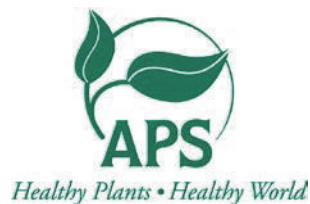# plant disease

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Bacterial Wilt of Cucurbits: Resurrecting a Classic Pathosystem

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Erwinia tracheiphila (E. F. Smith) Holland, the causal agent of bacterial wilt of cucurbits, was one of the first bacterial plant pathogens ever described (Smith 1911). In 1893, pioneering plant pathologist Erwin F. Smith witnessed "entire fields of cucumbers, cantaloupes, and of winter squashes" destroyed by the disease in Michigan, United States. More than 120 years later, bacterial wilt still menaces many cucurbit crops, causing yield losses of up to 80% (Latin 2000; Saalau Rojas et al. 2011; Smith 1911). Smith's belief that striped and spotted cucumber beetles (*Acalymma vittatum* Fabricius and *Diabrotica undecimpunctata howardi* Barber, respectively) were vectors of *E. tracheiphila* was confirmed by Rand and Enlows in 1916 (Rand and Enlows 1916). Rand and colleagues also indicated that bacteria could overwinter inside the adult striped cucumber beetle, which is the most important vector species of cucurbit bacterial wilt (Bassi 1983; Ellers-Kirk et al. 2000; Rand and Cash 1920).

After this initial flush of discovery, scientific knowledge of *E. tracheiphila* and bacterial wilt advanced very little for nearly 80 years, in part because the pathogen can be challenging to isolate and bacterial wilt epidemics occur only sporadically. However, a wave of research progress has gathered momentum for the past two decades, yielding many new insights into bacterial wilt ecology, genetics, etiology, and management. This article—the first review of the bacterial wilt

pathosystem—integrates current and historical information about the host, vectors, and pathogen. We highlight recent research in areas that are especially promising in understanding bacterial wilt epidemiology and improving the effectiveness of disease management.

Biogeography and Symptomology

Production of bacterial wilt-susceptible cucurbit crops currently exceeds 65,000 ha in the eastern half of the United States, representing about half of the total cucurbit crop acreage in the nation (USDA-NASS 2012). Bacterial wilt is regarded as a major threat for cucurbit production in much of this area—including the Midwest, Mid-Atlantic, and Northeast regions—as well as extreme southern portions of Ontario and Quebec, Canada (Fig. 1). Interestingly, the disease appears to be rare or unknown in other areas of the world.

E. tracheiphila is a xylem pathogen. Transmission occurs when cucumber beetles feed on plants and deposit infested frass onto fresh feeding wounds on leaves or flower nectaries (Leach 1964; Sasu et al. 2010a). Once inside the xylem the bacteria multiply, produce extracellular polysaccharides (slime), and obstruct xylem vessels. Strands of bacterial slime are sometimes visible when the cut ends of wilting stems are slowly pulled apart (Fig. 2) (Latin 2000). This diagnostic sign of the disease is useful in cucumber (*Cucumis sativus* L.) and muskmelon (*C. melo* L.), but is not seen consistently in squash (*Cucurbita maxima* Duchesne, *C. moschata* Duchesne, *C. pepo* L.) or pumpkin (*C. pepo* L.) (B. Bruton and E. Saalau Rojas, *unpublished data*).

Bacterial wilt derives its name from the characteristic wilting of leaves and stems (Fig. 3), which is usually followed by foliar necrosis and plant collapse (Fig. 4). Smith (1911) noted that symptoms begin in leaf areas having visible cucumber beetle damage; he described a darker green area that develops around the wounds, with the entire

plant eventually changing to a dull green color. Affected leaves become flaccid, followed by wilting of stems and vines. Initial wilt symptoms occur from 4 to >21 days after infection (de Mackiewicz et al. 1998; Saalau Rojas et al. 2013), but symptom expression varies among cucurbit crops and with host phenology (Brust 1997b). As with other cucurbits, pumpkin displays different symptoms depending on host phenological stage. Seedlings that become infected early in the season may exhibit relatively rapid wilting, soon followed by collapse. Since bacterial wilt symptoms are easily confused with aboveground symptoms of root rots and other vascular diseases, isolation of the pathogen followed by phenotypic identification, PCR, or other molecular assay is necessary to confirm a diagnosis. With the exception of watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) and a few cultivars of *Cucurbita* species (e.g., *C. pepo*, *C. maxima*, *C. moschata*), infected plants rarely recover or yield marketable fruit.

