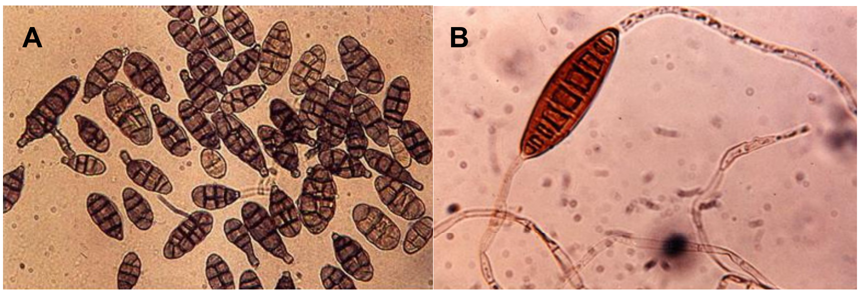

Fig. 4 (**A**) Conidia of *Alternaria alternata*. (**B**) Germinating conidium of *Bipolaris sorokiniana*. Reprinted with kind permission from Eds. A. Arya and C. Mónaco. Natural plant extracts: an alternative control of seed borne fungi, Sisterna and Dal Bello. In: Seed Borne Diseases: Ecofriendly Management, Scientific Publisher, ©2007.

was confined to the germ end of the kernel. The authors observed that natural infection of wheat grain by *F. proliferatum* was unexpected because in other surveys of *Fusarium* species in wheat with symptoms of FHB *F. proliferatum* was rarely detected (Wilcoxon *et al.* 1988; Clear and Patrick 1990). In other studies, Desjardins *et al.* (2007) determined that *F. proliferatum* produced significant symptoms of kernel BP, decreased kernel yield and contaminated ker-

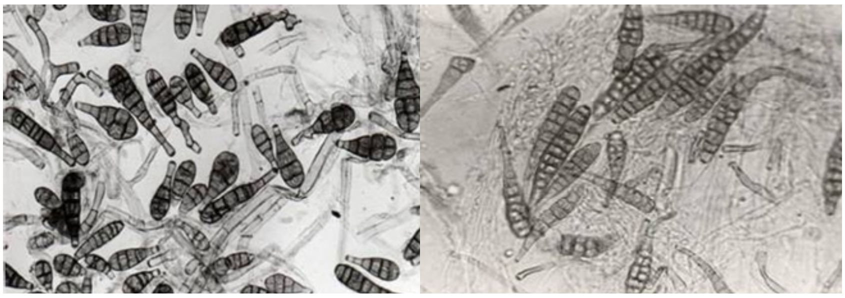

Fig. 5 Conidia of *Alternaria infectoria*. Reprinted with permission from **Perelló AE, Moreno MV, Sisterna MN** (2008) *Alternaria infectoria* species-group associated with black point in Argentina. *New Disease Reports*. Available online: www.bspp.org.uk/ndr/july2007/2007-30.asp web-site

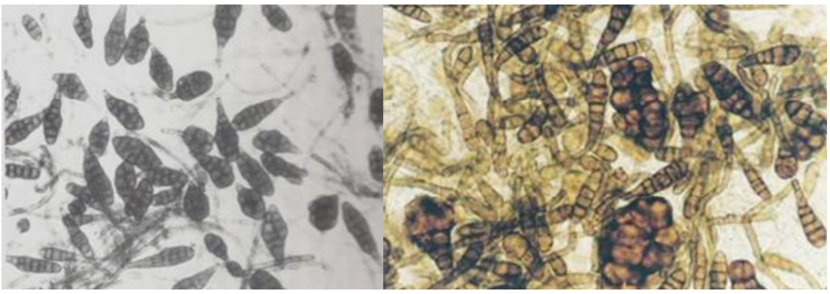

Fig. 6 Conidia of *Alternaria triticina*. Reprinted with permission from **Perelló AE, Moreno MV, Sisterna MN** (2006) Leaf blight of wheat caused by *Alternaria triticina* in Argentina.. *New Disease Reports*. Available online: www.bspp.org.uk/ndr/july2005/2005-35.asp web-site

nels with fumonisins B_1, B_2 and B_3, important mycotoxins. These data indicate that there is a significant potential for fumonisin contamination of wheat in which this fungus is present.

