Sunflower Stalk Diseases Initiated Through Leaf Infections

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Stalk diseases in sunflower (*Helianthus annuus* L.) can be broadly categorized into two major groups: those that are initiated through leaf infections that progress to the petiole attachment on plant stems, and those diseases caused by soilborne pathogens infecting plants through the roots. This article will concentrate on comparing and contrasting three stalk diseases on sunflower that originate via leaf infections: *Phoma* black stem, *Phomopsis* stem canker, and bacterial stalk rot.

Host: Sunflower (*H. annuus* L.)

Disease: *Phoma* black stem.

Pathogen: *Phoma macdonaldii* (syn. *Leptosphaeria lindquistii* Frezzi) (Frezzi 1968). Originally classified as *Phoma oleracea* var. *helianthi-tuberosi* Sacc. (MacDonald 1964), which is a synonym of the saprophyte *Phoma herbarum* Westend. *P. macdonaldii* is tentatively being reclassified as *Plenodomus lindquistii* (Frezzi) Gruyter, Aveskamp & Verkley (Aveskamp et al. 2008). Other species of *Phoma*, all saprophytes or opportunistic pathogens, have been isolated from various *Helianthus* species on live or overwintered tissue, including *Phoma nebulosa* (Fr.) Mont., *P. septicalis* Boerema (*Coniothryium telephii* [Allescher] Verkley & Gruyter), *P. acutata* Fuckel (*Leptosphaeria dolium* [Fries] Cesati & de Notaris), and *P. sanguinolenta* Grove (*L. purpurea* Rehm) (Boerema et al. 2004).

Taxonomy: Kingdom Fungi; phylum Ascomycota; subdivision Pleosporomycetidae; class Dothideomycetes; order Pleosporales; family Leptosphaeriaceae; genus *Leptosphaeria*; and species *L. lindquistii*.

Symptoms and Signs

Stem lesions most frequently result from leaf infections that progress down the petiole to the stem (MacDonald 1964; Maric and Schneider 1979) (Fig. 1). Airborne ascospores of *L. lindquistii* germinate in guttation drops at leaf margins and colonize major leaf veins, turning them necrotic, with little or no chlorosis, and thus the foliar disease phase is often overlooked. Once the pathogen reaches

the petiole, it becomes noticeably black, and the entire leaf blade wilts as the water supply is curtailed. The initial infections usually occur on lower leaves of the canopy, thus further escaping notice. As the pathogen reaches the base of the petiole, a black circular to elliptical lesion will form that can eventually encircle the stem (Fig. 2). The margin is often distinctly delimited and seldom exceeds 5 to 6 cm in length. In contrast, a *Phomopsis* stem canker lesion is light to dark brown in color and can achieve 15 to 20 cm in length (Fig. 3). *Phoma* lesions are also usually superficial, and there is no pith degradation (Fig. 4), whereas a *Phomopsis* lesion will result in pith degradation to the point of producing a hollow stem and eventual lodging at that point (Fig. 5). Once mature, the small, black pycnidia, mostly buried in the epidermis, may be observed with a hand lens of 5 to 10×. The fruiting structures of the sexual stage (*L. lindquistii*), properly termed pseudothecia, will form after the stems have overwintered (Donald et al. 1986; Frezzi 1968).

Basal stem lesions (Fig. 6), occurring at the soil line, can occur with *Phoma*-contaminated seeds, or owing to early seedling infection via roots (Al Fadil et al. 2009; Donald et al. 1987; Masirevic et al. 2014). Basal stem lesions are similar in appearance to those occurring at leaf axils. When stem-feeding insects such as *Apion* and *Cylindrocopturus* stem weevils oviposit in leaf axils, a stem lesion will form without leaf infection. When the eggs hatch, the *Phoma*-contaminated larvae will burrow upward into the pith, carrying *Phoma*, and cause extensive pith degradation, premature maturity, and death of the plant (Donald and Venette 1983; Gaudet and Schulz 1984; Gulya and Charlet 1984).

Host Range

The primary host of *P. macdonaldii* is cultivated sunflower (*H. annuus* L.). The pathogen has also been isolated from lesions on annual and perennial wild *Helianthus* species, all native to North America. It does not infect other Asteraceae genera, but there are many other *Phoma* species reported on other Asteraceae (Boerema et al. 2004).

