Chapter 2 : Bacterial Leaf Streak and Black Chaff Caused by *Xanthomonas translucens*

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Bacterial leaf streak (BLS) of cereals, also known as bacterial leaf stripe, is caused by *Xanthomonas translucens* (ex Jones, Johnson and Reddy 1917) Vauterin, Hoste, Kersters and Swings 1995. The disease, called black chaff when on the glumes, is seed-borne and a constraint for international germplasm exchange. Black chaff has been reported since the end of last century; however, some reports are misleading because disease symptoms on the ear are often confused with the abiotic stress known as pseudo-black chaff or brown melanosis (Broadfoot and Robertson 1933; Hagborg 1936).

The pathogen was first identified on barley (*Hordeum vulgare* L.) (Jones *et al.* 1917), and later on wheat (*Triticum aestivum* L.) (Smith *et al.* 1919), rye (*Secale cereale* L.) (Reddy *et al.* 1924), grasses (Wallin 1946a) and finally on triticale (X *Triticosecale* Wittmack) (Zillinsky and Borlaug 1971). Different names have been proposed, depending on the host plant, but the name *X. campestris* pv. *translucens*, first assigned to the barley pathogen originally described by Jones *et al.* (1917), has been used for any cereal streak pathogen even when the host range was undetermined (Bradbury 1986). As a result, data on host specialization, on the association (or non-association) of a pathogenic bacterium with black glumes (particularly in early reports), and on the importance of the disease have been confusing. BLS is usually considered to be widespread but unimportant (Lelliott 1987). However, accounts of this disease, particularly on wheat in the 1980s, have become more frequent and have aroused much concern (Duveiller 1989).

Names currently included in the International Society of Plant Pathology's (ISPP) list of plant pathogenic bacteria (Young *et al.* 1996) are used in this manual. Hence, the name *X. t.* pv. *undulosa* is used here to refer to the pathogen that causes BLS on wheat, except when the authors themselves have used another name. Since pathovar names are not used consistently by all authors, in this manual we use the designation "*X. translucens*" in a broad sense when it is not known which pathovar is involved or to refer to the group of pathovars that cause BLS on cereals and grasses.

Distribution

Most *Xanthomonas* strains causing BLS symptoms have been isolated from samples collected in experiment stations and not in commercial fields, where they are less commonly found. This may be because scientists who are familiar with bacterial problems make more intense observations in experiment stations. Whatever the reason, BLS is found mainly in breeding stations, where the problem has been ignored for years (Schaad 1987a).

Bacterial leaf streak occurs over a range of very different conditions such as sprinkler irrigated fields in temperate climates, high rainfall subtropical highlands, and warmer environments characterized by cool nights or frequent climatic changes and sudden temperature variations. Although reports are generally sporadic, they come mostly from warmer cereal-growing areas.

In North America, BLS has been reported in several states of the USA (Heald 1906; Jones *et al.* 1916; Smith 1917; Johnston 1929; Melchers 1930; Milus and Kirkpatrick 1990; Murray and Malloy 1990). Outbreaks have not been observed in Canada recently (Hagborg 1934; 1968; 1974). In Mexico, the disease was observed in the northeast in 1931 (Bamberg 1936) and is most severe today in areas of high elevation such as the temperate and humid central highlands of Toluca (2,650 m above sea level) (Duveiller 1989). It is often found in experiment stations due to the broad range of genotypes sown, some of which are quite susceptible. In South America, wheat is the most affected crop, although BLS is also found on other small grains and grasses (Mehta 1990; Duveiller *et al.* 1991). The disease occurs in Argentina, Bolivia, parts of Brazil, Paraguay, Peru and Uruguay (Mehta 1990; Duveiller,

1989; Pereira *et al.* 1983; Luzzardi *et al.* 1983; Mohan and Mehta 1985; Duveiller *et al.* 1991; Frommel 1986; Tessi 1949; Abadie 1988).

In Asia, the disease is known on wheat in China (Chen and Ding 1981; Sun and He 1986), Pakistan (Akhtar and Aslam 1985 and 1986) and Iran (Alizadeh and Rahimian 1989; Alizadeh *et al.* 1995), and on triticale in India (Richardson and Waller 1974). In the Near and Middle East, it affects durum (*Triticum turgidum* var. *durum* L.) and bread wheat in irrigated areas of Syria (Mamluk *et al.* 1990), Israel (CIMMYT 1977), Turkey (Sands and Fourest 1989; Demir and Üstün 1992), and Yemen (Maraite and Ferauge, personal communication). Gorlenko (1960) noted the occurrence of *X. t.* pv. *translucens* and *X. t.* pv. *secalis* in the Omsk region (Russia) and BLS was also reported in Krasnoyarsk (Dobretsov 1963) and the Novosibirsk area (Bushkova 1966). It has also been found in Malaysia (Sabah, as cited in Bradbury 1986). In Japan, *X. t.* pv. *translucens* and *X. t.* pv. *cerealis* have been found on several gramineae but not on wheat (Tominaga 1967; Miyajima 1980 and 1982; Miyajima and Tsuboki 1980).

In Europe, the first reports of black chaff came from France, Belgium and Russia (Millasseau 1928; Hocquette 1929; Marchal 1930 and 1932, Gorlenko *et al.* 1939). However, Marchal (1948) later denied that black chaff was present in Belgium. The disease currently seems to be absent from western Europe (Paul and Smith 1989), probably due to unfavorable environmental conditions, particularly low temperatures. Nonetheless, sporadic outbreaks on barley in Spain (Lopez *et al.* 1987; Noval 1989) and on triticale (Wolski, personal communication) and wheat (Arseniuk, personal communication) in Poland indicate that the risk in western Europe should not be underestimated.

In Africa, BLS has been found in Kenya (Burton 1930), Ethiopia (Korobko *et al.* 1985), South Africa (Vervoerd 1930; Smit and Van A. Bredenkamp 1988), Tanzania (Bradbury 1986), Libya and Madagascar (Bragard *et al.* 1995), and Morocco (Sands and Fourest 1989).

In Australia (Moffett 1983), BLS has been recorded on wheat and rye in New South Wales (Noble *et al.* 1934; Noble 1935), and *X. t.* pv. *cerealis* was identified on Japanese millet (*Echinochloa crus-galli* var. *frumentacea* L. Beauv.) (Moffett and McCarthy 1973) in Queensland.

Importance

Little quantitative information is available on losses caused by BLS. Measuring yield losses in commercial fields is not easy because the lack of an effective treatment makes it impossible to grow healthy control plots. Moreover, when favorable conditions are present, the disease may develop very fast within a region, making it difficult to observe different infection levels in a range of plots, particularly if few genotypes are planted.

Yield losses as high as 40% have occurred in the most severely diseased fields in Idaho, although losses are generally 10% or less (Forster 1982; Forster *et al.* 1986). In severe cases, 5-10% of the wheat spikes may be sterile due to infection (Forster and Schaad 1988), and the disease may attack a complete nursery so severely that nothing can be harvested (Burton 1931).

Using a modified single tiller approach over three seasons in the USA, Shane *et al.* (1987) calculated that 50% disease severity on the flag leaf resulted in an 8-13% loss in kernel weight and that 100% disease severity on the flag leaf resulted in a 13-34% loss. In Mexico, yield loss in wheat was evaluated in a high rainfall, temperate environment based on infection and yield in single tillers. Data indicated that, on average, losses below 5% can be expected when the percent infected flag leaf area is under 10%. However, up to 20% yield reduction can be anticipated, on average, if 50% of the flag leaf is diseased (Duveiller and Maraite 1993a).

Yield loss is a linear function of the percent infected flag leaf area, and even a small infected leaf area has an effect on yield (**Figure 2.1**). Although the disease is usually observed late in the growing season, the negative effect of the pathogen on yield can be determined as soon as lesions develop on the flag leaf because even a small percentage of diseased leaf area (DLA) has an immediate effect. Since similar effects on yield can be observed when leaves are detached from plants, the effect of BLS on yield is probably related to a reduction in photosynthesis resulting from the extent of DLA (Duveiller and Maraite 1993a).

Based on experimental observations in Mexico's high rainfall, temperate highlands, a formula has been proposed to calculate the expected yield loss based on disease severity at Zadoks' growth stage 73-83 and field incidence. Considering the different percentages of DLA occurring in a field, the expected

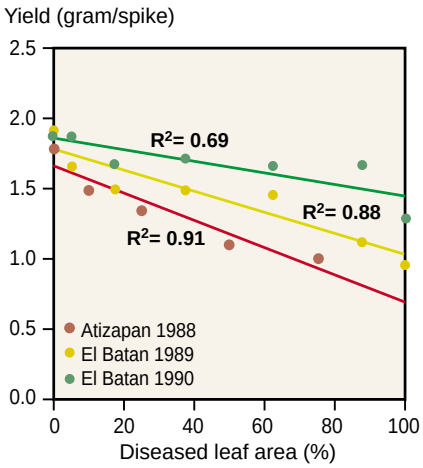


Figure 2.1. Relationship between yield and bacterial leaf streak severity on the flag leaf, at early milk-dough, in genotype Alondra, three years' observation data.

percent yield loss due to *X. t.* pv. *undulosa* in a commercial plot can be calculated as:

$$\text{Yield loss (\%)} = C \frac{\sum_{i}^{n} \text{\% DLA}}{n}$$

where n = number of flag leaves sampled and C = 0.397 on average (Duveiller and Maraite 1993a). A random sample of fertile tillers should be used when applying the formula. For plots of up to 0.004 ha, a sample of 10 primary fertile tillers is suggested. For larger plots and fields, up to 50-80 tillers should be selected at random while walking diagonally across the plot, but sample size is determined depending on disease variability and the desired accuracy (James 1971; Kennedy 1990).

BLS mainly affects grain filling, but grain number was significantly correlated with BLS severity levels two out of three years under Mexican conditions (Duveiller and Maraite 1993a). This confirms a previous report by Waldron (1929) but not results obtained by Shane *et al.* (1987).

In triticale, both yield and test weight may be reduced (Zillinsky and Borlaug 1971). When the average of infected genotypes was compared to the mid-variety in the test, yield losses ranged from 12 to 43% and test weight was reduced from 2 to 13% (Fuentes 1974).

Symptoms

Leaf streak and black chaff

Typical symptoms on the leaf consist of elongated, light brown lesions, several centimeters long, which are initially distinct but later coalesce to cover larger solid areas. Early symptoms are characterized by translucent stripes that are easily seen under incident light. Initially lesions are water-soaked and produce honey-like exudates under humid conditions (Smith 1917; El Banoby and Rudolph 1989; Duveiller and Maraite 1993b). If undisturbed, the exudates harden into yellowish, resinous granules studding the surface of the lesions and are easily detachable (**Figure 2.2 a and b**). Frequently, these droplets coalesce when there is dew, rain or guttation water to form conspicuous milky drops that may later spread over the leaf surface and dry down as thin, grayish, almost transparent flakes (Jones *et al.* 1917) (**Figure 2.3 a and b**). In commercial wheat fields, particularly without sprinkler irrigation, lesions with no exudate can be observed (**Figure 2.4**); this makes it difficult to identify the cause of the symptoms without isolating the pathogen.

Dickson (1956) reported that many leaf lesions start at the apex and extend downward; the

Figure 2.2 a. Bacterial streak of wheat: translucent stripes and exudates. b. Wheat peduncle with resinous granules resulting from severe bacterial leaf streak infection.

