SEED TRANSMITTED BACTERIAL DISEASES OF CEREALS: EPIDEMIOLOGY AND CONTROL

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Abstract

Sands, D.C., G. Mizrak V.N. Hall, H.K. Kim, H.E. Bockelman and M.J. Golden 1986. Seed transmitted bacterial diseases of cereals: epidemiology and control. Arab J. Pl. Prot. 4: 127 - 125

Two bacterial diseases of cereal grains in the Middle East are bacterial leaf streak, also known as black chaff, caused by *Xanthomonas campestris* pv. *translucens* and bacterial leaf blight caused by *Pseudomonas syringae*. The former is a seed transmitted pathogen, virulent on almost all cultivars of wheat, barley, and triticale. Control is best implemented by use of clean seed (hot water treatment at 53°C for 10 minutes, immediately cooled and dried) and subsequent isolation of breeder and foundation seed fields. The genetics of resistance in barley is only partially defined, but promising resistant material is from Ethiopian sources. The second disease is only a problem if there are prolonged periods of moisture with near freezing temperatures. Seed treatment is not useful. Semi-selective media for both pathogens are described. Both bacteria are ice nucleation active and this phenomenon will be discussed in terms of their epidemiology and dispersal.

Additional key words: bacterial leaf streak, bacterial leaf blight, seed treatment, cereals.

Introduction

Bacterial diseases are usually ignored by cereal pathologists because the rusts, smuts, leaf spotting fungi, and powdery mildews elicit obvious symptoms that appear to be more important in terms of disease losses. In addition, most plant pathologists are not trained in bacteriology, and these pathogens cannot be identified solely by colony type or morphology. There appears to be a resurgence of these diseases either because their symptoms are more obvious as we now have better control of the afore mentioned fungi, or because of the curtailment of mercurial seed treatments. Perhaps the recent awareness of these diseases is due to the new and simpler methodology developed in phytobacteriology in the last 15 years. We have worked on some of the bacterial diseases of cereals for the past nine years and this report will review our findings with *Pseudomonas syringae*, an epiphytic microbe with a leaf blighting phase, and *Xanthomonas campestris* pv *translucens*, the leaf streaking and black chaff pathogen.

***Pseudomonas syringae:* Descriptive Methodology and Symptoms** *Pseudomonas syringae* is the specific nomen of a large group of phytopathogenic bacteria. Computer analysis of a wide array of these bacteria indicates that some strains are host specific, whereas others can infect a wide range of hosts (7). Epiphytic ranges have not been studied, but it is apparent that symptoms are not elicited by many epiphytic strains, unless very high population (10^7 per leaf) are reached. These bacteria are gram negative, polarly flagellated, fluorescent on King's Medium B, oxidase negative, and have a maximum temperature growth of about 32° C (8). They are cool temperature pathogens, can grow slowly at 4°C, and a majority of cereal isolates initiate ice formation at - 3 to - 5° C. Symptoms are difficult to define because of their diffuse nature, and their variability dependent on host cultivar. Leaf symptoms start as watersoaking along tip margins and in curved sections of the leaf where free water remains the longest. Apparently epiphytic growth during moist periods leads to internal population buildup, resulting in either watersoaking or intercellular invasion. Symptoms are hypersensitivity, a white bleaching of the leaf, or watersoaking followed by light brown necrotic spots with chlorotic margins, or a combination of these symptoms. The flag leaf is often the most seriously affected, perhaps because it is formed predisposed by mobilization of plant formed nutrients into seed production.

***Pseudomonas syringae:* Isolation Techniques**

Moisture at heading can lead to shrunken, darkened kernels, that are sometimes fluorescent with black light (long wave length uv). These bacteria can be isolated in high numbers (10^6 / gram of leaf) on selective media during the watersoaking phase, but if the leaves have dried, isolation is much more difficult. Small leaf pieces are soaked in a drop of ster-

ile distilled water and the water is streaked-out after 1/2 hour, 4 hours, and overnight. The selective medium is BCBRVB (8), a highly selective medium containing 10 mg bacitracin, 0.5 mg rifampicin, 6 mg vancomycing, 250 mg benomyl, and 75 mg cycloheximide per liter. The base agar medium is medium B of King, et al. (5), containing per liter of distilled water 20 g Difco protease peptone number 3,17 ml glycerol, 2.5 g K_2HPO_4 $3H_2O$, 6 g $MgSO_4$ $7H_2O$, and 15 g Agar. The antibiotics are sterilized with ethanol after cooling. Iron in the water will prevent or quench the fluorescence of these bacteria. Fluorescence is observed with a long wavelength uv light.