The bacterium *Pseudomonas syringae* pv. *atrofaciens* may cause BP symptoms on wheat. This bacterium also cause a disease called "basal glume rot", in which the glumes become darkened or streaked towards the base (McCulloch 1920). Glume discoloration is often more visible on the inner glume surface and stained brown to black at the embryo end. Kernels are usually badly shrunken. The disease arises when heads emerge and mature accompanied by excessive moisture (Wiese 1987).

Research on BP was initially focused on the assumption that this discoloration was the result of a saprophytic infection. However, evidence of fungal association with BP is often contradictory within the literature (Kaan *et al*. 1995; Williamson 1997; Jacobs and Rabie 1987). Some biochemical results rule out the hypothesis of the occurrence of Maillard browning reaction (Vidal 1974). Williamson (1997) found no direct link between the presence of *Alternaria alternata* and the development of BP symptoms. He also did not recognize a greater density of hyphae of this fungus in discolored tissue of inoculated wheat kernels at the embryo end of the grain. Similarly, studies in barley have also failed to establish any causal fungal association with BP (Jacobs and Rabie 1987). Studies on the cause of BP are now focusing on the biochemistry of the discoloration (Sulman *et al*. 2001; Hadaway 2002). Numerous works on plant cells and tissues suggest that many brown discoloration reactions may be due to phenolic compound oxidation (Kaan *et al*. 1995). Black point symptoms are usually found at the embryo end of the grain and it has been suggested that this is because enzymes such as peroxidases, which are known to catalyse the formation of coloured phenolic products, are located in this region (Williamson 1997). A single layer of cells, the germ aleurone, which is the only living tissue in mature grains outside the embryo, is responsible for the production of peroxidases. In barley, it has been observed that the germ aleurone peroxidases brings about the oxidation of phenols during the process of germination and that they are responsible for the shiny golden-brown material which accumulates in/on the walls of the cells torn when the tissue is ruptured by the germinating embryo (Cochrane 1994a, 1994b). Black point was reproduced *in vitro* when grains were soaked in a phenolic acid solution and then transferred into hydrogen peroxide (Williamson 1997). The requirement for hydrogen peroxide suggests peroxidase enzymes were involved in the discoloration reaction. Peroxidase enzymes are able to catalyse the oxidation of a range of substrates into dark coloured end-products including ferulic and *p*-coumaric acid, provided hydrogen peroxide reduces the substrate first (Rasmusen *et al.* 1997). Ferulic and *p*-coumaric acid are the main phenolic acids found in monocotyledonous plants with ferulic acid constituting more than 90% of total phenolic acid in wheat flour (Sosulski *et al.* 1982). Within cereal endosperm and pericarp (flour, semolina, and bran products), the main phenolic compound is ferulic acid esterified and related to the cell wall, particularly in pericarp tissues (Pussayanawin and Wetzel 1987).

Comparisons between a BP susceptible and tolerant durum wheat variety have revealed that the susceptible variety contained higher levels of ferulic acid and increased activity of phenylalanine ammonia lyase (PAL), an enzyme involved in phenolic acid biosynthesis (Regnier and Macheix 1996). Considerable differences in the level of peroxidase enzyme activity between BP resistant and susceptible wheat varieties grown under similar conditions have been determined by isoelectric focusing (Sulman *et al.* 1999). It remains unclear whether the processes responsible for the production and distribution of hydrogen peroxidase and the phenolic substrates contribute to BP resistance/susceptibility or whether this is entirely controlled by the presence of peroxidase enzyme. A better knowledge of discoloration phenomena may be obtainable through dynamic studies of phenolic compound metabolism during grain development, filling and drying (Kaan *et al.* 1995). McCalum (1989) and McCallum and Walker (1990) demonstrate that phenolic soluble and insoluble compound content are at a maximum at the milk stage in bread wheat. Tabusse (1986) reports similar results in durum wheat. Black pointed seed of different samples was found to be richer in insoluble esterified ferulic acid than in healthy kernels (Kaan *et al.* 1995)

According to March *et al*. (2007) BP is associated with physiological changes within the grain and understanding precisely what environmental conditions induce BP will be of significant benefit in further understanding the molecular mechanism of the browning. The authors suggest that future research will concentrate on the location of the identified proteins within BPed and healthy grain using immunolocalisation. This knowledge will aid in understanding what is happening within the grain in relation with BP development.