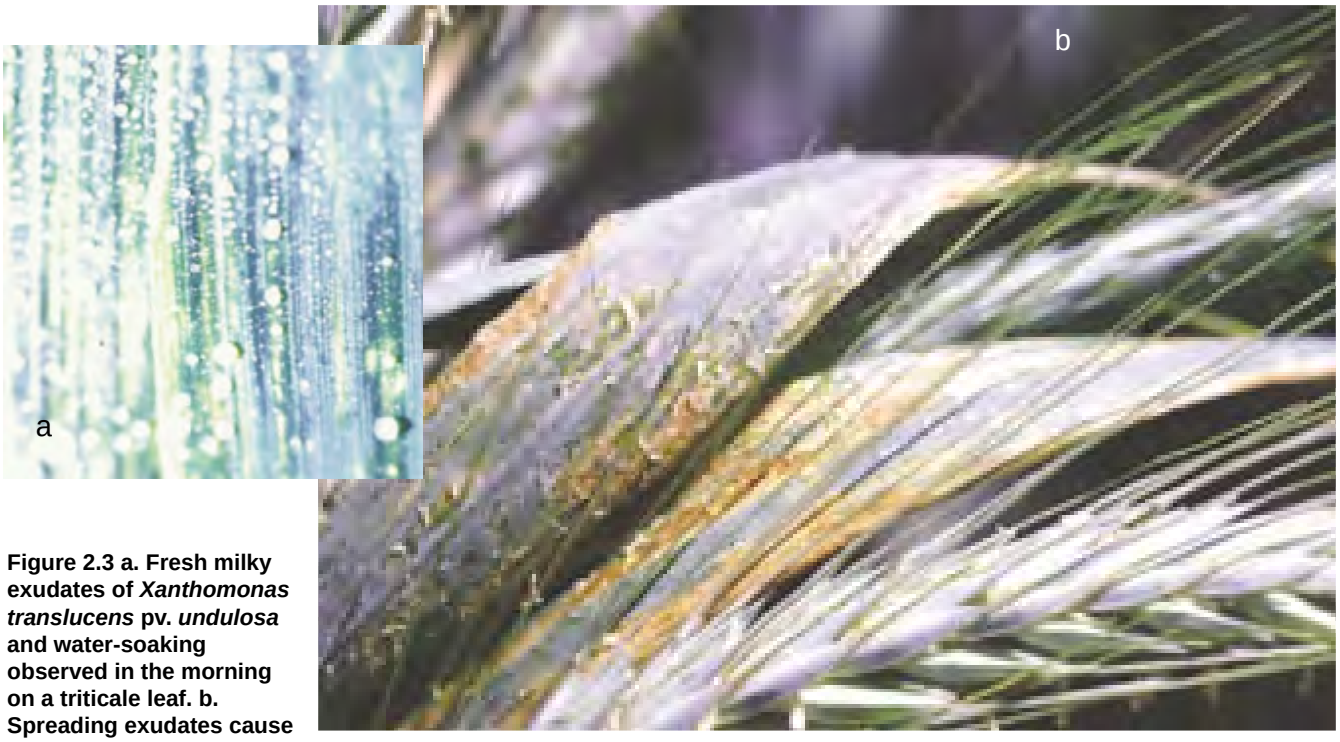


Figure 2.3 a. Fresh milky exudates of *Xanthomonas translucens* pv. *undulosa* and water-soaking observed in the morning on a triticale leaf. b. Spreading exudates cause flakes to form on triticale leaves with bacterial leaf streak lesions.

Figure 2.5. Translucent lesion in the middle of a durum wheat leaf where dew remains longer in the morning.

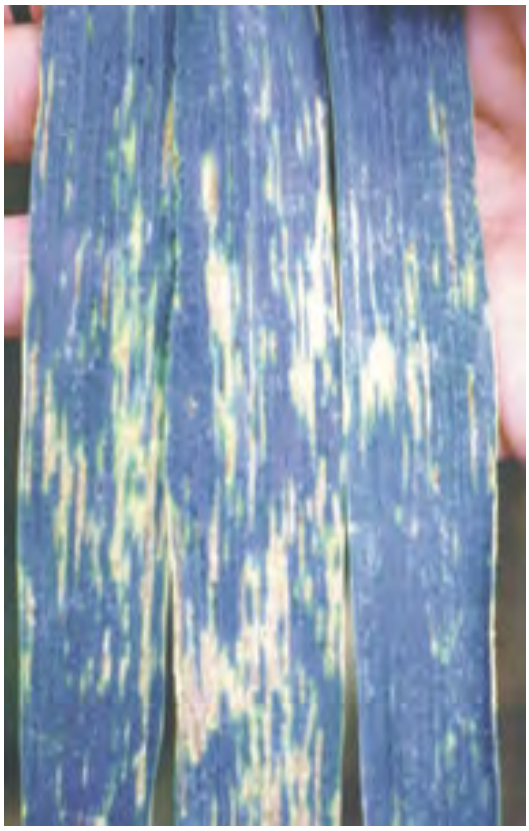


Figure 2.4. Inconspicuous blotches caused by *Xanthomonas translucens* pv. *undulosa* on wheat leaves.

assessment scale proposed by James (1971) suggests similar disease development. This pattern, however, is not typically observed under subtropical field conditions (Duveiller 1994a). Symptoms often develop in the middle of the leaf, where dew remains longer in the morning (**Figure 2.5).** Streaks are more usual on triticale than on wheat.

Culms, leaves, rachis, glumes, and awns may become infected, and symptoms on wheat have been reported to vary with the environment, variety, disease severity, and interaction with fungi (Bamberg 1936; Boosalis 1952). However, extensive experience with different genotypes in Mexico indicates that variations of BLS symptoms on wheat leaves are limited (Duveiller 1994a). Most so-called variations are probably due to melanic reactions on ears and nodes under abiotic stress being mistaken for disease reactions.

Although Forster *et al.* (1986) observed early infection in the field, symptoms often go undetected in seedlings. Bamberg (1936) could not find seedling infection on thousands of plants, and there was no evidence of infection in the field before booting. Similar evidence was obtained in the field and in the greenhouse under Mexican conditions (Duveiller 1994b).

How to distinguish black chaff from brown melanosis

When on the glumes, BLS is characterized by black, longitudinal, more or less parallel stripes that are more numerous and conspicuous on the upper parts (Smith 1917). BLS can be recognized by a greasy appearance

Figure 2.6. Wheat spike showing typical black chaff symptoms: discoloration of the peduncle and alternating bands of healthy and diseased tissue on the awns.

or alternating bands of diseased and healthy areas on the awns (**Figure 2.6**). Purple-black symptoms may extend to the peduncle between the inflorescence and the flag leaf, and may sometimes present a yellow center (Forster *et al.* 1986). On triticale, the bacterium causes moist gray to whitish lesions on the glumes, and discoloration is rarely seen on the peduncle (Duveiller 1989) (**Figure 2.7**).

Several authors have found that susceptibility to melanism on the spike, also referred to as black chaff, is often inherited from stem rust-resistant parents (Goulden and Neatby 1929; Waldron 1929). Bamberg (1936) emphasized the risk of black chaff in progeny of crosses involving the hard red spring wheats H-44 and Hope. Johnson and Hagborg (1944) showed that high temperature conditions, especially when combined with high humidity, favored the development of melanic areas on the glumes, lemmas, peduncles and internodes of rust resistant varieties. As brown

Figure 2.7. Triticale spike infected by *Xanthomonas translucens* pv. *undulosa* showing moist gray lesions on the glumes.

melanosis is known to be associated with the *Sr2* gene for stem rust resistance (McIntosh 1988), it is possible that what early reports of black chaff were really describing was pseudo-black chaff not caused by bacteria.

Sharp discolored interveinal streaks on the glumes suggest the presence of *Xanthomonas*, particularly if also irregularly distributed on the spike, and if there is abundant BLS on the leaves. In contrast, melanosis on the peduncle, which occurs on the same side of most culms in a field as a result of exposure to sunshine (UV light), is indicative of brown melanosis.

Besides this physiological disorder, other very similar symptoms on the spike that are not caused by *Xanthomonas* may be induced by *Bipolaris sorokiniana* (Sacc.) Shoem., *Alternaria* spp., *Stagonospora nodorum* (Berk.) Castellani and E.G. Germano (syn. *Septoria nodorum* (Berk.) Berk. in Berk. and Broome), and *Pseudomonas syringae* pv. *atrofaciens* (McCulloch) Young, Dye and Wilkie 1978.

Epidemiology and Biology

Survival

Seed. Seed is the most important source of primary inoculum, and large-scale transmission of BLS is due to its seed-borne nature (Jones *et al.* 1916, 1917; Smith *et al.* 1919; Tsilosani *et al.* 1969). Depending on storage conditions, it is estimated that the bacterium will die in 63-81 months (Forster and Schaad 1987, 1990). However, in two seed lots infested with 1.3×10^7 and 8.7×10^5 bacteria per gram, respectively, Klykov (1945) (as reported in Neergaard 1977) showed that recovery of the bacterium was reduced by 93% and 79% after only six months of storage, and that more than 99.5% of the bacteria was not detectable after three years.

Black chaff of wheat has a very low transmission rate, i.e., low levels of seed contamination will not result in field disease (Schaad 1988b). In Idaho, more than 60% of all spring wheat seed lots were found to be contaminated, and seed lots with less than 1000 colony forming units (cfu) per gram do not cause field epidemics. This suggests that methods for detecting the pathogen on the seed do not have to be very sensitive (Schaad 1987a; Forster and Schaad 1987). However, the situation may vary from one environment to another, and the pathogen's multiplication capacity should not be underestimated. In the wheat genotype Anahuac, the number of bacteria per lesion increases from about 10^4 to 10^8 in less than 48 h (Duveiller 1992). In the field, seedlings may present symptoms very early in the season, and secondary lesions can start when plants are 3-4 cm (Jones *et al.* 1917; Forster *et al.* 1986). Natural injuries may facilitate the induction of primary lesions on seedlings. Hagborg (1936) and Gorlenko (1941) obtained 81% and 42% infected seedlings, respectively, from artificially wounded seeds infested with the bacterium.

Testing procedures may recover mainly external bacteria, which may not be important for disease induction. So far, the precise location of the pathogen in the wheat seed is not known, but it seems to be located mostly (88.9%) in the external seed coats (Duveiller 1992). Wallin (1946b) intended to show the path the bacterium follows when moving from the seed surface to the aerial portion of the plant. The plumule is infected through wounds or through the stomata on the coleoptile. The pathogen invades the coleoptile, reaches the enclosed foliage, and infects it before the first leaf emerges from the coleoptile.

Soil. *Xanthomonas t.* pv. *undulosa* that survives in soil and crop debris seems not to be a major cause of primary inoculum. The bacterium survives poorly in soil but does better when crop debris is present (Boosalis 1952). Free bacteria cannot survive more than 14 days in air-dried soil and no more than 57 days when infected triticale leaves are mixed into moistened soil (Cunfer 1988). Also, plant stubble usually decays very fast in warm, humid climates, and wheat pathogenic bacteria cannot survive in decomposing debris.

Survival on grasses and overwintering. *Xanthomonas t.* pv. *undulosa* can survive on weeds and grasses due to its broad host range; however, this is probably not significant on annual hosts. In Uruguay, clear streak symptoms caused by *X. t.* pv. *undulosa* were found on canarygrass (*Phalaris canariensis* L.) used as border rows in experimental wheat plots (**Figure 2.8**). Epiphytic populations (i.e., microorganisms living on the surface of the plants) of the pathogen have been detected in Idaho near spring wheat fields on *Poa pratensis*, *Festuca arundinacea*, *F. rubra*, *Hordeum leporinum* and *Medicago sativa* (Thompson *et al.* 1989).

Wallin (1946a) gathered evidence that "*X. translucens*" can overwinter on perennial hosts such as smooth brome (*Bromus inermis* Leyss.) and timothy (*Phleum pratense* L.), which gives the pathogen the opportunity to spread to nearby cereals. The bacterium also seems to overwinter on winter wheat and rye (Boosalis 1952).

Conditions conducive to epidemics

Humidity and temperature. Bacterial leaf streak outbreaks are characterized by sporadic epidemics and higher incidence in breeder's plots (Kamel 1981; Schaad 1987a); they are usually observed by farmers relatively late in the growing season (Forster *et al.* 1986). These factors make gaining a better understanding of the epidemiology of this disease more difficult.

BLS is thought to occur during the wet season or in sprinkler irrigated fields where humidity is high (Forster *et al.* 1986; Bamberg 1936). However, the relative importance of dew, rainfall or irrigation—by sprinkler or gravity—versus such environmental conditions as temperature is not well documented.

Moisture facilitates the pathogen's release from the seed and contributes to leaf colonization and invasion of leaf tissue. Free water allows the pathogen to spread in the field and to disperse on the leaf, thus increasing the number of lesions. Bacteria enter through the stomata and multiply in large masses in the parenchyma. This causes elongated streaks limited by the veins, which act as barriers. Later milky or yellow exudates form on the surface of lesions. Rain and wind greatly influence the spread of these exudates and of the disease from leaf to leaf throughout the field (**Figure 2.9**). However, brief but intense rainstorms, frequently observed in more tropical regions, can also wash the inoculum down the plant. Micro-injuries to awns and leaves caused by hail or wind may contribute to bacterial penetration of the blades.

The BLS-inducing pathogen tolerates a wide range of temperatures (15-30°C) (Duveiller *et al.* 1991) and grows best when temperatures are above 26°C (Forster *et al.* 1986). Recent studies (Duveiller and Maraite 1995) have shown that temperature has a major impact on epidemics. Pathogen multiplication in leaf tissue is directly dependent on temperature, and dry air conditions (< 30%) do not limit disease progress. Symptoms only occur when temperature allows the bacterial population to reach an estimated threshold of 10^8 cfu/leaf (**Figure 2.10**). Low temperatures retard the multiplication of the pathogen and disease progress.