Geographic Distribution

P. macdonaldii, originally identified in North America (MacDonald 1964), has been recorded from many countries in North and South America (Bruni 1965; Gulya and MacArthur 1984; Kandel and Gulya 2016), Europe (Maric and Schneider 1979; Penaud and Peres 1994), Asia, Africa, and Australia (Miric et al. 1999; Wu et al. 2012). In contrast to its impact on the U.S. crop (Carson 1991), unless vectored by stem-boring insects, *P. macdonaldii* is felt to be a significant pathogen on sunflower in Europe, where significant yield losses have been reported from France (Penaud and Peres 1994).

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Pathogen Isolation

Isolation from diseased plants, whether the lesion be on stems, petioles, or leaves, is best done with a semiselective medium because *P. macdonaldii* can be overgrown with saprophytic *Alternaria*,

Rhizopus, and other genera. One semiselective medium (Donald and Venette 1983), specifically tested with *P. macdonaldii*, utilizes several fungicides, whereas another generic *Phoma*-selective medium relies on manganese chloride to exclude other fungi (Sørensen et al.

FIGURE 1

Early stem lesions forming at petiole attachments.

FIGURE 2

Multiple lesions on sunflower stems due to Phoma black stem. Note some have completely encircled stems.

FIGURE 3

Infected sunflower stem exhibiting lesions caused by both *Phomopsis* stem canker (top) and *Phoma* black stem (bottom).

FIGURE 4

Sunflower field after harvest showing degree of infection by *Phoma* black stem. Note lack of stalk lodging.

2009). Fungal growth of pure cultures can be done on conventional mycological media, including acidified potato dextrose agar (APDA, pH is adjusted to 4.5 to 5.0 using lactic acid), potato dextrose agar (PDA), potato carrot agar, oatmeal agar (OA), and malt agar (MA) (Boerema et al. 1994; Larfeil et al. 2002; Maric and Schneider 1979).

Pathogen Identification

P. macdonaldii belongs to the section *Plenodomus*, as currently categorized by Boerema et al. (1994) and other *Phoma* taxonomists. The 26 species of *Phoma* in this section are characterized by thick-walled pycnidia, with a resultant small lumen bearing the pycniospores. The standard media for *Phoma* identification are APDA and OA, optimal for pycnidia production, and MA, optimal for crystal production (Noordeloos et al. 1993). On APDA, ivory-white colored colonies that have a fluffy appearance are produced in

3 days after incubation. A large number of brown-colored pycnidia will be formed on APDA, either separately or in clusters; from the ostiole of the pycnidia, a pale pink gelatin-like substance (pycnidiospores) may be exuded (Fig. 7). On OA, *P. macdonaldii* generally displays a 7-day growth rate of 25 to 45 mm and has whitish, cottony aerial mycelium. There is a great deal of variability in *P. macdonaldii* colony morphology, however, and colony color may vary from white to gray to olive green (Fig. 8). The colony reverse is generally citrine to olive green, often with white (occasionally apricot) granular crystals. Old cultures may display a pink or red color (Fig. 9), which characteristically turns blue or purple with a drop of 1 N NaOH. Pycnidia production varies considerably between isolates, ranging from 15 to 50/mm² (Roustae et al. 2000). Pycnidia in culture are subglobose, spherical, or oblate with very short or no necks, and they range in size from 70 to 10 µm, while on dead stems they may reach 300 µm in diameter. Conidia (pycniospores) are highly variable in size, generally in 5 to 9 × 1.5 to 3 µm range, single-celled, oval-shaped, and are eguttulate (lacking oil droplets, which are characteristic of other *Phoma* species).

Pathogen Storage

For a thorough discussion of various long-term storage methods, consult Dhingra and Sinclair (1995). Agar slants, covered with sterile mineral oil and held at 4°C, will preserve viability of *P. macdonaldii* for up to a year. Another method, tested with *P. macdonaldii*, involves growing the fungus on sterilized sunflower stem pieces until pycnidia form and then storing the colonized tissue under calcium chloride granules at 6°C (Larfeil et al. 2002). Longer term viability is best accomplished with storage at -80°C or in liquid nitrogen.

Pathogenicity Tests

Although root immersion with a mycelial suspension can successfully initiate *Phoma* premature ripening (Donald et al. 1987), it is time consuming, and all current inoculation methods use conidia (10⁶ conidia/ml; Larfeil et al. 2002; Seassau et al. 2010) or mycelial plugs on stems, with or without wounding (Dedić 2012; Larfeil et al. 2002; Roustae et al. 2000; Seassau et al. 2010). Greenhouse inoculations of 12-day-old plants may be evaluated within 1 to 2 weeks postinoculation, and the results agree with field observations of mature plants. Lesion length, rated on a 1 to 9 scale (Seassau et al.