Predisposing factors

Environmental conditions have been confirmed as triggers for BP formation in cereals and have a major impact on its incidence (Wang *et al*. 2002). High humidity and rainfall during grain development have been linked to increased incidence of BP in barley (Petr and Capouchova 2001). In wheat, rainfall during the milk to dough grain development stage is most critical for the development of BP (Conner 1989; Moschini *et al*. 2006). Also, its incidence increases with increasing duration of the dew period (Southwell *et al*. 1980a).

Fungal infection levels have been related to the **cultivar characteristics** (Gooding *et al.* 1993; Fernández *et al*. 1994), although it can be very influenced by environmental conditions (Ellis *et al.* 1996). Also, the differences among cultivars can be greater than differences between contrasting seasons and management practices (Gooding *et al.* 1993). According to Conner and Davidson (1988) the differences in BP incidence in susceptible cultivars at different locations may have depended on whether or not conditions were favourable for disease development at certain stages of plant development. These authors found that no cultivar was completely free of disease. The different susceptibility among cultivars has been associated with differences in grain size and weight (Waldron 1934; Brentzel 1944; Lorenz 1986; Sisterna and Sarandón 2005; Toklu *et al.* 2008) but Ellis *et al.* (1996) suggested that there is not a strong causal link between cultivar grain size and BP severity.

There appears to be little consensus on **the stage of plant development** at which BP infection occurs. The factors responsible for this stage effect have not been determined (Conner *et al*. 1990). Machacek and Greaney (1938) speculated that infection in the field occurs either at anthesis or during the late stages of kernel development. Adlaka and Joshi (1974) reported high rates of infection at anthesis and low rates of infection prior to anthesis and at the dough stage. Southwell *et al*. (1988a) found that susceptibility gradually increased from anthesis to the late milk stage. According to Conner and Thomas (1985), inoculations at anthesis or the mid-dough stage produced the most BP.

Black point incidence variations have also been attributed to **cultural practices** such as nitrogen fertilization (Conner *et al*. 1992) and changes in tillage systems (Sisterna and Sarandón 1996). Nitrogen application can cause an increase (Conner *et al.* 1992; Gooding *et al*. 1993) or a decrease (Melegari *et al*. 1998; Sisterna and Sarandón 2005) in this disease incidence, suggesting that the fertilization effects would also depend on the interaction with other factors. Also, tillage methods change soil biological activity affecting nutrient release, especially nitrogen availability, and crop susceptibility to pathogens could be modified (Pearson *et al*. 1991). Sisterna and Sarandón (1996) reported differences in the percentage of contaminated grains under different tillage methods (no-till *vs*. conventional tillage) after artificial inoculations with *B. sorokiniana.* Irrigation also increases the incidence of BP (Kilpatrick 1968), making it a frequent problem in soft white spring wheat, which is grown exclusively under irrigation to maximize yield and keep protein levels low. Conner (1987) found that surveys of soft white spring wheat fields showed that high incidences of BP occurred in fields even in relatively dry summers and that timing of irrigation has a critical role in the epidemiology of BP in those wheats. Conner and Kuzyk (1988a) found no consistent relationship between disease incidence and type of irrigation system, while Maloy and Specht (1988) registered a significant difference in the amount of BP between different types of irrigation.