It is therefore not surprising that BLS prevails in warmer, non-traditional wheat growing areas where rainfall is limited but humid night-time conditions (dew) are

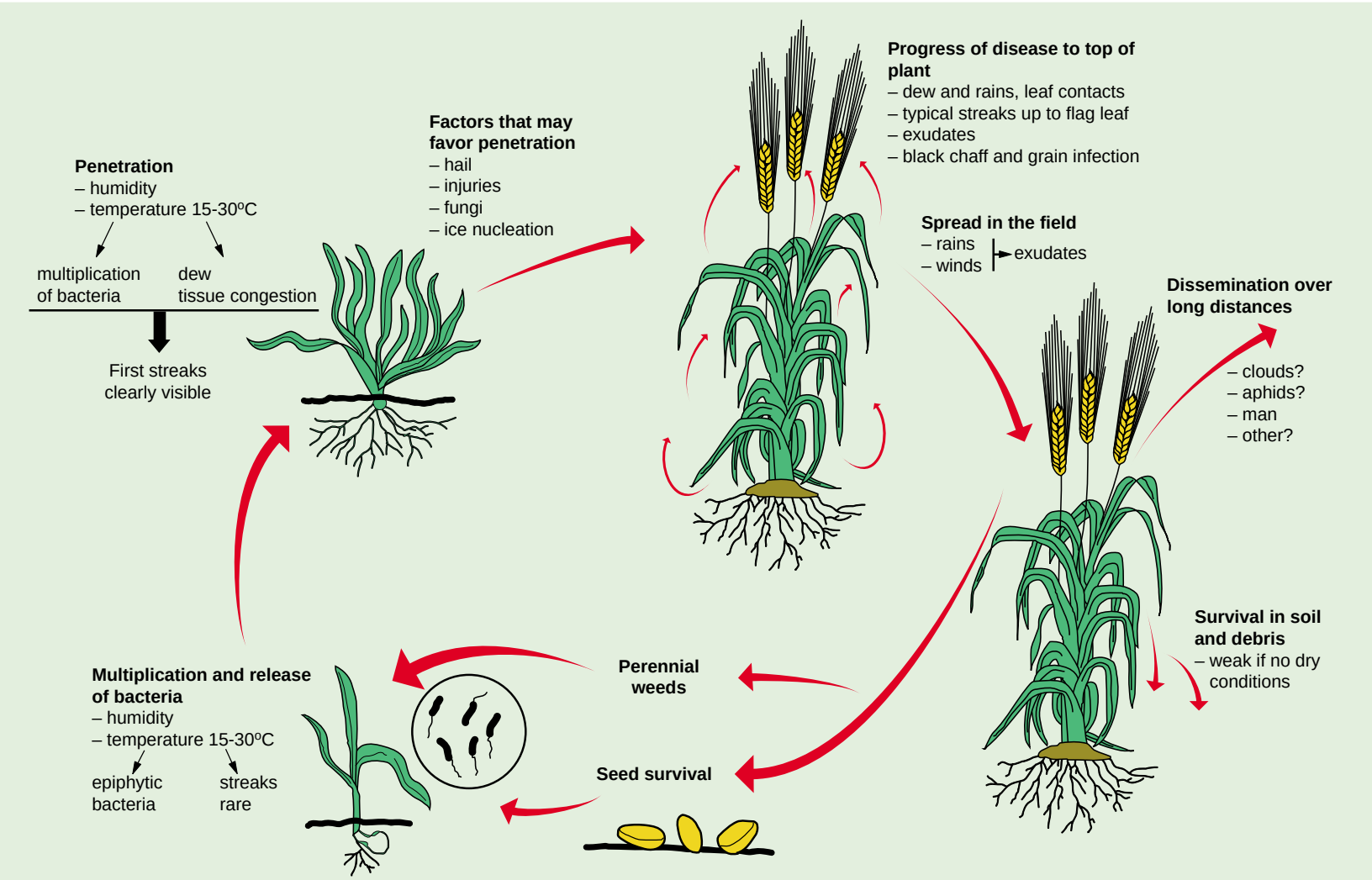
Figure 2.8. Streaks caused by *Xanthomonas translucens* pv. *undulosa* can be found on other Gramineae such as canarygrass.

enough to favor penetration of the parenchyma. Once inside the leaf, the bacterial population can still grow, and leaf moisture does not constrain that growth even under dry air conditions.

Epiphytic populations may be important for understanding the etiology of BLS and discovering why the disease is sporadic. *Xanthomonas t.* pv. *translucens* can multiply and persist on tomato leaves for several weeks, which suggests that survival does not depend on host plant infection and that the bacterium may reside on non-host species (Timmer *et al.* 1987). Forster and Schaad (1988) reported high epiphytic populations on wheat leaves after Zadoks' growth stage DC50, and a detectable level of the pathogen was observed at DC30. In Mexico, it was possible to monitor a *X. t.* pv. *undulosa* population in plots of symptomless genotypes contrasting in their field resistance to the pathogen (Duveiller 1994c). The population of pathogenic bacteria decreased after a heavy rainfall, which suggests that epiphytic *X. t.* pv. *undulosa* are present on wheat leaves before they actually penetrate the parenchyma.

Frost damage and ice nucleation. Bacteria exhibiting the ice-nucleation phenotype have the ability to trigger ice formation at temperatures between 0° and -10°C. Strains of *X. t.* pv. *translucens* express ice nucleation activity at temperatures from -2°C to -8°C (Kim *et al.* 1987; Zhao and Orser 1988). Damage caused to plant tissue by the ice provides conditions suitable for pathogen invasion and multiplication. Frost conditions may thus explain the frequent incidence of BLS in high elevation environments or in regions such as southern Brazil, where wheat is grown during the winter season. Waller (1976) reported that a slight frost precipitated a BLS outbreak in the Toluca Valley of Mexico (2600 meters above sea level) in 1973. However, frost conditions are not common in this area during the summer season when plants present BLS symptoms, and ice nucleation is thus not necessary to induce an epidemic (Duveiller *et al.* 1991a).

Figure 2.9. Disease cycle of *Xanthomonas translucens* pv. *undulosa* and possible ways disease may spread.



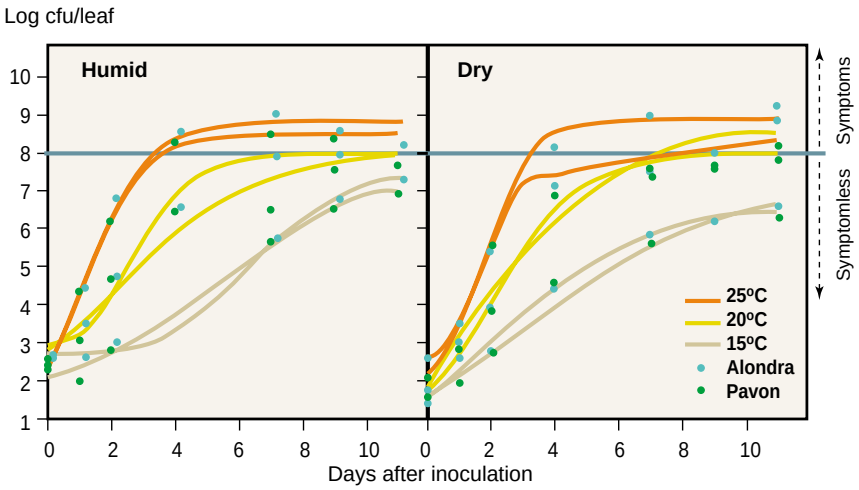


Figure 2.10. Effect of temperature on the multiplication of *Xanthomonas translucens* pv. *undulosa* in wheat genotypes Alondra and Pavon, under humid and dry air conditions; symptoms are visible when the pathogen population reaches 10^8cfu/leaf.

Wheat, barley, maize and bean plants sprayed to runoff with suspensions containing 10^8 cfu/ml of *X. t.* pv. *translucens* suffered greater frost damage than when sprayed with water alone. Bacterial suspensions containing as little as 30 cfu/cm^2 resulted in increased frost injury (Azad and Schaad 1988a).

Interaction with fungal diseases. Wheat with severe root rot infection may be even more infected with "*X. translucens*", which suggests that fungi may have a role in the epidemiology of BLS. Experimental evidence also indicates that root rot and leaf spot fungi, such as *Bipolaris sorokiniana*, predispose wheat to infection by *Xanthomonas* (Boosalis 1952).

Contradictory results have been obtained from studies carried out under greenhouse and field conditions to investigate the interaction between *Septoria nodorum* and *X. t.* pv. *undulosa* (Jones and Roane 1979; Jones *et al.* 1981; Jones and Roane 1981).

It is highly significant that *X. t.* pv. *undulosa* was easily isolated in Mexico from vacuum flasks containing rust uredospores collected in fields where black chaff was present (Duveiller, unpublished). Rust uredospores are used by breeders to infect spreader rows used in selecting for rust resistance. The abundance of sticky exudate and the huge amount of bacteria associated with it facilitate the infestation of rust inoculum. As a result, there is higher incidence of BLS in spreader rows inoculated with rust inoculum if conditions are favorable to the pathogen. This is probably one of the reasons *Xanthomonas* streak problems are observed more frequently in breeding stations.

Spread of the pathogen in the field

Transmission by rain and wind. Pathogen transmission by rain and dew and plant-to-plant contact explains local dissemination (Boosalis 1952). In addition, visitors to demonstration plots, particularly in the morning when dew is at its maximum, increase the spread of bacteria. The disease may spread in the direction of the prevailing wind and driven rain; however, the movement of the disease in space under other conditions proved to be limited. In the USA, in 3-m triticale rows whose borders were inoculated using a grass clipper, diseased plants were found 1 m down the rows but not further down (Cunfer *et al.* 1980). In Brazil, Mehta (1990) indicated that the spread of BLS from one field to another is limited, and disease spread through splashing rain is restricted to distances as short as 4-5 m. This is in agreement with observations made in Mexico (Duveiller 1992).

Role of insects. According to Leach (1940, in Dickson 1956), insects play a role in disease dissemination and infection; however, very little research has been conducted on the topic. Insects may occasionally be trapped in sticky exudates if the latter are abundant (Jones *et al.* 1917). Under favorable conditions such as water-congested tissues, contaminated aphids can transmit *Xanthomonas* to wheat and barley and aid in long distance dissemination (Boosalis 1952). However, the role of aphids in long distance transmission of the disease is probably limited.

The Pathogen

Isolation and identification

Media. *Xanthomonas t.* pv. *undulosa* grows fastest *in vitro* at 28-30ºC. Growth is possible but slower at room temperature (17-27ºC) and will stop at around 36ºC. The bacterium can be cultivated on common media such as nutrient agar, YPGA, GYS, KB and Wilbrink's medium (see Appendix). These culture media are not semi-selective and can be used for a wide range of bacteria. Semi-selective media

include KM-1, XTS and WBC. When no selective medium is available, Wilbrink's medium (Sands *et al.* 1986) is preferred, given the pathogen's typical yellow colony is best distinguished from saprophytes on this non-selective medium (**Figure 2.11**). Wilbrink's medium is particularly useful for massive inoculum production from a pure strain, since it induces an abundant and fast-growing culture.

Pathogenicity tests. Soil inoculation of the pathogen does not induce the disease in cereal seedlings (Hagborg 1936). Seed inoculation using injured, unsprouted grains coated with a bacterial slime or suspension is workable but tedious and unsatisfactory for testing pathogenicity (Hagborg 1936).

Forcing a bacterial suspension into the leaf whorl of young plants (4-5 leaves) or into the boot of older plants with a hypodermic syringe is a very effective inoculation method (Bamberg 1936). This was confirmed at CIMMYT, where plants are usually incubated for five days in a humid chamber after inoculation (Duveiller 1994b). In some cases, water-soaking is observed as early as 3-4 days.

Boosalis (1950) obtained good infections using a partial vacuum. Hagborg (1970) proposed a device for injecting solutions and suspensions into thin leaves of plants. The device consisted of tongue seizing forceps, soft rubber stoppers, and a hypodermic needle and syringe (see **Figure 3.9**). Several injections per minute can be applied with this apparatus, but the size of stomatal openings, the amount of pressure exerted and how long pressure is applied affect the amount of tissue infiltrated. Sun and He (1986) used leaf clippings under conditions optimal for expressing typical symptoms at 22°C. Also, Colin *et al.* (1990) used an inoculation technique where detached leaves are infiltrated to test pathogenicity. However, it is better to use whole plants for inoculation and for pathogenicity tests.