FIGURE 5

Sunflower field at harvest badly affected by *Phomopsis* stem canker. Note the numerous lodged plants.

FIGURE 6

Phoma black stem lesions girdling stems at the soil surface.

FIGURE 7

Mature pycnidia characteristic of *Phoma* with conidia oozing out.

2010), rather than the rate of lesion expansion, is the best indicator of plant resistance (Schwanck et al. 2016), but the authors also noted that leaf number and plant height were highly correlated with *Phoma* resistance, and thus morphological characters are predictive of resistance as well. Owing to its low economic impact on U.S. sunflower, there is scant information on the performance of public germplasm. In contrast, the bulk of research on resistance evaluation is being done in France and other EU countries.

Host: Sunflower (*H. annuus* L.)

Disease: Phomopsis stem canker.

Pathogens: *Diaporthe helianthi* Muntañola-Cvetkovic, Mihaljcevic and Petrov (syn. *Phomopsis helianthi* Muntañola-Cvetkovic, Mihaljcevic and Petrov) (Hajdu et al. 1984; Herr et al. 1983; Mathew et al. 2015a; Meyer et al. 2009; Yang et al. 1984), *Diaporthe gulyae* Shivas, Thompson and Young (Mathew et al. 2015a; Thompson et al. 2011), *Diaporthe stewartii* Harrison (Olson et al. 2017), *Diaporthe kongii* Shivas, Thompson and Young (Thompson et al. 2011), *Diaporthe*

kochmanii Shivas, Thompson and Young (syn. *Diaporthe sojae* Lehman; Thompson et al. 2011), and *Diaporthe novem* Santos, Vrandečić and Phillips. Four other species of *Diaporthe* have been isolated either from seeds, stems, roots or residue of sunflower in the United States or Australia (*Diaporthe longicolla* (Hobbs) Santos, Vrandecic and Phillips, *Diaporthe masirevicii* Shivas, Morin, Thompson and Tan; *Diaporthe miriciae* Shivas, Thompson and Tan [syn. *Diaporthe sojae* Lehman], *Diaporthe sackstonii* Shivas, Thompson and Tan [F. Mathew and T. Olson, unpublished; Thompson et al. 2015]). *Diaporthe serafinae* Shivas, Thompson and Tan was isolated from the seed of ornamental sunflower, *H. annuus* (Thompson et al. 2015).

Taxonomy: Kingdom Fungi; division Ascomycota; subdivision Pezizomycotina; class Sordariomycetes; order Diaporthales; family Diaporthaceae; genus *Diaporthe*; and species *D. helianthi*, *D. gulyae*, and other species of *Diaporthe*.

Symptoms and Signs

Lesions initially appear as brown spots with a yellow chlorotic margin at the edge of older leaves of the sunflower plants (Fig. 10). As the disease spreads through the leaf veins and petioles, they become necrotic and cause death of the entire leaf. The infection spreads through the petioles, where moisture accumulates, eventually reaching the main stalk of the sunflower plant. Lesions on the stem develop a wet appearance, appear tan to brown in color (Fig. 11), have a sunken border, increase in length (often reaching 15 to 20 cm), and eventually girdle the stalk, whereupon plants wilt and lodge (Fig. 12). Phomopsis stem canker causes the pith to degrade to the point that the stalk is easily crushed under moderate thumb pressure. These symptoms appear between bud initiation and flowering growth stages of the sunflower field (Herr et al. 1983; Masirevic and Gulya 1992).

Symptoms of Phomopsis stem canker can be easily confused with those produced by other pathogens. For example, lesions produced by *P. macdonaldii* on sunflower are usually smaller, black colored, entered around the nodes of the plant (Figs. 3 and 4), and do not cause pith degradation or lodging. Lesions produced by *Sclerotinia sclerotiorum* (Lib.) de Bary, the causal pathogen of Sclerotinia midstem rot, resemble those produced by species of *Diaporthe*; however, the sunflower plant has black sclerotia forming in the stem (Gulya et al. 1997).