Control

The effectiveness in controlling BP diseases is limited by the multiplicity of its causes. The development of resistant cultivars is generally considered the most practical way to control BP, but no variety is fully resistant (Conner and Thomas 1985; Conner and Davidson 1988). The incidence of discolored kernels was significantly lower in resistant cultivars than in susceptible cultivars. Peterson (1965) speculated that breeding for BP resistance may be difficult because resistance must be effective at least against two fungi. Now, with the new understandings on the etiology of this phytopathological problem that include abiotic stress conditions, it is even more difficult to achieve efficient control of so many causes involved.

Fungicide application is another alternative but few studies have been done on the impact of fungicide application on the incidence of BP and results differed. Application of chemicals such as sulphur dust has been effective in controlling the disease, but it is not economically feasible in most wheat growing areas (Machacek and Greaney 1938; Parashar 1970). Conner and Kuzyk (1988b) were unable to obtain reliable control of blackpoint on soft white spring wheat from the foliar application of propiconazole, triadimefon, fenpropimorph, oxycarboxin, chlorothalonil or mancozeb, nor for seed treatment with triadimenol. In other studies, prochloraz applied at flag leaf and ear emergence increased BP severity, while fenpropimorph applied at the same timings increased and decreased severity depending on the previous nitrogen fertilizer application rate (Gooding *et al*. 1993). Mellado *et al*. (1990) applied fungicides to the seed and the foliage of a spring wheat but without success. Ellis *et al.* (1996) indicated that fungicide applications leading to large increase in kernel mass could increase BP infection. It is widely reported that fungicide applications increase kernel mass (Morris *et al*. 1989; Entz *et al*. 1990; McCabe and Gallagher 1993; Gooding *et al*. 1994). At two different locations, Wang *et al*. (2002) carried out studies applying tebuconazole and chlorothalonil at different growth stages from stem elongation to head emergence, on three spring common wheat and three durum wheat. They found that fungicide applications from stem elongation to flag emergence could increase BP infection and it was, in many cases, associated with an increase in kernel mass. Fungicide applications at or after head emergence could reduce the incidence of BP, although this was not consistent.

The need of minimizing the release of pesticides to the environment in a sustainable agricultural context requires a better understanding of genotype × environment interactions of this disease in order to design adequate management strategies. As no durable resistance to the disease currently exists, the control of BP relies on an integrated combination of cultural management (Conner *et al.* 1992; Gooding *et al.* 1993), fungicides (Conner and Kuzyk 1988b; Ellis *et al.* 1996) and the use of partially resistant or tolerant cultivars (Conner and Davidson 1988; Sisterna and Sarandón 2000). A complementary strategy within the integrated management is the possibility of biological control. Several biological antagonists of *Bipolaris sorokiniana* and *Alternaria alternata*, isolated from leaves (Fokkema *et al*. 1975; Hodges *et al.* 1994) and soil (Turham 1993) have been identified. In this sense there are some records on the potential biocontrol of *Trichoderma* isolates on *B. sorokiniana* (Biles and Hill 1988). The role of mycoflora as a control factor in BP development, warrants further consideration, as it may be possible to reduce disease incidence by manipulating populations of beneficial saprophytes on the head of wheat.

EXPERIMENTAL RESULTS IN ARGENTINA

Over the past several years, there has been an increase in the wheat area cultivated under conservation tillage systems in Argentina and, consequently, an increase in the use of nitrogen fertilizers. Though grain discoloration has been reported for many years, the natural incidence of this disease has increased, probably due to an interaction effect between nitrogen fertilization and tillage systems. In spite of this, few studies have been carried out on this pathology.

In Argentina, the experiences conducted comprise several aspects:

1) Incidence and etiology

Marchionatto (1934) mentioned this discoloration for the first time as "mancha del escudete". It has been frequently observed since 1927, with a great epiphytic in 1929 in "San Martín" variety. Associated with this disease, the researcher isolated an *Alternaria* species (similar to *A. peglionii*, cited by Curzi in 1926, for the "puntatura"). He referred also to investigations (Christensen 1922; Henry 1923) where the fungus *Helminthosporium sativum* (= *B. sorokiniana*) was isolated from wheat grains from Argentina.