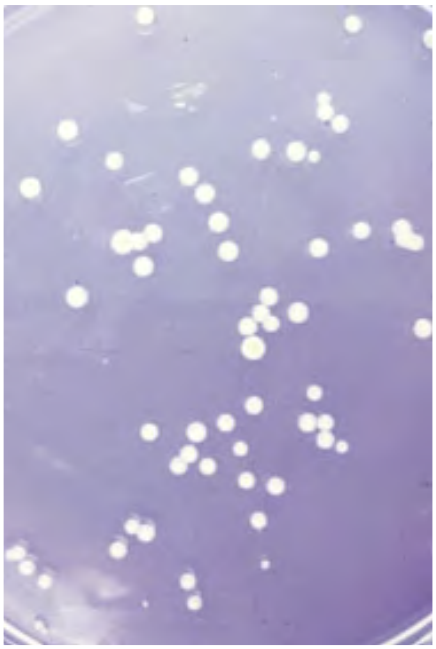


Figure 2.11. Colonies of *Xanthomonas translucens* pv. *undulosa* on Wilbrink's medium present a typical yellow color that easily distinguishes them from saprophytic bacteria isolated from a bacterial leaf streak lesion.

Diversity of *X. translucens* strains that attack wheat and other small grains

Host range and other host/pathogen relationships. Since BLS was first identified on wheat, several pathovar names have been used for *X. translucens* strains isolated from small grains; however, these strains have not always been subjected to differential host range pathogenicity tests. This has caused confusion: in the first place, strains having different names (based on the host plant from which they were isolated) may be similar; second, many authors use the name *X. t.* pv. *translucens* in a general sense for any cereal streak pathogen (Bradbury 1986) although the names of four BLS-inducing pathovars of *X. translucens* are currently included in the most recent ISPP list of plant pathogenic bacteria (Young *et al.* 1996):

Xanthomonas campestris pv. *translucens* (Jones, Johnson and Reddy 1917) Vauterin, Hoste, Kersters and Swings 1995
X. t. pv. *cerealis* (Hagborg 1942) Vauterin, Hoste, Kersters and Swings 1995
X. t. pv. *secalis* (Reddy, Godkin and Johnson 1917) Vauterin, Hoste, Kersters and Swings 1995
X. t. pv. *undulosa* (Smith, Jones and Reddy 1919) Vauterin, Hoste, Kersters and Swings 1995

If the ISPP rules are followed correctly, the pathovar name *X. t.* pv. *translucens* should be reserved for strains pathogenic on barley only (Jones *et al.* 1917) and the name *X. c.* pv. *hordei* should be considered a synonym. This was indicated at the 9th Congress on Plant Pathogenic Bacteria held in Madras, India, in 1996.

Xanthomonas t. pv. *undulosa* designates strains pathogenic on wheat and triticale and can be isolated from several hosts including wheat, barley, triticale and rye. Hence, its host range is not only broader than that of *X. t.* pv. *translucens*, but also covers the host range of *X. t.* pv. *cerealis* as defined by inoculation tests conducted on oat, rye and *Bromus* (Boosalis 1952; Bragard 1996; Bragard *et al.* 1995).

Xanthomonas t. pv. *secalis* has been described as pathogenic on rye (Reddy *et al.* 1924). However, strains with this pathovar name have been reported to infect barley, oat and wheat, although they should not have done so according to the pathovar concept (see Chapter 1) (Bradbury 1986; Cunfer and Scolari 1982; Bragard *et al.* 1995) (**Table 2.1**).

The issue of correctly naming and distinguishing pathovars may appear irrelevant given that different pathovars may induce similar symptoms on the same crop (i.e., wheat). However, studies on pathogenic specialization (Hall and Cooksey 1986; Mellano and Cooksey 1988) are important when searching for disease resistance. Host specific virulence (*hsv*) genes that could expand the host range of *X. t.* pv. *translucens* have been cloned (Waney and Gabriel 1990; Waney *et al.* 1991). Based on RFLP analysis, Bragard *et al.* (1995) showed that strains pathogenic on barley, but not on wheat, clustered in a genetically different group.

Clear differences in aggressiveness were noted among *X. t.* pv. *undulosa* strains from various geographical origins. Whereas typical strains induce extensive stripe symptoms on wheat and barley, strains from some areas induce limited symptoms (Bragard and Maraite 1994). No evidence of strong race specialization has as yet been found on wheat, as indicated by highly non-significant cultivar x strain interaction (Milus and Chalkley 1994).

Biochemical and physiological traits. The BLS pathogen is non-sporing, rod-shaped, Gram negative, and motile by a single polar flagellum. It is further characterized by rods 0.4-0.8 x 1.0-2.5 µm, singly or in pairs, except in peptonized nutrient broth with 2% NaCl, in which long non-motile chains are formed (Jones *et al.* 1917; Dowson 1939).

It is easy to distinguish *X. translucens* from other wheat pathogenic bacteria using several key tests (see Chapter 1). This bacterium is oxidative, i.e., it always produces acid from glucose under aerobic conditions. No nitrate to nitrite reduction is observed (Dye 1962). The reaction for Kovacs' oxidase and arginine dihydrolase is also negative. There is no 2-ketogluconate production, and esculin hydrolysis is positive (Miyajima 1980; Bradbury 1984). Hypersensitivity on tobacco is positive (Mohan and Mehta 1985). Unlike *X. campestris, X. translucens* strains do not hydrolyze starch and do not use lactose (Schaad 1987b).

Within the same bacterial species (e.g., *X. translucens*), pathovars group strains that can only be recognized based on host range. Therefore, very few biochemical and physiological tests are useful for differentiating strains at the pathovar level. Hence, metabolic fingerprinting obtained with the BIOLOG MicroPlates™ system and based on the use of

Table 2.1. Comparison of the host range of *Xanthomonas translucens* strains from cereals and grasses after inoculation by water injection plus pricking of wheat (cv. Alondra), barley (cv. Corona) and oat (cv. Alfred) plants at the four-leaf stage.

				Host range		
Strain[a]	Name	Origin	Species where first isolated	Wheat[b]	Barley	Oat
NCPPB2821	*X.t.* pv. *undulosa*	Canada	*Triticum turgidum* var. *durum* L.	+	+	C
UPB480	*X.t.* pv. *undulosa*	Pakistan	*Triticum turgidum* var. *durum* L.	+	+	C
UPB513	*X.t.* pv. *undulosa*	Mexico	X *Triticosecale* Wittmack	+	+	C
UPB605	*X.t.* pv. *undulosa*	Brazil	*Triticum aestivum* L.	+	+	C
UPB645	*X.t.* pv. *undulosa*	Syria	*Triticum turgidum* var. *durum* L.	+	+	C
NCPPB973	*X.t.* pv. *translucens*	USA	*Hordeum vulgare* L.	(+)	+	–
UPB684	*X.t.* pv. *translucens*	Iran	*Hordeum vulgare* L.	–	+	–
UPB780	*X.t.* pv. *translucens*	Spain	*Hordeum vulgare* L.	–	+	T
NCPPB2820	*X.t.* pv. *translucens*	India	*Hordeum vulgare* L.	–	+	C
NCPPB2822	*X.t.* pv. *secalis*	Canada	*Secale cereale* L.	+	+	C
UPB676[c]	*X.t.* pv. *secalis*	South Africa	*Secale cereale* L.	–	+	T
NCPPB1944	*X.t.* pv. *cerealis*	USA	*Bromus inermis* L.	+	+	C

[a] NCPPB = National Collection of Plant Pathogenic Bacteria, Harpenden, England; UPB = Unité de Phytopathologie Bacterial collection, Louvain-la-Neuve, Belgium.
[b] + = positive reaction, compatibility; (+) = weak positive reaction; - = negative reaction; C = chlorosis; T = translucens spot.
[c] Received as pathovar *translucens* from J. Smith, Small Grains Centre, Bethlehem, South Africa.

carbohydrates and amino acids by the pathogen should not be expected to aid in identification at the pathovar level within the same species. Moreover, all biochemical and physiological tests are not of equal taxonomical value; many of them may give variable results for a population of strains of the same pathogen.

Other differential traits. Based on host range and electrophoretic patterns of cell proteins, it was confirmed that *X. translucens* strains collected from wheat and barley in Iran were different (Alizadeh and Rahimian 1989; Alizadeh *et al.* 1996). However, Kersters *et al.* (1989) could not distinguish *X. t.* pv. *undulosa* from *X. t.* pv. *translucens*. Fatty acid profiles do not clearly differentiate between *Xanthomonas translucens* pvs. *translucens*, *cerealis*, *secalis* and *undulosa* (Stead 1989).

Bacteriophages specific to *X. t.* pv. *undulosa* and *X. t.* pv. *secalis*, as well as phages polyvirulent for several "*X. translucens*" strains, were isolated by Katznelson and Sutton (1954). However, their early attempts to use pathovar-specific phages to identify cultures of *X. t.* pv. *undulosa* or *X. t.* pv. *translucens* were unsuccessful because available phages were strain-specific. More recently, a bacteriophage specific to *X. t.* pv. *undulosa* from wheat and triticale, which, in association with other determination tests, may prove to be suitable for quick identification of the bacterium, was obtained from severely infected leaves of the susceptible genotype Alondra (Mohan and Mehta 1985). Similarly, phages that are highly specific to pathogenic strains of *X. translucens* have been isolated from wheat by Forster and Strausbaugh (1994).

Immunological methods do not help to differentiate among the various pathovars of *X. translucens* from cereals. The high degree of serological homogeneity within this group was confirmed in several studies based on polyclonal antibodies (Hagborg 1946; Elrod and Braun 1947a, 1947b; Azad and Schaad 1988b; Samson *et al.* 1989), although Fang *et al.* (1950) grouped five forms of "*X. translucens*" into four serotypes and observed that the immunological closeness between *X. t.* pv. *undulosa* and *X. t.* pv. *secalis* coincided with their pathogenicity. Research on monoclonal antibodies specific to "*X. translucens*" corroborated that pathovars within this group could not be differentiated, which confirms their close serological similarity. However, using these monoclonal antibodies, all strains virulent on wheat, barley, rye and triticale could be differentiated from less aggressive, deviating *X. campestris* strains isolated from wheat (Bragard and Verhoyen 1993).

Control Strategies

Rotations

There is little information on the role rotations play in reducing black chaff epidemics. Since the major source of inoculum is infected seed, rotations may not play a key role in controlling the disease. Straw can harbor viable inoculum from season to season and cause initial infection in the field, but the number of viable bacteria in infested, overwintered straw is reduced when the straw is incorporated into the soil (Boosalis 1952).

At CIMMYT, Mexico, wheat is grown in the highlands during the wet season, and two growing cycles are separated by a six- to seven-month interval during which vetch is grown as a winter crop. *Xanthomonas t.* pv. *undulosa* is able to survive for that length of time on naturally infected straw kept under dry conditions in the laboratory. In the field, wheat straw from the previous season is sometimes found at planting. However, survival of the pathogen seems improbable due to rotation with a non-host crop and its extreme susceptibility to antagonistic bacteria (Schaad and Forster 1985), especially saprophytic fluorescent *Pseudomonas* (Duveiller, unpublished).

Seed health

Detecting the pathogen through seed washing. The best way to limit BLS is to avoid sowing infected seed. A seed wash test on a semi-selective medium after dilution (tenfold) plating (**Figure 2.12**) is the normal non-destructive procedure used in pathogen-free seed certification. The method has the advantage of detecting living pathogens. The number of colony forming units per gram of seed gives an estimate of the number of bacterial cells present in the sample. The number of single colonies growing on the agar medium has to be counted, and representative colonies have to be cloned and proven pathogenic on wheat (**Figure 2.13**). The best estimate is obtained by counting colonies on plates where the number of colonies ranges between 50 and 300. Hence, when *x* grams of seed are washed in *x* ml of saline solution

(w:v = 1:1) and 0.1 ml is plated onto agar medium,

$$\text{cfu/ gram} = n_d \times 10^{(d+1)},$$

where n represents the number of colonies counted on the medium at a dilution fold *d*.