Host Range

Between the two predominant pathogens (*D. helianthi* and *D. gulyae*) causing Phomopsis stem canker of sunflower in the world, *D. helianthi* infects sunflower (Herr et al. 1983; Mathew et al. 2015a), wild sunflower (Mihaljčević et al. 1985), *Xanthium italicum* (Vrandecic et al. 2010), *Xanthium strumarium* (Vrandecic et al. 2010), *Arctium lappa* (Vrandecic et al. 2010), and grapevine (*Vitis vinifera*) (van Niekerk et al. 2005). *D. gulyae* infects sunflower (Mathew et al. 2015a, b; Thompson et al. 2011), soybean (*Glycine max* L.) (Mathew et al. 2018; Thompson et al. 2015), and saffron thistle (*Carthamus lanatus*) (Ash et al. 2010).

Geographic Distribution

D. helianthi has been confirmed causing Phomopsis stem canker of sunflower in eastern Croatia, France, South Africa, and the United States based on culture characteristics and DNA sequence analyses (Mathew et al. 2015a; Says-Lesage et al. 2002; Udayanga et al. 2011). *D. gulyae* was confirmed causing Phomopsis stem canker of sunflower in the United States (Mathew et al. 2015a) and

FIGURE 8
Culture of *Phoma macdonaldii*.

FIGURE 9
Close-up image of older culture of *Phoma*.

Canada (Mathew et al. 2015b). In Australia, among the several species of *Diaporthe* reported on sunflower, *D. gulyae*, *D. kochmanii* (syn. *D. sojae*), and *D. kongii* cause Phomopsis stem canker (Thompson et al. 2011).

FIGURE 10
Leaf lesion characteristic of infection by Phomopsis stem canker pathogens.

FIGURE 11
Light brown to tan lesion on stem of plant affected by Phomopsis stem canker.

Pathogen Isolation

Species of *Diaporthe* can be easily isolated from infected sunflower stalks on PDA (Becton, Dickinson and Company, Franklin Lakes, NJ, 39 g/liter, 200 mg of streptomycin, pH 6 or following the protocol of Leslie and Summerell [2006]). Stem samples are washed in tap water for 2 min and cut into pieces (approximately 1 cm long). The pieces are surface sterilized in sodium hypochlorite (0.05%) and ethanol (70%) for 1 min each, rinsed in autoclaved distilled water, blotted between sterile filter papers, and placed on PDA. Plates are incubated at 25°C for 7 to 14 days under 12 h of alternating light and dark conditions. Cultures are scored for presence of *Diaporthe* based on morphological characteristics such as color and appearance of colony, arrangement and shape of pycnidia, and size of spores (Barnett and Hunter 1972; Mathew et al. 2015a; Thompson et al. 2015, 2011).

Pathogen Identification

For *D. helianthi*, colonies on PDA form less abundant white mycelium along with narrow greenish-yellow areas (Fig. 13, left). Reverse of culture on PDA appears white to tan color and has light brown scattered spots initially that later turn dark brown. The pycnidia formed are usually aggregate, rarely solitary, measuring 240 to 450 × 230 to 380 μm. They contain two types of conidia (Fig. 14): alpha-conidia, which are unicellular, colorless, elliptical, and 8 to 21 × 1.7 to 5.5 μm; and beta-conidia, which are unicellular, filiform, and 16 to 42 × 0.5 to 6.9 μm. The proportions of alpha- and beta-conidia can vary between *D. helianthi* isolates and with production under natural conditions or in culture. For example, *D. helianthi* isolates from Minnesota, North Dakota, and South Dakota have only beta-conidia formed in culture, and they do not germinate (at least under laboratory conditions) (Mathew et al. 2015a). The ascoma (perithecia) of *D. helianthi* are 350 to 450 μm in diameter with a prominent beak 350 to 600 μm long on overwintered crop debris. Asci are colorless, 8-spored, sac-like, and 44 to 67 × 7.5 to 12.5 μm. Ascospores are colorless, elliptical, uniseptate, and 9.2 to 19 × 2.2 to 7.5 μm.

For *D. gulyae*, colonies on PDA have scant aerial mycelium and slightly darker centers (Fig. 13, right). Pycnidia appear scattered on PDA (Fig. 15), subglobose, up to 3 mm diameter, and occasionally have ostiolate beaks up to 1 mm long. Alpha conidia are globose, subglobose, ellipsoidal, oval or obovoid, hyaline, and 6.0 to 10.0 × 2.5 to 3.5 μm. Beta conidia have not been observed in culture on PDA (Mathew et al. 2015a; Thompson et al. 2011).