Studies have been performed on rye grain with different discoloration degree, called "tostado" since 1961/62, by Carranza (1972). Similar symptoms on wheat and barley were also observed. Several fungi were isolated and inoculated to confirm pathogenicity. Carranza and Goñi (1974) referred to the 1972/73 crop in the SE of Buenos Aires Province, main durum wheat cropping area in Argentina, where they recorded this discoloration in some punished samples of exported wheat. They could isolate different fungi genera associated with BP: *Alternaria*, *Cephalosporium*, *Cladosporium*, *Fusarium*, *Helminthosporium*, *Stemphylium*. Also they remarked the lack of concern of phytopathologists and breeders about this disease that frequently affected small grains crops. Carranza and Luttrell (1967) reported an undescribed species of *Exosporium*: *Exosporium pampeanum*, that was isolated from grains of rye affected with kernel smudge. Because it was found in the "pampeana" region of Argentina, it is given the specific epithet *pampeanum.*

In a more extensive survey that tested seeds of 15 crops, Winter *et al*. (1974), found several fungi, *A. alternata*, *Cladosporium cladosporioides*, *Epicoccum purpurascens* and *B. sorokiniana*, associated with discolored wheat grains:

Since Marchionatto's (1934) first report on BP in the 1920s followed by studies by Carranza (1967, 1972, 1974), few have been the attempts to deepen on the etiology and other aspects of this pathology. In the 1990s, the importance of this disease prompted the study of its incidence and etiology associated with wheat seed discoloration.

Sisterna and Sarandón (2000, 2005) and Melegari *et al.* (1998) agreed with the results that the main fungi isolated from discolored grains were *A. alternata* and *B. sorokiniana.* These fungi had the highest values in BP natural incidence, on both bread and durum wheat. Although *B. sorokiniana* was observed at lower levels than *A. alternata*, it caused a greater effect on seed germination (Sisterna and Sarandón 2000) (**Fig. 7**). The authors also registered other organisms as: *Bipolaris spicifera*, *Curvularia lunata*, *Drechslera siccans*, *Fusarium graminearum*, *F. oxysporum*, *F. equiseti*, *F. moniliforme*, *F. acuminatum* and *F. poae* but at levels lower than 1.5% (Sisterna and Sarandón 2000). Disease incidence differed for years and locations for durum wheat. In 1996 the average incidence was higher (17.5–47%) than in 1997 (0–1.5%) (Sisterna and Sarandón 2000). For bread wheat under different tillage systems, Melegari *et al*. (1998) found a higher disease incidence in conventional tillage (14%) than in no-till (5%), while Sisterna and Sarandón (2005) did not observe statistically significant differences between tillage systems. The authors also found that lower levels of disease incidence were observed in fertilized plots as compared with no fertilized ones, independently of timing application.

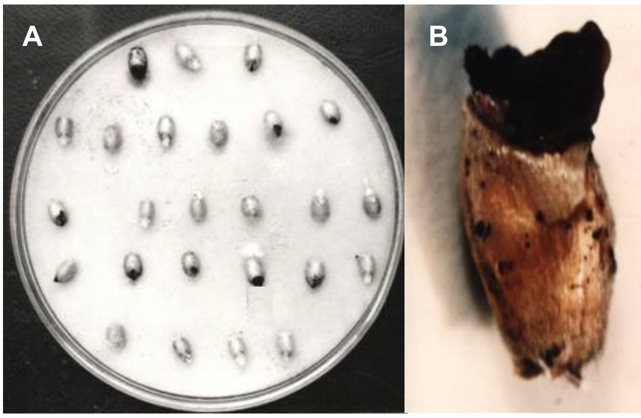

Fig. 7 *B. sorokiniana* effect on wheat seed germination. (**A**) Blotter test showing inhibition of germination. (**B**) Necrosis of embryo with black mass of conidia. Reprinted with kind permission from Eds. A. Arya and C. Mónaco. Natural plant extracts: an alternative control of seed borne fungi, Sisterna and Dal Bello. In: Seed Borne Diseases: Ecofriendly Management, Scientific Publisher, ©2007.