Several semi-selective media have been developed:

- Kim *et al*. (1982) developed the KM-1 medium, which exhibited high selectivity on soil samples and barley leaf debris (see Appendix). Compared to Wilbrink's medium, the plating efficiency (i.e., the number of colonies on semi-selective medium/number of colonies on general medium) ranged from

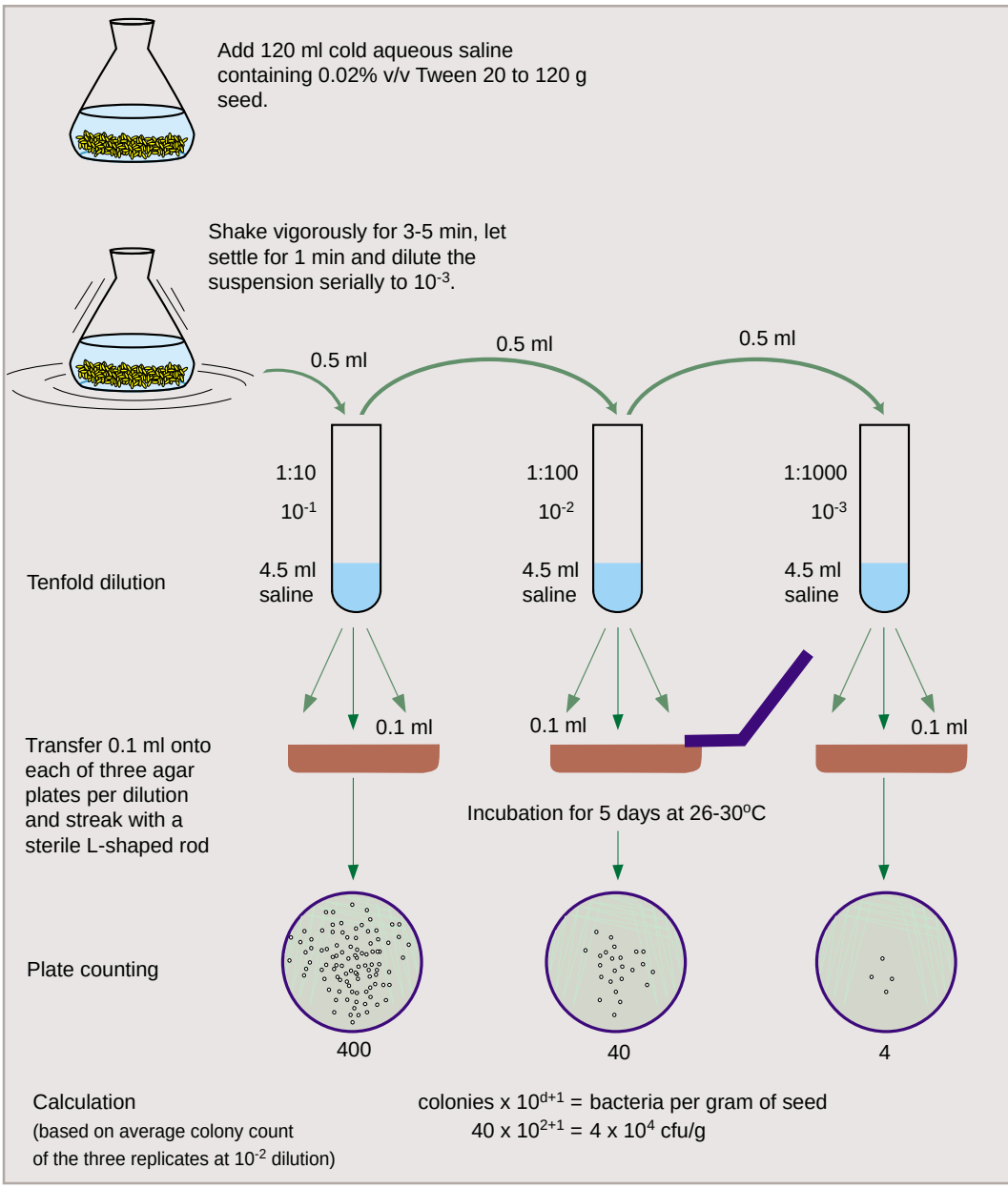


Figure 2.12. Pathogen detection using seed washing and the dilution plating technique.

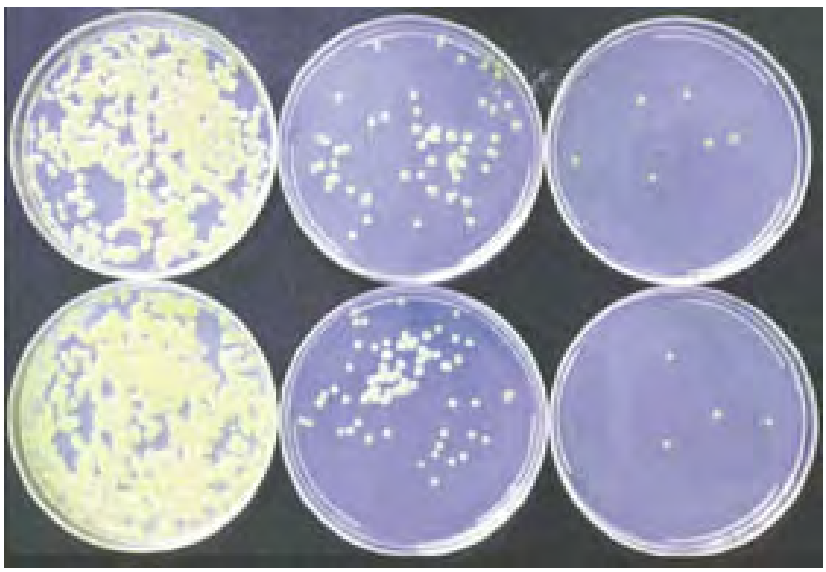


Figure 2.13. Growth of *Xanthomonas translucens* pv. *undulosa* in two replicates of WBC agar Petri dishes after wash water of infected seed was plated and diluted tenfold.

0.91 to 2.13 for strains of *X. t.* pv. *translucens*. However, many "*X. translucens*" strains from Idaho were found to grow poorly on this medium (Schaad and Forster 1985).

- Schaad and Forster (1985) developed the XTS medium (see Appendix) and tested it for isolating the pathogen from wheat seed. To perform the test, take 120 ml of sterile, cold 0.85% NaCl (saline) containing 0.02% v/v Tween 20 and add to 120 g seed (about 3,000 seeds). After shaking vigorously for 3-5 min, let settle for 1 min and prepare tenfold dilutions to 10^{-3} using cold sterile saline. Transfer 0.1 ml of each dilution onto each of three plates of XTS agar and spread with an L-shaped rod. Examine plates after five days' incubation at 30°C. Colonies of "*X. translucens*" are 1-2 mm in diameter, yellow, clear, round, convex and smooth (Schaad and Forster 1993). Streak a known culture onto XTS for comparison. Positive colonies are tested for pathogenicity by injecting a bacterial suspension (approximately 10^5 cfu/ml) into leaf whorls of susceptible wheat seedlings. Disease symptoms appear after incubating for 5-7 days in a dew chamber at 26°C.
- Claflin and Ramundo (1987) used XTS medium with 2 mg/L instead of 8 mg/L gentamycin to increase pathogen recovery. According to Schaad and Forster (1989), antagonistic bacteria may occasionally act synergistically with gentamycin to inhibit the growth of *X. t.* pv. *translucens*. The use of XTS without gentamycin is recommended in such cases. Under Idaho conditions, results of laboratory seed tests using XTS were in agreement with the development of black chaff in the field; levels of 1000 cfu/g or less in seed washes are likely to result in little or no disease (Schaad and Forster 1985). Zero tolerance is not necessary where BLS is endemic (Schaad 1988b), but infested seed should not be used for germplasm exchange.
- Another seed test which proved to be effective under Mexican and other conditions is WBC medium, a modification of Wilbrink's medium (Duveiller 1990b; Duveiller and Bragard 1992; Bragard *et al.* 1993). WBC medium is Wilbrink's medium amended with boric acid (0.75 g/L) and cephalexin (10 mg/L). It does not contain gentamycin but includes cycloheximide to reduce fungal growth (see Appendix). The protocol used in seed washing and dilution plating is similar to the method used with the XTS medium (see above). Duveiller (1990b) pointed out that bacteria recovery may vary among samples from the same seed lot; this sometimes leads to poor correlation between laboratory test results and field detection. The variation could be due either to saprophytic microorganisms that show activity antagonistic to *X. t.* pv. *undulosa* or to uneven distribution of the pathogen among subsamples. As a rule, when using antibiotics, each new flask should be tested prior to utilization, given that antibiotic activity may vary from one lot to another or may be reduced after storage.

Recently Maes *et al.* (1996) developed a method for recognizing BLS-causing *Xanthomonas* pathogens using rDNA spacer sequences and PCR (Maes and Garbeva 1994). The tests proved to be quick (results can be obtained in 5 h, compared to several days using the dilution and plating method) and relatively sensitive (2×10^3 cfu/g of seed), indicating the technique might be useful for detecting those pathogens in seed without isolation. However, this method also detects five other *X. campestris* pathovars with a host range restricted to forage and some ornamental grasses. No data are available on the survival of these grass pathogens on non-host plants, especially in seeds of cereals such as wheat. In addition, there is a risk of false positive PCR detection of dead BLS bacteria.

Serodiagnostic assays. As early as 1939, Gorlenko *et al.* suggested that serological methods could be adapted for detecting black chaff in seed.

Using rabbit polyclonal antibodies for detecting *X. t.* pv. *translucens* in wheat seed, Claflin and Ramundo (1987) were only able to obtain positive readings with a dot-immunobinding assay (DIA) (Lazarovits 1990) when cell concentration was 10^5 cfu/ml or higher. According to these authors, the DIA would be most valuable for identifying *X. t.* pv. *translucens* when used in conjunction with plating on a semi-selective medium.

Frommel and Pazos (1994) used polyclonal antibodies for detecting *X. t.* pv. *undulosa* in naturally infected wheat seed. Using ELISA after enrichment in a semi-selective liquid medium (nutrient broth 8 g/L, glucose 5 g /L, pH 7, gentamycin 5 µg/ml, cephalexin 6 µg/ml, tyrothricin 150 µg/ml, ampicillin 5 µg/ml, cycloheximide 200 µg/ml,

benomyl 80 µg/ml, and Tween 20 at 0.02%), these authors were able to detect the pathogen in samples that originally had less than 5 x 10^2 cfu/ml. However, detection using ELISA without enrichment did not significantly correlate with the potential seedling infection rate determined by growing naturally infested seedlings in the greenhouse.

Bragard and Verhoyen (1993) developed monoclonal antibodies from hybridoma cell lines produced by fusing splenocytes from *X. t.* pv. *undulosa*-immunized Lou rats with IR983F myeloma cells. The monoclonal antibodies reacted positively with *X. t.* pv. *undulosa*, *X. t.* pv. *cerealis*, *X. t.* pv. *translucens*, and

Protocols:

Wash 10 g seed by shaking (200 rpm) in 100 ml sterile distilled water for 30 min at room temperature. Centrifuge to decant debris. Then use either of the following techniques.

Dot-immunobinding assay (DIA)

- Secure a pure nitrocellulose membrane (pore size 0.45 µm) Bio-Rad Trans blot transfer medium that has been previously immersed in TRIS-buffered saline (TBS; 20 mM Sigma 7-9, 500 mM of NaCl, pH 7.5) in a Bio-Dot® microfiltration apparatus (Bio-Rad, Richmond, CA).
- Pipette 200 µl of seed wash water into each well of the apparatus (**Figure 2.14 a**).
- Apply vacuum and wash the membrane with 200 µl of TTBS (TBS 0.05%, Tween 20, pH 7.5) per well.
- Flood the membrane (200 µl per well) for 30 min with a blocking solution (TBS containing 1% bovine serum albumin).
- Add monoclonal antibody AB3-B6 diluted 500 times in the blocking solution (200 µl per well) and let stand for 60 min.
- Wash the membrane twice with TTBS (200 µl per well, eliminated by vacuum).
- Incubate the membrane for 60 min in peroxidase-labeled Mab MARK-PO (IMEX, UCL, Brussels) diluted 500 times in the blocking solution.
- Remove the membrane coated with *X. t.* pv. *undulosa*-(AB3-B6)-MARK-PO, wash with TBS, and expose to solution containing 50 ml TTBS and 30 mg 4-chloro-1-naphtol dissolved in 10 ml methanol.
- The test is positive if a gray precipitate is observed several minutes after adding 50 µl of perhydrol 30% H_2O_2 (**Figure 2.14 b and c**).