Taxonomic and Host Range Considerations

We include all these bacteria in the *Pseudomonas syringae* group without designating pathovars. They do not seem to be confined to monocots (3). Host range within the Gramineae is not restricted and includes all wheats, rye, oats, barley and triticale, and many native grasses. Wheat and barley symptoms in years that are wet and cool range from 1 to 7 on a scale of increasing severity of 0 - 9, depending on cultivar. Temperatures over 33° C on dry sunny days can sharply curtail disease development.

Ice Nucleation and Its Effects

We have found that these bacteria can cause ice to form at - 3 to - 5° C, and that dew condensation activity (10) is also associated with the presence of these bacteria on a leaf surface. These unique characteristics may be important in frost injury and dissemination of these bacteria. We have reported these bacteria in ice clouds 1700 meters above infected wheat fields (9). The possibility indicates that these bacteria are involved in a proposed Bioprecipitation Cycle (9) where they are swept into the atmosphere leading to downwind ice nucleation and hence rainfall. The desertification of areas may arise from overgrazing of plants, leaving few epiphytic bacteria to initiate condensation and rainfall. Our current research is directed to finding nurse crops that harbor high numbers of these bacteria, with the idea that these could enhance precipitation.

Bacterial Leaf Streak

This disease of cereals is caused by *Xanthomonas campestris* pv *translucens*. First described as *Bacterium translucens* by Erwin F. Smith in the early 1900's, this bacterium has changed nomenclature several times. Hagborg (2) designated several forma speciales based on host specificity, however, the current nomenclature, *Xanthomonas campestris* pv *translucens* denotes only that the hosts are in the Gramineae.

***Xanthomonas:* Symptoms**

Symptoms on triticale and barley are similar - a yellow watersoaked streak of the leaves. Symptoms on the wheat leaves are less distinct than spots with rust colored margins. Head symptoms are termed black chaff, actually a brown shellac on the glumes and on the seed. Characteristic is a thin transparent layer of bacterial ooze, that when dry can be peeled off the leaves or glumes.

These bacteria are gram negative, aerobic rods, polarly flagellated, slow growing, with a maximum temperature near 33° C. Distinguishing features are their slow growth, even at their temperature optimum of 26 - 30° C, and their orange carotenoid pigments, termed xanthomonadins. They are always ice nucleation positive, but they vary considerably in maximum temperature of activity.

***Xanthomonas:* Isolation**

A commonly used medium for growth of this bacterium is Wilbrink's medium (1) with high peptone where yellow orange colonies are visible after 48 - 72 hours. Wilbrink's medium contains 5 g Bacto peptone, 10 g sucrose, 0.5 g K_2HPO_4, .25 g $MgSO_4$, 7 g H_2O, 0.05 g Na_2SO_3 (anhydrous), 15 g Bacto agar, 1 liter distilled water, and 75 mg cycloheximide or 250 mg benomyl (Dupont) all added in 2 ml ethanol after cooling.

The X - Gal Test for *Xanthomonas*

A new diagnostic tool developed in our laboratory is the «X-gal» test. A colorless substrate 5 - bromo - 4 - chloro - 3 - indolyl ß - D - galactopyranoside (Sigma Chemical Co.) or «X - gal» is cleaved by *Xanthomonas campestris* pv. *translucens* to form a blue dye. In Wilbrink's agar with lactose instead of glucose with X - gal at 20 mg/1, the blue reaction is sometimes yellow-green. The reaction develops slowly and is more reliable if the grown plates are refrigerated for a few days. Few other bacteria associated with plants give this reaction. A small piece of infected tissue placed in one drop of a 50 mg/1 solution of X - gal will give a blue reaction in 2 hours or longer.