FIGURE 12
Lodging of sunflower plants severely affected by Phomopsis stem canker.

Pathogen Storage

Diaporthe isolates can be stored on PDA slants for the long term at 4°C; PDA is prepared in 60 ml glass jars following the protocol of Leslie and Summerell (2006). After autoclaving and cooling, PDA is inoculated with the mycelial plugs of *Diaporthe* isolates. The mycelial plugs should be taken from the leading edge of fresh *Diaporthe* isolates. After 2 weeks of incubation at 25°C, contents of glass jars can be placed at 4°C for up to a year.

Pathogenicity Tests

In a study by Mathew et al. (2015a), a stem-wound method was adopted to compare aggressiveness among *D. helianthi* and *D. gulyae* isolates. Mycelial plugs (4 to 6 mm in diameter) taken from the margin of the growing colony cultured on PDA were used as inoculum. The stems of sunflower plants between the V4 and V6 growth stages (four to six true leaves) were wounded on the second internode and a *Diaporthe*-infested mycelial plug placed on the wound. The mycelial plug was attached to the stem wound with Parafilm to avoid dehydration. The pots were placed on the greenhouse benches at 22 to 25°C under a 16-h light/dark cycle. Disease severity was evaluated at 14 days after inoculation using a rating scale of 0 to 5 (Thompson et al. 2011). Symptoms caused by

D. helianthi and *D. gulyae* appeared on the sunflower plants within 3 to 7 days. The Mathew et al. (2015a) study showed that *D. gulyae* produced brown-colored lesions on the stem, causing the sunflower plant to wilt, lodge, and die 3 days after inoculation. In contrast, *D. helianthi* caused tan-colored, elongated lesions at 7 days after inoculation with no plant mortality (Mathew et al. 2015a).

Host: Sunflower (*H. annuus* L.)

Disease: Bacterial stalk rot.

Pathogens: *Pectobacterium carotovorum* subsp. *carotovorum* (Jones 1901) Hauben et al. 1998 emend. Garden et al. 2003 (formerly known as *Erwinia carotovora* subsp. *carotovora* [Jones 1901] Bergey et al. 1923) and *Pectobacterium atrosepticum* (van Hall 1902) Garden et al. 2003 (formerly known as *Erwinia carotovora* subsp. *atroseptica* [van Hall 1902] Dye 1969). Further information on current bacterial taxonomy can be found (Bull et al. 2010).

Taxonomy: Kingdom Bacteria; phylum Proteobacteria; class Gamma Proteobacteria; order Enterobacteriales; family Enterobacteriaceae; genus *Pectobacterium*; and species *P. carotovorum* and *P. atrosepticum*.

Signs and Symptoms

Typical symptoms of bacterial stalk rot include green-black to black discoloration forming on the outside of stalks, and infection is aided by any type of mechanical injury. Infection often begins at entry points centered on petiole axils (Fig. 16). This is a site that collects water and serves as a favorable environment for bacterial residence and insect activity. Breakdown of tissues is odorless unless stems are in an advanced state of decomposition. In advanced stages of disease, a wet, slimy rot of internal tissues appears with a characteristic unpleasant odor of rotting potatoes. Infected stalks soften and dry up, becoming dark brown to black (Fig. 17); flowers and seeds may also be discolored. Stalks often lodge under the weight of maturing heads (Fig. 18) (Harveson et al. 2012).

Under conditions of high humidity, signs of the pathogen may be seen exuding out of wounds (Fig. 19), and severely affected plant tissues will eventually disintegrate. If the weather becomes warm

FIGURE 13

Culture growth characteristic of *Diaporthe helianthi* (left) and *D. gulyae* (right).

FIGURE 14

Both alpha and beta conidia characteristic of *Diaporthe helianthi*.

FIGURE 15

Pycnidia with masses of alpha conidia oozing out through ostioles, characteristic of *Diaporthe gulyae*.

and dry, the affected tissues will dry out and turn black. As fermentation of the sugars in the plant occurs owing to bacterial activity, exudates and gases are formed. This action may then be followed by a foam appearing on infected stems (Fig. 20). This disease often occurs after extended wet periods late in the growing season, which suggests that only stressed or senescing plants are highly susceptible. The bacterial stalk rot pathogen is readily disseminated in and among sunflower fields by splashing rain and irrigation water, and it survives between sunflower crops in infected crop debris and as a pathogen on many other plants (see Host Range below) (Harveson et al. 2012; Schwartz and Harveson 2016).