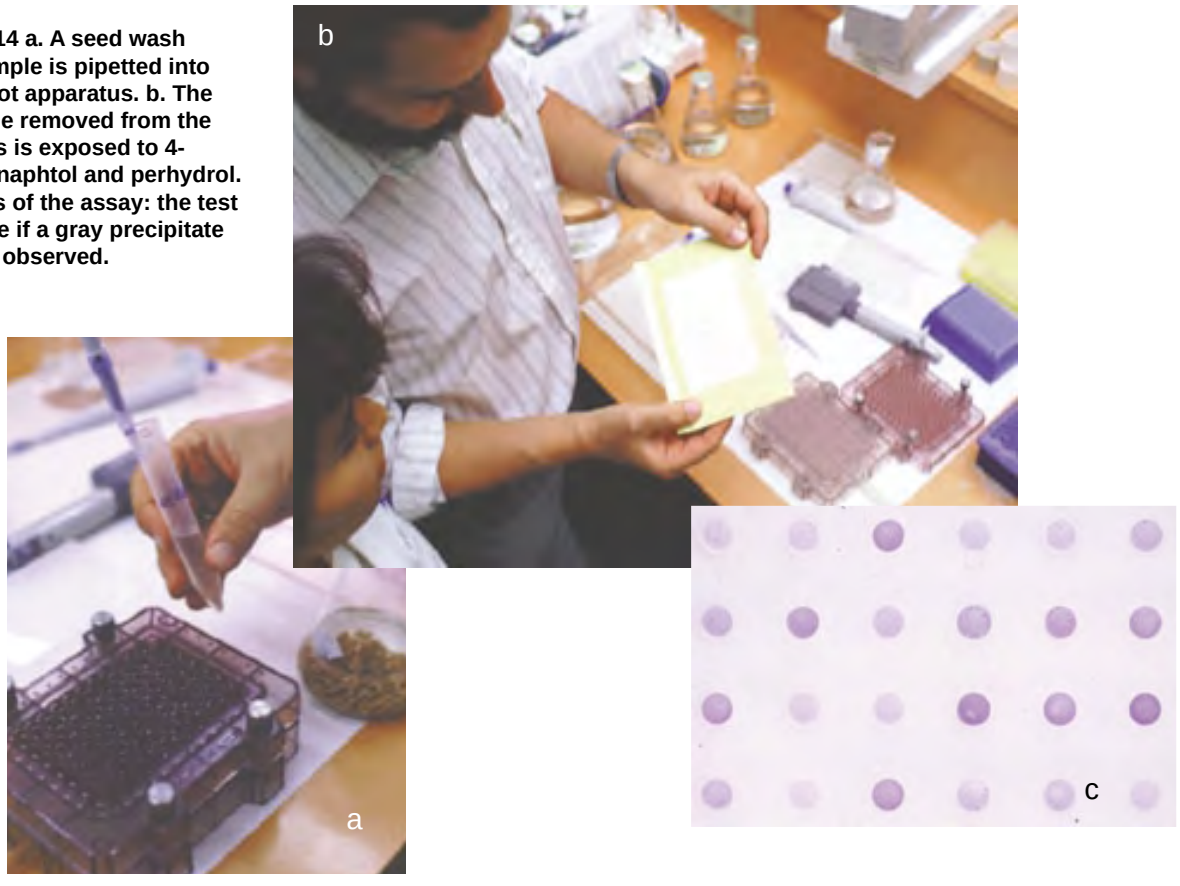
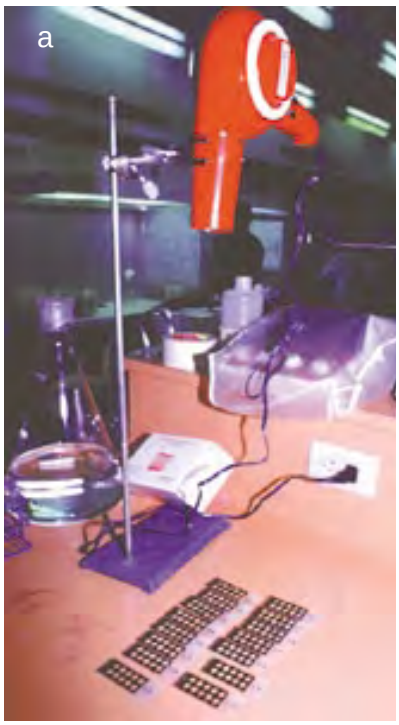


Figure 2.14 a. A seed wash water sample is pipetted into the Bio-dot apparatus. b. The membrane removed from the apparatus is exposed to 4-chloro-1-naphtol and perhydrol. c. Results of the assay: the test is positive if a gray precipitate is clearly observed.

Immunofluorescence (IF)

- Pipette 40 µl of seed wash water directly from the flask into a 6-mm well on a multiwindow slide and then fix with hot air from a hair dryer (**Figure 2.15 a**).
- Expose wells for 60 min to Mab AB3-B6 diluted 100 times in phosphate-buffered saline (PBS; 8 g of NaCl, 2.7 g of $Na_2HPO_4 \cdot 2\ H_2O$ and 1 L of distilled water, pH 7.2); use 20 µl per well.
- Rinse wells with PBS and expose them for 30-60 min in the dark to a mouse anti-rat Mab conjugated with fluorescent isothiocyanate MARM4-FITC (IMEX, UCL, Brussels) diluted 1:100 in PBS. The optimum dilution will vary with each batch of conjugated antibody and therefore must be determined by trying a range of dilutions (usually 1:20 - 1:200 of commercial preparations) on a known positive sample. Use 20 µl per well.
- Rinse with PBS.
- Add three drops of buffered glycerine (100 mg of diphenilamine in 10 ml of PBS, pH 9.6, and 90 ml of glycerol); slip on a coverglass.
- Observe under immersion oil using a microscope equipped with a high-pressure mercury ultraviolet lamp HBO-50 and Carl Zeiss filter combination 10 (x1000) (**Figure 2.15 b and c**). If they cannot be observed on the same day, the multiwindow slides can be stored in the dark for later observation.



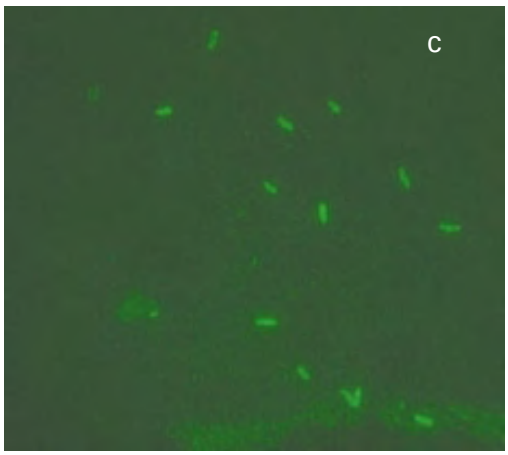


Figure 2.15 a. Seed wash samples are fixed onto multiwindow slides with the help of a hair dryer. b. Epi-UV microscope for the observation of immunofluorescence. c. Fluorescent rods of *Xanthomonas translucens* pv. *undulosa* are observed under immersion oil (x1000).

X. t. pv. *secalis*, and proved more specific than polyclonal rabbit antisera (see Chapter 1).

Serological methods are very useful for identifying strains grown as pure cultures and are also a potential tool for seed indexing procedures. Monoclonal antibody AB3-B6 was used in both immunofluorescence (IF) and DIA to detect pathogens in aqueous seed extracts. These techniques were compared to dilution plating of a seed wash (see p. 37). Seed lots contaminated with a high (>10^4 cfu/g) population of bacteria were consistently identified with all three methods. Immunofluorescence was more sensitive than DIA and gave more reproducible results. The DIA method is simple and requires inexpensive equipment, but the detection threshold is high (10^5 cfu/ml), making it more appropriate for the identification of pure strains. Also, it is not easy to recognize false positive reactions due to dust associated with the seed. On the other hand, although IF requires more expensive equipment, it is relatively quick and sensitive. The detection threshold is 10^3-10^4 cfu/ml. IF positive seed lots should not be used for sowing in areas that favor disease development, since a pathogen concentration of 1 x 10^3 cfu/g of seed is likely to induce an epidemic (Duveiller and Bragard 1992).

Techniques using seedlings. Mehta (1986b) obtained infected seedlings after growing naturally infected seed at 22-25°C, in 20 x 2-cm tubes containing 20 g of sterilized soil and 4 ml of water.

Determining the percent infected seedlings after growing naturally contaminated seed on sterile soil in a moist chamber (100% HR; 22 ± 3°C) did not prove workable under Mexican conditions. No symptoms were found on more than 13,000 seedlings grown from heavily infected seed (>10^6 cfu/g) after four weeks' incubation in trays where each seed was put in a 1-cm deep hole in sterile soil (Duveiller 1994b).

The modified injection technique proposed by Mehta to detect the presence of *X. t.* pv. *undulosa* on wheat, triticale and rye seed and *X. t.* pv. *translucens* on barley seed may be used for quarantine purposes (Mehta 1986a and 1990). Shake seeds (20 g) thoroughly for 90 min in 20 ml of sterile saline; remove seeds and inoculate the suspension into 20-day old seedlings with a hypodermic syringe. *Xanthomonas* streak symptoms are assessed 7-12 days after inoculation. This method is recommended when immuno-fluorescence microscopy is not available (Bragard *et al.* 1993).

X-gal. Sands *et al.* (1984) used X-gal (the substrate 5-bromo-4-chloro-3-indolyl ß-D-gluco-pyranoside) (20 mg/L), from which "*X. translucens*" forms a blue dye, to quickly detect contaminated seed (Sands and Fourest 1989). X-gal solubilized in 1 ml dimethyl formamide can be combined (20 mg/L) with Wilbrink's medium, where lactose replaces sucrose, to show blue colonies of "*X. translucens*" after 4-5 days at 28°C. The major problem with this method is lack of specificity, given that several saprophytic bacteria can induce the blue coloration. Also, the solution used in this technique may be harmful to human health.

Seed treatments

Since no pesticide effectively controls the disease in the field, research on chemical control focuses on seed disinfection. However, the disease cannot be controlled by seed treatments alone, although several studies report partial effectiveness of various compounds (Sands *et al.* 1981).

Braun (1920) indicated that seedling infection was greatly reduced by the use of formalin or copper sulfate. However, results of chemical seed treatments are contradictory. It was believed that the high BLS incidence recorded during the 1980s was due to the ban on mercurial compounds (Forster 1982; Duveiller 1989), but as shown by Forster and Schaad (1988), these products proved to be ineffective for controlling the disease. Cupric hydroxide (Kocide SD), non-volatile mercury (Mist-O-Matic) and volatile mercury (Panogen 15) are also unsatisfactory (Jons *et al.* 1982; Forster 1982).

Mehta (1986c) reduced transmission of *X. t.* pv. *undulosa* by 80% with 300 ml/100 kg seed of Guazatine Plus (syn. Panoctine Plus) in an experiment with naturally infected seed of wheat genotype IAPAR-Caete. When the dosage was increased to 350 ml/100 kg, a 24% reduction in germination was observed (Mehta 1986c). The fungicide substantially reduces disease severity in plots sown with treated seed as compared to plots sown with untreated seed. The treatment is effective if applied at least five months before sowing, but usually ineffective if applied a month before sowing. Also, a few heavily contaminated seeds may escape the product during the procedure and remain contaminated (Mehta and Bassoi 1993).

Seed treatment with acidified cupric acetate (0.5%) at 45°C for 20 min significantly reduced the amount of black chaff in the field (Forster and Schaad 1985 and 1988). Bacteria were isolated from both seed wash water and naturally contaminated seed plated onto XTS after treatment with methoxyethylmercury acetate, ethylmercury-toluene sulphonanilide, phenylmercury acetate, cupric hydroxide, calcium hypochlorite, sodium hypochlorite and calcium propionate but not with hot cupric acetate (Forster and Schaad 1988). Stand count can be reduced significantly compared to other treatments when seed treated with acidified cupric acetate is planted; however, this level of phytotoxicity is considered acceptable for a foundation seed health program (Forster and Schaad 1988).

Recently, an old method based on dry heat seed treatment (Atanasoff and Johnson 1920) was proposed for reducing the amount of bacteria in infected seed. Fourest *et al.* (1990) recommend treating the seed at 72°C for seven days. This method allows treating larger amounts of seed, but experiments conducted at CIMMYT indicate that the method is not completely effective, particularly on seed samples larger than 100 g (Duveiller 1992).

It should be pointed out that the detection technique used in assessing bacterial infestation may make it difficult to evaluate a seed

treatment's effectiveness. When seed is washed and the seed wash is plated on selective agar medium, the bacterium may be rinsed out with the wash water and killed by the bactericide. Also, it is not known exactly where bacteria are located in the seed. Most are located in the external parts of the seed, so they can be reached by a chemical compound. However, bacteria located in the internal layers of the seed coat may be unaffected. When pathogen extraction is non-destructive (e.g., seed washing), bacteria associated with the embryo are sometimes not recovered during testing.