***Xanthomonas:* Field Inoculation**

An inoculum of 2 X 10^6 cells per ml is sufficient for field inoculation. The inoculum is Wilbrink's broth, without cycloheximide, diluted after growth at room temperature in shake culture. Field inoculation methods developed by Gurbuz Mizrak (6) involve mowing and spraying spreader rows planted every third row in a test plot after inoculation. Our most reliable inoculation methods involved evening inoculation on wet days. Sprinkler irrigation and inoculation early in the season are keys to confluent infection. Symptoms appear in 10 to 14 days, and several infection cycles are required during the growing season. Warm (26 - 30° C) moist conditions favor the disease, especially during heading.

***Xanthomonas:* Resistance**

H.K. Kim (4) identified several resistant cultivars in barley, mostly of Ethiopian origin, and Gurbuz Mizrak followed resistance through the first backcross. The F_3 plants are now being studied for segregation. Several strong sources of resistance are apparent from these studies and these will be reported in greater detail. A barley recurrent selection population with strong selection for *Xanthomonas* resistance is in its fifth year cycle.

***Xanthomonas:* Seed Transmission and Dissemination**

Seed transmission is the major mode of dissemination of this pathogen. Seed treatment was found to be an effective control-immersion 10 minutes at 53° C followed by immediate cooling. In the 50's and 60's, mercurial seed treatments were very effective against this pathogen, however, these

treatments are no longer available because of their acute human toxicity. Secondary spread from a single infected plant can increase to thirty square meters during a growing season. This disease is termed a breeder's disease because most field infections can be traced to infestation of crossing blocks, yield trials, or foundation seed.

Acknowlegement

This work supported in part by a cooperative Montana State University-ICARDA contract with USAID.

الملخص

سانـدز، د. س.، خ. ميـزراك، ڤ. ن. هـول، أش. ك. كيم، أش. أ. بوكلمان وم. ج. غولدن. 1986. الانتشار الوبائي ومكافحة أمراض الحبوب البكتيـرية التي تنتقـل بواسـطة البذور. مجلة وقـاية النبات العربية 4: 125 - 127

إن المرضين الناتجين عن إصابة محاصيل الحبوب بالبكتريا في منطقة الشرق الأوسط هما تخطط الأوراق البكتيري الناتج عن الإصـابـة بـالبكتـريـا *Xanthomonas campestris* pv. *translucens* ولفحـة الأوراق البكتريـة الناتـج عن الإصـابـة بـالبكتـريـا *Pseudomonas syringae* . إن البكتـريـا المسببـة للمـرض الأول تنقل بـواسطة البـذور وتصيب جميـع أصنـاف القمح والشعير والترتيكالي. إن أفضـل الطرق لمكـافحة هـذا المرض عن طريق استعمال البذور الخالية من البكتريا. ويمكن التخلص من البكتـريا في البـذور عن طريق معـاملتها بـالمـاء الساخن عند درجة حرارة 53° م لمـدة عشر دقـائق ثم تبريـدها وتجفيفهـا بسرعـة. يضـاف إلى ذلـك ضـرورة عـزل الحقـول المستعملة بواسطة مربى النبات وحقول إكثار البذور المختارة.

إن كيفية توريث صفة المقاومة في الشعير ليست معروفة كلياً، والأصـول الوراثيـة التي عندهـا صفة المقـاومة هي من أصـل حبشي. أما المرض الثـاني فهو يسبب مشكلة فقط عنـد توفـر فترات طويلة من الرطوبة عند درجـات حرارة قـريبة من درجـة التجمـد. إن معالجـة البـذور لمكـافحـة هـذا المـرض ليست مفيـدة. سـأتعـرض في هـذه الـدراسـة إلى البيئـات النصف متخصصة بالنسبة لنوعي البكتـريا المـذكورين أعـلاه. ويجدر الإشارة بأن هذين النوعين هما من الأنواع التي تكون نواة ثلجية على سطح الورقة وسنتعرض لهذه الـظاهرة وعـلاقتها بـانتشار المرض.

كلمـات مفتاحيـة : تخطط الأوراق البكتيـري، لفحـة الأوراق البكتيرية، معاملة البذور، نجيليات.

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المراجع