Host Range

Species of *Pectobacterium* have been reported as pathogens of species in 16 different dicot plant families in 11 orders, and of species in 11 monocot families in 6 orders (Ma et al. 2007). An exception to the wide-host-range nature of *Pectobacterium* spp. is *P. atrosepticum*, which has been reported only on potato (*Solanum tuberosum* L.) and sunflower (Baştaş et al. 2009; Ma et al. 2007). Conversely, *P. carotovorum* causes soft rot of vegetable and ornamental plants including potato, sunflower, broccoli (*Brassica oleracea* L.), and calla lily (*Zantedeschia* spp.) (Arsenijevic 1970; Bradbury 1986; Gašić et al. 2013; Toth et al. 2003; Wright 1998). However, most plant species are rotted by *P. carotovorum* post-harvest (Pérombelon and Salmond 1995).

Geographic Distribution

Bacterial stalk rot of sunflower has been observed occurring sporadically throughout the Great Plains of the United States (Gudmestad et al. 1984; Gulya et al. 2012), but it is generally not considered to be of major concern for production. It is also known to occur throughout Europe, Russia, Turkey, South Africa, and several other central African countries (e.g., Tanzania and Uganda) (Arsenijevic 1970; Baştaş et al. 2009; Gulya et al. 1997; Harveson et al. 2016; Raj and Wati 2005).

Pathogen Isolation

The pathogens are easily isolated from leaves or from blighted petioles and stalks. Tissue pieces taken from the margins between diseased and healthy areas can be lightly surface disinfested

utilizing 70% ethyl alcohol and/or 0.05% bleach (sodium hypochlorite) followed by rinsing in sterile water. Tissue pieces are then plated on various media, including nutrient broth–yeast medium, nutrient agar, yeast dextrose agar, or yeast extract peptone agar (Harveson et al. 2012; Schwartz and Harveson 2016).

FIGURE 17
Sunflower stalk affected by bacterial rot.

FIGURE 16
Bacterial stalk rot lesions beginning at attachments sites of petiole axils with stalk.

FIGURE 18
Severely diseased stalk lodging from weight of unaffected head.

Pathogen Identification

The two species of *Pectobacterium* are in the soft-rot-causing group of plant pathogenic bacteria. Both pathogens are gram-negative rods with peritrichous flagella, catalase positive and oxidase negative, fermentative, facultatively anaerobic, and pectolytic (Schaad et al. 2001; Schwartz and Harveson 2016). They produce grayish white to creamy white colonies on most media and can be distinguished based on several physiological and biochemical properties. *Pectobacterium carotovorum* can grow at 37°C and does not utilize keto-methyl glucoside, whereas *P. atroseptica* cannot grow at 37°C but does utilize keto-methyl glucoside (Schaad et al. 2001).

Pathogen Storage

Long-term storage can be achieved from suspending lyophilized cultures in sterile glycerol (30 to 50%) or 10% dry milk and then placing at –70°C (Harveson and Schwartz 2007). Another relatively new method of storage can be employed through commercially available Microbank kits (PRO-LAB Diagnostics, Austin, TX). This technique utilizes sterile vials filled with a liquid growth medium and porous beads as carriers. Vials are inoculated with young (18- to 24-h) cultures. Vials are closed and inverted four

FIGURE 19

Bacterial growth emerging from infected stalk lesions.

FIGURE 20

Foamy substance emerging from infected stalks after fermentation of sugars by pathogen.

FIGURE 21

Retrieving bacterial pathogen after storage on beads.

to five times, and the bacteria will adhere to the beads. The vials are stored at –70°C until needed. Inoculated beads may then be removed and used to directly streak to solid media (Fig. 21) or dropped into an appropriate liquid medium (see Pathogen Isolation above).

Pathogenicity Tests

For testing bacterial pathogens for pathogenicity, only young, healthy and vigorously growing plants should be used. Inoculum is prepared by growing cultures in nutrient broth and adjusting to 10^6 to 10^7 CFU/ml. After wounding stems, adjusted inoculum is sprayed onto plants, or it can also be injected into the stem with a syringe and 26-gauge needles (Richeson 1981). Inoculated plants are placed into a mist chamber at temperatures ranging from 25 to 28°C or, alternatively, are covered with plastic bags for 12 to 14 h. Inoculated plants can then be incubated in the greenhouse until symptom development (internal decay, external blackening of the stalk).

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