The amount of bacteria in the seed, as well as its heterogeneous distribution, may partly explain contradictory results, particularly if the samples used are too small. If a seed lot contains 10^5 cfu/g, a 99% effective bactericide will still allow 1,000 cfu/g to survive, which is the minimum amount needed to cause an epidemic.

Bactericide seed treatments were evaluated in Mexico using naturally infected wheat genotype Alondra, which is very susceptible to BLS (**Table 2.2**). Laboratory tests were carried out using 10-g samples and kernels individually soaked in 3 ml sterile saline. In the field, the percentage of infected plants in plots (5 x 5 m; 3 replicates) sown with treated seed was determined at Zadoks' growth stage DC45. Results indicated that hot cupric acetate, Panoctine Plus (a.i. guazatine 300 g/L and imazalil 20 g/L), formaline and dry heat consistently reduced the amount of bacteria in the seed as determined using the dilution plate method with WBC agar (Duveiller 1990b). This is in agreement with earlier observations by Mehta (1986d), Forster and Schaad (1988) and Fourest *et al.* (1990). However, although the effects of bactericide treatments were significant (P=0.05), control of BLS in the field was not possible (Duveiller *et al.* 1991). When seed with high levels of *X. t.* pv. *undulosa* is used, bacteria surviving the treatments can multiply to reach the threshold for symptom induction (>10^8 cfu/leaf). The incomplete effect of Panoctine Plus and dry heat was confirmed using heavily contaminated seed (100-g samples), but Panoctine Plus was used only a few days before planting (Duveiller 1992) (**Figure 2.16**). Nevertheless, even if not completely satisfactory, seed treatment with dry heat or a product such as Panoctine Plus is recommended.

Seed multiplication in a disease-free area

Clean seed should be used to produce foundation seed, and multiplication should be conducted in a disease-free area under dry

Table 2.2. Disinfection of heavily contaminated seed of genotype Alondra harvested in Toluca, Mexico in 1988. Infection levels compared by seed washing and dilution plating; average percentage (three 20-m² plots) of plants from that seed which showed symptoms in the field after booting the following season (1989) in Toluca and El Batan.

Seed treatment	Log cfu/g[1]	% infected plants Toluca	El Batan
Dry heat 72°C, 7 days	BDT[2]	12.0 ab[3]	1.6 b
Rolitetracycline 1%, soaking 4 h[4]	5.1	0.0 c[5]	4.3 ab
Cupric acetate 0.5%, 40°C, 20 min[4]	5.0	7.3 abc	4.6 ab
Bordeaux mixture (1 lb:1 lb:100 gal), 20 min, 40°C[4]	5.7	3.6 bc	6.7 ab
Formaline 0.8%, 1 h[4]	BDT	9.4 abc	9.8 ab
Panoctine Plus, 400 ml/100 kg seed	5.2	12.5 ab	10.0 a
Cupric acetate 0.5%, 45°C, 20 min[4]	BDT	14.2 ab	12.3 a
Kasugamycin 2%, slurry	6.2	22.1 ab	14.6 a
Copac E, 0.5 ml/50 g seed	6.1	11.7 ab	15.0 a
Control	6.5	17.0 ab	15.5 a

1 cfu = colony forming unit.
2 Below detection threshold (dilution 10^3) on WBC agar.
3 F test (arcsin): Toluca = 2.47*; El Batan = 2.48*; LSD (P=0.05); treatments with the same letters are similar.
4 Seed was rinsed after treatment.
5 Data possibly not reliable due to poor emergence.

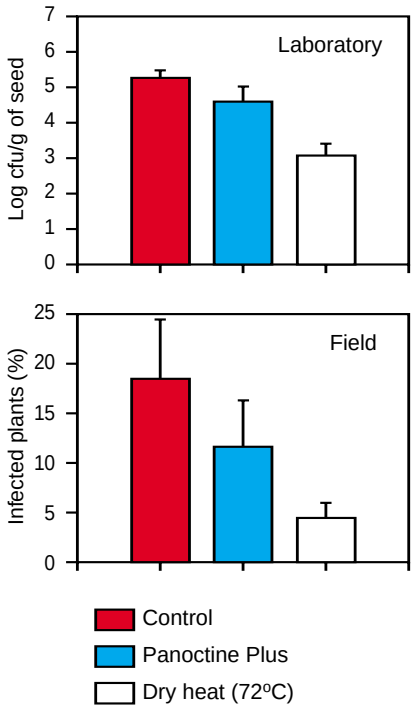


Figure 2.16. Comparison of the bactericidal effects of Panoctine Plus and dry heat (72°C) seed treatments in laboratory and field experiments at El Batan, Mexico, in 1991; the laboratory test was conducted a month before sowing.

conditions without overhead irrigation. Also, it is not a good idea to walk in the fields when leaves are wet. To avoid recontaminating plants produced from clean seed, a distance of 0.4 km is suggested for isolating a seed increase and certification program (Forster *et al.* 1986). Although a seed multiplication field may not show black chaff symptoms, the pathogen may increase on the leaf and head surfaces, resulting in contaminated seed. In contrast, a high percentage of disease in the field does not necessarily result in a higher amount of bacteria in harvested seed lots (Mehta 1990).

Breeding for resistance

Since controlling black chaff through seed treatment is not easy, breeding resistant genotypes appears to be the best way to reduce the risk of yield losses. Screening for resistance is essential for breeding. The material to be screened must be uniformly exposed to the pathogen, and this is only possible through artificial inoculation. Epidemics are sporadic, and natural homogeneous infection in the field is too unreliable to allow adequate evaluation. Lines identified as susceptible under natural conditions may be infected as a result of higher seed infection levels. Also, disease-free genotypes may not really be resistant but may have simply escaped infection.

Greenhouse testing. Greenhouse tests can be conducted on seedlings and young plants by infiltrating low cell concentrations into the leaf. It is important to use low bacterial concentrations to be able to detect measurable differences in resistance. A concentration of 10^4 cfu/ml of a young culture (24 h) on agar medium is usually appropriate. The concentration can be adjusted with the help of a Petroff-Hausser counting chamber (Figure 1.5). A drop of pure bacterial suspension is diluted in water so that a countable number of cells can be observed under the phase contrast microscope (x400). Since living bacteria move, they have to be immobilized to ease the counting. This can be achieved by adding a drop of 4% formaldehyde to the bacterial suspension before observing it in the counting chamber, but the dilution caused by this additional drop must be taken into account.

In another inoculation technique, the seedling pseudostem is filled with sterile water, and then a needle dipped in a young bacterial culture is passed through it. After 5-7 days' incubation in a humid chamber, ideally at 24-26ºC, disease is scored on the emerging leaf.

The major problem with screening at the seedling stage in the greenhouse is the fairly high degree of data variation. To minimize this variation, inoculum concentration, infiltration into confined portions of the leaf blade, and moisture distribution in the dew chamber have to be carefully standardized. Also, the correlation between disease scores on seedling and field data is not always clear, as shown by Duveiller (1992) on a set of 50 genotypes.

Milus and Mirlohi (1994) used a 0-6 scale based on percent water-soaking to evaluate disease reaction on seedlings, whereas Duveiller (1992) measured lesion length and used a 0-4 scale to evaluate the level of exudate production. The correlation between lesion length and degree of exudate production is significant ($P<0.01$) but R^2 is only 0.39. In view of the above, evaluating resistance based on adult plant response in the field is recommended.

Disease evaluation scale (Milus and Mirlohi 1994):

0 = no visible symptoms
1 = chlorosis but no water-soaking
2 = water-soaking less than 10%
3 = water-soaking 10-30%
4 = water-soaking 31-70%
5 = water-soaking 71-100%
6 = water-soaking extended beyond the infiltrated area

Scale for evaluating exudate production (Duveiller 1992):

0 = no symptoms
1 = water-soaking but no exudate
2 = water-soaking with little exudate
3 = water-soaking with readily detectable exudate
4 = water-soaking with abundant exudate

Field screening. Field inoculation can be done by spraying a concentrated (10^9 cfu/ml) bacterial suspension on plants at the tillering stage. This should be done in the afternoon to take advantage of night-time dew formation, which increases the chances of successful infection through leaf stomata. Approximately 200 Petri dishes containing a pure culture of a single *X. t.* pv. *undulosa* strain cultured on Wilbrink's medium are needed to inoculate half a hectare. After two days' incubation at 30°C, wash the agar and suspend the bacteria in water to produce highly concentrated inoculum (**Figure 2.17 a**). The inoculum can be prepared in the laboratory or in the

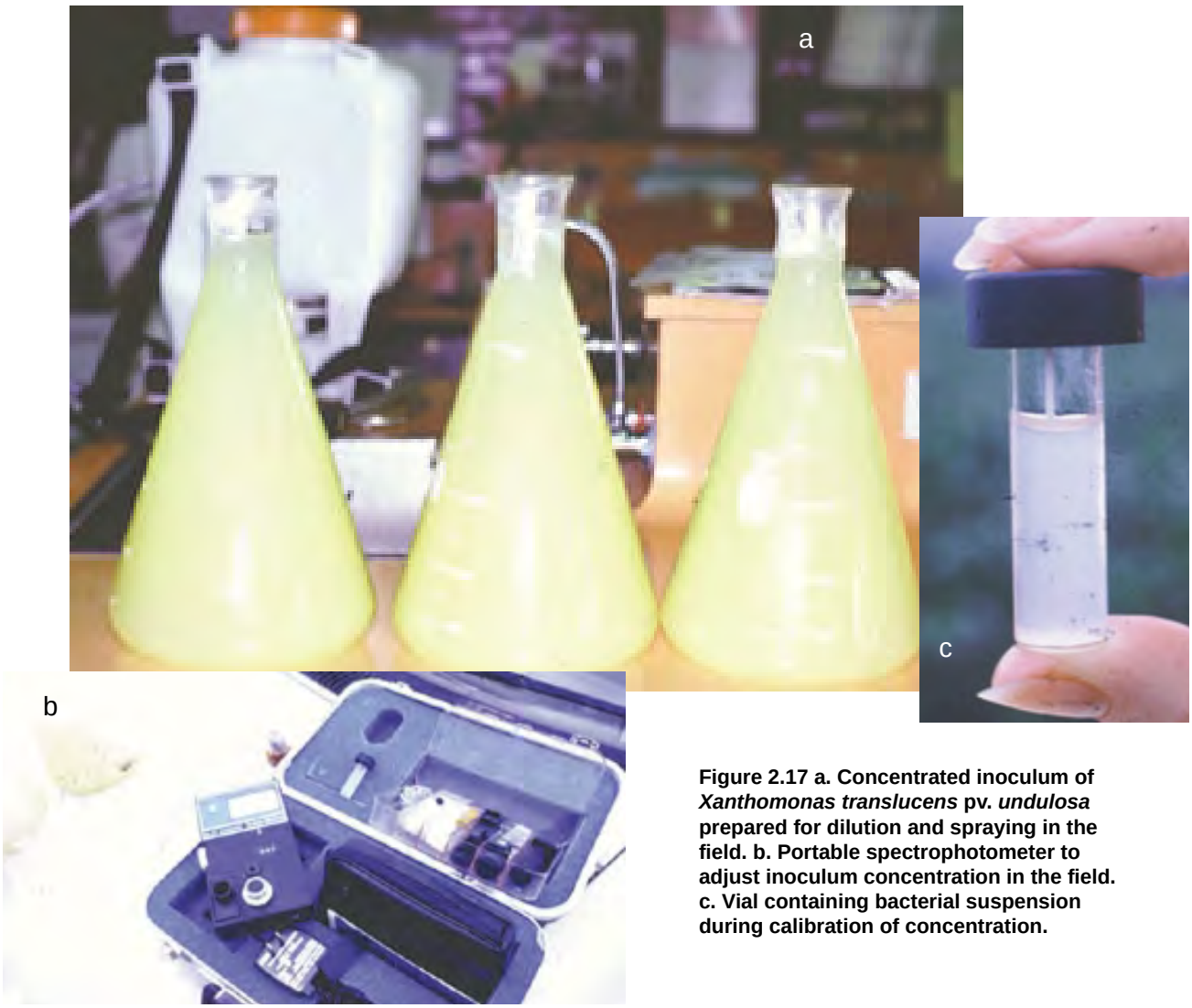
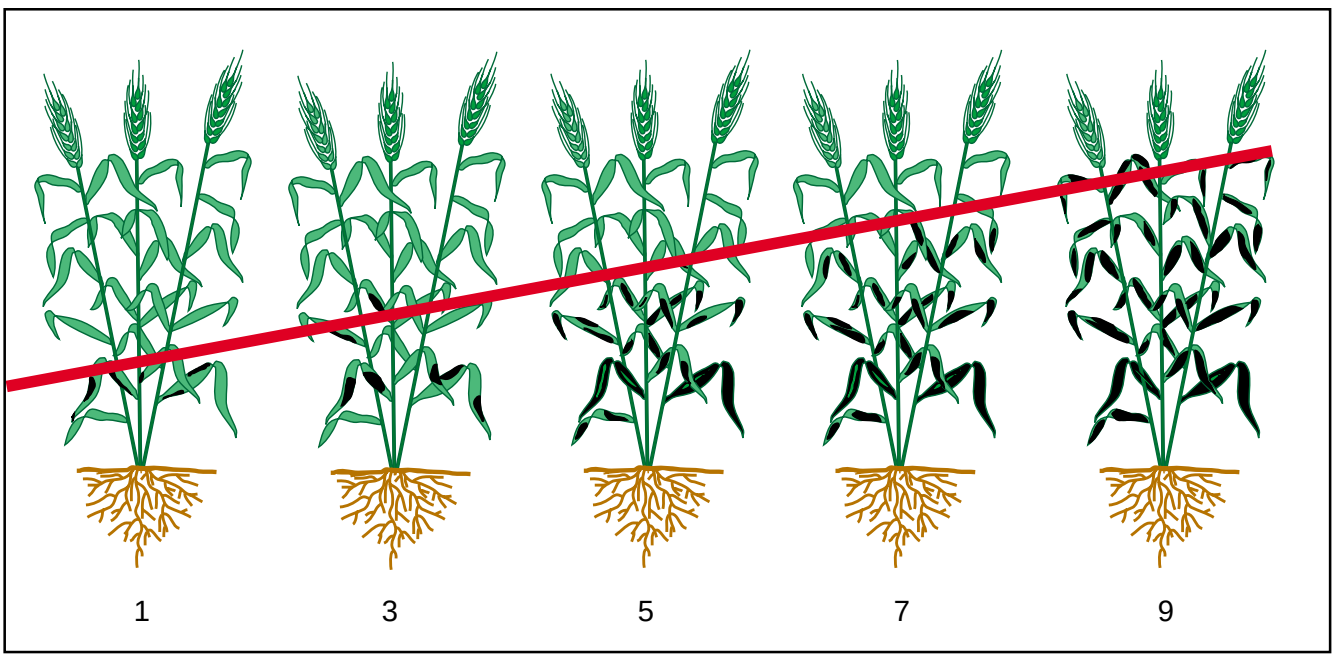