In Argentina, previous records of *Alternaria* spp. refer to *A. alternata* associated with BP in wheat (Marchionatto 1934; Winter *et al*. 1974; Sisterna and Sarandón 2000, 2005). Other Alternarias such as *triticina* were isolated. Although they have been recently detected in Argentina on wheat leaves and seeds (Perelló and Sisterna 2006), they have probably existed as a minor pathogen for many years without being noticed. However, in the last years the vast majority of *Alternaria* strains conformed to the *A. infectoria* complex. The *Alternaria infectoria*-group includes different species (*A. infectoria*, *A. oregonensis*, *A. triticimaculans*, *A. metachromatica* and *A. conjuncta*) and several taxa have not yet been determined (Simmons 1986, 1994). In Argentina, *A. triticimaculans* is the only species of the complex identified (Perelló *et al*. 1996). The incidence levels of this group are gaining importance and have increased probably due to changes in cropping systems in most of the different agroclimatic zones of Argentina (Perelló *et al*. 2005, 2008). This highlights the necessity to better understand the relationship of this group with the deterioration of wheat subproducts and the risk of harmful mycotoxins production.

Another mycobiota accompanies the main pathogens always present in discolored wheat grain. In Argentina, little information is available regarding these weak pathogenic fungi. A few genera were recorded by some researchers. Marchionatto (1948) mentioned *Cladosporium* and Winter *et al.* (1974) found *Cladosporium cladosporioides* and *Epicoccum purpurascens* associated with discolored grains of wheat and barley, reducing the commercial value of seeds for consumption. González *et al*. (1999, 2008) recorded *Cladosporium cladosporioides*, *E. nigrum*, *Nigrospora oryzae*, *Phoma* spp. and *Ulocladium* spp. on seed samples of wheat. In this sense another contribution was carried out by Sisterna and Lori (2005).

2) Influences of nitrogen fertilization, tillage systems, cultivars and locations

Crops susceptibility against diseases has been associated with nutrients availability, especially nitrogen (Huber 1980). This can be modified either by fertilizer application or by other cultural practices such as different tillage methods. It is known that tillage methods change soil biological activity, affecting nutrient release, especially N availability. Though N mineralization in both conventional and no-till systems could be enough to satisfy crop requirements during all crop cycle, the rate of N release is usually faster under conventional tillage than under no-till. In this case crop susceptibility to pathogens could be modified.

Sisterna and Sarandón (1993, 1996) carried out studies on the behavior of bread wheat cultivars artificially inoculated with *B. sorokiniana* under no-till and conventional tillage and nitrogen fertilization treatments (control and N 90 kg N/ha). It was found that tillage systems have an important effect on grain contamination. On the other hand, no effect of nitrogen fertilization was observed related to germinated, contaminated and the proportion of non-germinated or contaminated grains.

Regarding natural infections of BP disease, Sisterna and Sarandón (2005) conducted a field trial with two contrasting bread wheat cultivars growing under three different tillage methods and three nitrogen fertilization treatments. They found that BP natural incidence levels were mainly influenced by cultivars and nitrogen fertilizer application but not by tillage systems. However, in previous reports, Melegari *et al*. (1998) registered an effect of tillage practices on a colder cropping area. This contradictory result suggests that other environmental factors in addition to tillage systems could be involved in the disease expression.

3) Biological control

In Argentina Dal Bello *et al.* (1994) evaluated the suppression of wheat seedling blight caused by *B. sorokiniana* using some *Trichoderma* isolates. No previous records of antagonism between *Trichoderma* spp. and the more frequently pathogens associated to BP were found.