Figure 2.17 a. Concentrated inoculum of *Xanthomonas translucens* pv. *undulosa* prepared for dilution and spraying in the field. b. Portable spectrophotometer to adjust inoculum concentration in the field. c. Vial containing bacterial suspension during calibration of concentration.

Figure 2.18. The 0-9 scale proposed by Saari and Prescott (1975) for appraising the intensity of wheat foliar diseases.



field. The concentration can be determined with a portable spectrophotometer at 545 nm (Spectronic Mini 20, Milton Roy Co., Rochester, NY) (**Figure 2.17 b and c**). The aim is to establish the dilution factor necessary to prepare a 10^9 cfu/ml inoculum suspension for spraying. Alternatively, inoculum calibration can be done in the lab by doing a cell count with the help of a Petroff- Hausser counting chamber, or by estimating the number of Petri dishes covered with a 48-h culture that are necessary to prepare the final inoculum. After adjusting the concentration, add Tween 20 (0.02%) to the inoculum to facilitate the spread of the liquid over the leaf. The inoculum suspension (approximately 20 ml/m^2) is applied using a back pack sprayer at 3 lb/cm^2 pressure. Inoculation can be carried out at Zadoks' DC30-35 stage (Zadoks *et al.* 1974; Duveiller 1990a), and may be repeated if necessary.

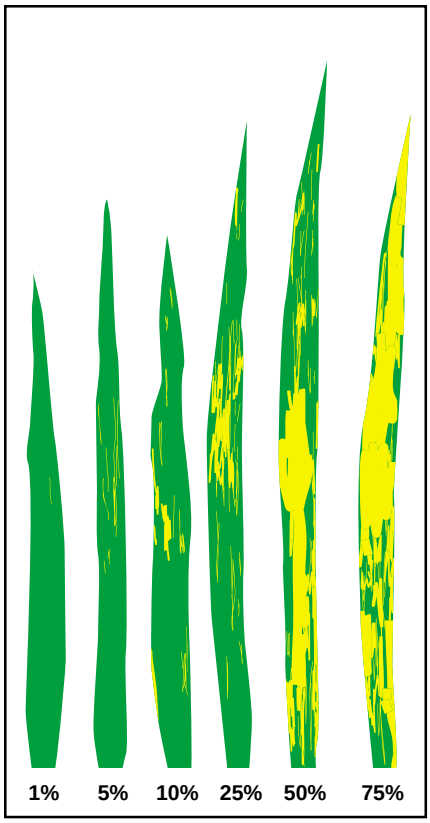


Figure 2.19. Standard disease assessment key showing percentages of leaf surface covered by bacterial leaf streak in bread wheat (Duveiller 1994a).

The disease progresses up a vertical gradient as shown by a smaller damaged leaf area on the flag leaf than on the flag leaf minus one (F-1). The disease progresses upward, and disease severity is assessed at flowering (Zadoks' DC64). The scale proposed by Saari and Prescott (1975) (**Figure 2.18**) for evaluating the intensity of foliar diseases in wheat and barley can be used for screening purposes; it may be modified by adding a second digit for scoring damaged leaf area. This scale is not appropriate for a precise assessment of quantitative resistance or for doing epidemiological studies, for which scales based on actual percent severity are preferred. New scales have been proposed to score severity of leaf damage in wheat and several other small grain cereals such as triticale, barley and rye (Duveiller 1994a) (**Figure 2.19**).

To evaluate disease severity in F_3 populations where a single row represents the offspring of a single F_2 plant, Duveiller *et al.* (1993) considered doing three measurements per row to distinguish segregating lines from non-segregating ones and to identify entries expressing more resistance or more susceptibility than either parent in the cross:

- The lowest and highest severities observed on individual plants within a row were recorded as S_{Min} and S_{Max}, respectively.
- The relative frequency of these two scores was estimated using the following 1-6 scale for assessing distribution of severity within a row:

1 = 1-15% plants with S_{Max} (and 85-99% plants with S_{Min});
2 = 16-35% plants with S_{Max};
3 = 36-65% plants with S_{Max}, indicating more or less equal distribution of S_{Min} and S_{Max};
4 = 66-85% plants with S_{Max};
5 = 86-99% plants with S_{Max};
6 = even severity (no segregation) within the row, $S_{Min} = S_{Max}$.

Disease rating can be done at flowering and again at early dough (Zadoks' DC80). Scores within the disease severity range of either of the respective parents are not considered different from that parent (Figures 2.18 and 2.19).

Immunity does not occur with BLS. Since resistance is incomplete, it is not easily observable under strong disease pressure. Disease may occur even in seemingly resistant parents, provided inoculum pressure is sufficiently strong and the disease has enough time to develop. Although BLS resistance has been identified globally in wheat (Akhtar and Aslam 1985; Bamberg 1936; Boosalis 1952; Thompson and Souza 1989; Duveiller 1990a and 1992; El Attari *et al.* 1996; Hagborg 1974; Milus and Mirlohi 1994; Milus *et al.* 1996), very little information is available on its mode of inheritance (**Table 2.3**).

In the past, resistance to the bacterium was sometimes thought to be controlled by a single genetic factor, *BcBc*, by people who erroneously interpreted and cited

work by Woo and Smith (Nelson 1973). In fact, what Woo and Smith (1962) studied under greenhouse conditions was the inheritance of melanism on the glumes, which they misleadingly referred to as "black chaff." Inoculation with bacteria was not mentioned in the article. Moreover, their study was conducted on a genotype that was highly resistant to stem rust and thus may have carried the *Sr2* gene for stem rust resistance associated with the expression of pseudo-black chaff under stress conditions. Hence, this work was in fact not a study on the inheritance of BLS resistance. Similarly, genetic studies of black chaff resistance by Waldron (1929) and Pan (1940) are misleading because these authors used parents such as Hope and H44 under natural conditions. The observed response was probably caused by pseudo-black chaff and not by the bacterial disease known as black chaff.

Recent research conducted in the field in Mexico involved a full combination of crosses and analysis of data obtained on two dates during an artificially induced epidemic (Duveiller *et al*. 1993). A single strain of *X*. *t*. pv. *undulosa* was used to reduce pathogen variability and enhance the reliability of this study. Results showed that BLS or black chaff resistance in five wheat lines (Turaco, Alondra, Angostura, Mochis and Pavon) is conditioned by five genes for which the names *Bls1/bls1*, *Bls2/bls2*, *Bls3/bls3*, *Bls4/bls4*, and *Bls5/bls5* have been proposed (**Figure 2.20)**. Genotypes Pavon and Mochis showed the highest level of resistance. None of the five genotypes contained the full set of identified resistance genes, which suggests there are cultivars with more resistance than Pavon and Mochis. It is likely that an accumulation of diverse genes associated with one or more resistance mechanisms will confer increased resistance levels in the field.

A study conducted on triticale indicated the presence of a single dominant gene in each of three BLS resistant lines: Siskiyou, M2A-Beagle and OK 77842. The three genes are either closely linked or the same gene (Johnson *et al*. 1987). The generally higher susceptibility of triticale compared to wheat is not due to the presence of the 6D/6A substitution favored by the empirical selection of complete triticales (Duveiller 1992).

Table 2.3. Wheat genotypes reported to possess resistance to bacterial leaf streak.

Winter wheat	
• Magnum, Bayles, Sawyer Terral 101	Milus *et al*. (1996)
Spring wheat	
• McMurachy Sonora 67/Tezanos Pintos Precoz	Hagborg (1974)
• Sonalika, Blue Silver Jou-Har-79 C-273	Akhtar and Aslam (1986)
• Thornbird	Duveiller (1994)
• Pavon 76, Mochis T88, Nanjing 8331	Duveiller *et al*. (1993)

Figure 2.20. Distribution of postulated genes for resistance to bacterial leaf streak among five bread wheat genotypes (Duveiller *et al*. 1993).

Conclusions

Bacterial leaf streak is a sporadic but widespread disease of wheat that can cause significant losses. The major problem is that the disease is seed-borne. Although zero tolerance of bacteria in the seed is not required due to its low transmission rate, there is a very real possibility that primary inoculum may increase during seed multiplication. The risk of disease is variable in many wheat growing areas of the world, but the possibility of it occurring in areas where it is not usually found should not be overlooked. Fortunately, a specific succession of events is necessary to induce an epidemic. If one of the events required for disease development does not occur, the epidemic may not materialize. Black chaff incidence, severity and distribution may thus vary from year to year, even in disease-prone areas.

Epidemics of bacterial leaf streak may occur in various scenarios. This explains why the disease has a global distribution and is sporadic in areas as different as sprinkler-irrigated wheat fields in the USA, Mexican highlands characterized by marked daytime temperature changes, and the Southern Cone countries of South America, where warm and cloudy days may occur alternately. Because disease occurrence is sporadic, research on epidemiology and resistance is particularly difficult and, consequently, advances in controlling BLS are slow.

Discarding infected seed prior to planting should be the primary control measure, since sowing pathogen-free seed is the first logical step in avoiding an outbreak. Seed indexing procedures are not routinely practiced in many places but should be encouraged. The apparent absence of races and the widespread distribution of the pathogen are not convincing reasons for not implementing seed health procedures to limit the initial inoculum. Foundation seed should be multiplied in disease-free areas where climatic conditions are unfavorable for the development of epidemics. Seed should be disinfected before sowing even if currently available seed treatments are not fully satisfactory. Wheat growers should keep in mind that dry environments do not hamper the multiplication of *X. t.* pv. *undulosa* once it is in the leaf and that temperature has a major effect on pathogen multiplication in leaf tissue.

The most economical and environmentally friendly way of controlling BLS is through genetic resistance, and sources of incomplete genetic resistance have been identified. Differences in the degree of susceptibility are more easily observed in the field in disease-prone areas where artificial epidemics allowing the consistent differentiation between susceptible and resistant genotypes can be induced. Screening for resistance should be encouraged in areas where pathogen populations present the most variation.