In a preliminary study, Mónaco *et al*. (2004) reported selected strains of *Trichoderma harzianum* and *T. koningii* that were assessed to act as biocontrol agents against *Bipolaris sorokiniana* and *Alternaria alternata*, major members of the complex of BP in Argentina. Both "*in vitro*" and field assays were carried out to determine the antagonism of the *Trichoderma* spp. against the pathogens. For field artificial infection experiments, one bread and one durum wheat varieties were tested. There were significant differences in the percentage inhibition of the mycelia growth of both pathogens among some *Trichoderma* isolates tested. The results of these tests conducted "*in vitro*" suggest that the competition could be the mode of action of the *Trichoderma* strains tested. They quickly colonized Petri dishes, overgrowing the pathogen colonies, with the ability to exclude them. Microscopical observation of the dual cultures revealed morphological effects of the antagonists on the pathogens, such as vacuolization of hyphae, plasmolysis of mycelium and mycelium showing torulose aspect. In the field assay no antagonistic effect on *A. alternata* and *B. sorokiniana* incidence was evidenced for any of the *Trichoderma* spp. strains tested either on bread or durum wheat. In most of the combinations the values registered were higher than in the control. Differences between "*in vitro*" and field results suggest that the efficiency of antagonists could be modified by environmental conditions. On the other hand, disease expression could be explained by other factors that caused several plant stresses, beyond the phytopathological ones.

4) Predictive models

Different approaches have been used to develop forecasting methods. Through the fundamental–empirical approach, the identification of meteorological variables highly associated with the disease could be helpful for analysis of short-range weather forecasts throughout the wheat critical period (Carranza *et al*. 2007).

Due to the assumption that the BP expression has a strong relationship with the environmental factors, Moschini *et al*. (2006) carried out a study to identify the meteorological variables most closely related to disease incidence and to derive prediction models, in southern Buenos Aires province (Argentinean Pampas region). The assays were conducted during 3 growing seasons at 5 locations using 9 durum wheat varieties in 1995 and 1997 and 16 in 1997. Strong links between meteorological variables and wheat BP were found during the period approximated the milk to dough kernel development stage (Zadoks scale: 71–87) (Zadoks *et al*. 1974). The beginning of the critical period occurred approximately 30 days after heading and extended for 14–20 days (depending on location and year). A positive relationship was found between disease incidence and simple moisture variables such as the total amount of precipitation and the precipitation frequency. The empirical models developed in this study included real-world factors that accounted for the influence of environment on disease, and these variables served as the primary predictors for the critical period length. This study helps to clarify and quantify the environmental effects on BP disease, showing the effect of a warm, rainy period during the grain filling stage in durum wheat. These results can be useful to assist farmers in rational, tactical and strategic disease management. Though chemical control of BP is difficult to analyse because of the concurrence of many interactive factors, the authors concluded that reliable chemical control would be more likely if fungicides were sprayed on the wheat heads after anthesis. Available disease forecasting systems would be needed for predicting those levels of BP that economically justify late fungicide applications.

CONCLUDING REMARKS

Despite the potential economic importance of BP, little is known on the implications of many husbandry factors on the severity and prevalence of the symptoms that can cause downgrading of the grain at marketing. Many investigations have linked the appearance of BP to the presence of fungi on the grain such as *Alternaria* spp., *Bipolaris* spp., *Cladosporium* spp., *Curvularia* spp., *Drechslera* spp., *Epicoccum* spp., *Fusarium* spp., etc. However, several studies found no evidence proving a direct causative association between fungal infections and BP, explaining this discoloration from a biochemical point of view. The environment has a major impact on the incidence of BP. Heavy rain, high humidity, and extreme temperature during the grain filling duration, particularly at the milk and soft dough stages, are the main factors predisposing the disease. Little can be done to prevent this problem. Studies on the impact of fungicide application have contradictory results. Besides, the use of chemicals, in general, has generated several problems causing contamination and safety risks for humans and domestic animals. In this sense, sustainable agriculture is adopting increasingly other eco-friendly options, to minimize the risks for producers, consumers and the environment. The significant genotype difference in susceptibility to BP infection indicates that selecting cultivars resistant to kernel discoloration would be an efficient measure to control this disease. There is an urgent need to identify novel sources of BP resistance that can be used in breeding programs.

In the past decade, there was an increase in the incidence of grain discoloration diseases probably due to changes in the cropping systems. Argentine is not an exception. This pathology has gained in importance and, though some efforts were done to study several aspects related to the disease, further research is needed to confirm these results and to understand the molecular mechanism of the browning.

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