Host Range, Susceptibility, and Etiology

Within the family Cucurbitaceae, cucumber and muskmelon are highly susceptible to bacterial wilt whereas squash and pumpkin are moderately susceptible and watermelon is apparently highly resistant (Brust 1997b; Yao et al. 1996). During Smith's early work (Smith 1911), inoculating cucumber and muskmelon produced wilting "with certainty and regularity of clock-work," but inoculation of squash varieties often resulted in inconsistent disease development. The ability of watermelon to serve as a host of *E. tracheiphila* is unclear. Natural occurrence of the disease on watermelon was reported only an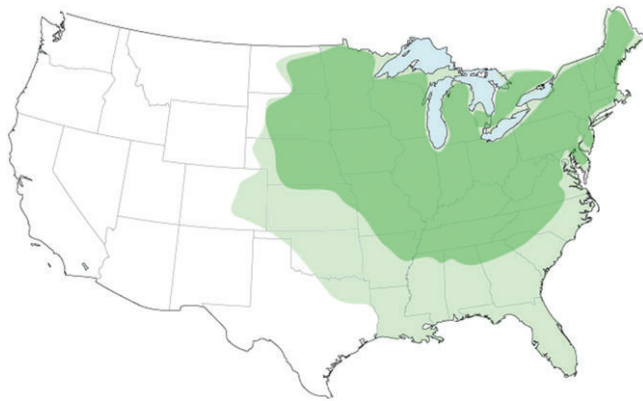ecdotally until recently, when it was confirmed in two commercial fields in New Mexico (Sanogo et al. 2011), which is located well outside the presumed geographic range of the disease.

In addition to commercially important cucurbit crops, some wild or noncultivated cucurbit species are also susceptible (Rand and Enlows 1920; Sasu et al. 2010b). Inoculations performed more than a century ago by Smith (1911) confirmed that buffalo gourd (*Cucurbita foetidissima* (Kunth.)), coyote gourd (*C. californica* Torr. ex S. Watson), burcucumber (*Sicyos angulatus* L.), and wild cucumber (*Echinocystis lobata* (Michx.) Torr. & A. Gray) succumb to bacterial wilt.

Many key details of bacterial wilt etiology remain unclear. Wild cucurbit species have been presumed to serve as reservoirs of *E. tracheiphila* inocula that could be transmitted to crop fields (de Mackiewicz et al. 1998), but there is no clear evidence supporting this idea. Serological assays suggested the presence of *E. tracheiphila* in noncucurbit herbaceous weed species, but these positive reactions may have been due to nonviable cells, as researchers were unable to re-isolate the pathogen from any of the species investigated (de Mackiewicz et al. 1998). Therefore, adult striped cucumber beetles are assumed to be the primary overwintering reservoirs of *E. tracheiphila*, linking infections in successive cucurbit growing seasons.

The impact of plant host phenology on susceptibility is gradually becoming clearer. Pumpkin cultivars rapidly developed wilt symptoms and died when inoculated at the cotyledon stage, but showed symptoms more slowly, and even recovered, within weeks after inoculation at the first- and second-true-leaf stages (Brust 1997b). A survey of 59 cucurbit species inoculated with *E. tracheiphila* showed results similar to those reported on pumpkin (Watterson et al. 1971). Liu et al. (2013) demonstrated that wilt symptoms progressed considerably faster in muskmelon seedlings inoculated 2 weeks after emergence than those inoculated at 6 or 8 weeks, which may be attributable in part to ontogenic resistance. Inoculum dose, age-related resistance, soil water

Fig. 1. Cucurbit bacterial wilt poses a major threat to cucurbit production in the Midwest, Mid-Atlantic, and Northeast regions of the United States and southern portions of Ontario and Quebec, Canada. Areas in which cucurbit bacterial wilt is a significant economic concern (dark green) overlap with the geographic distribution of the striped cucumber beetle (light green), which is the main vector of the disease.
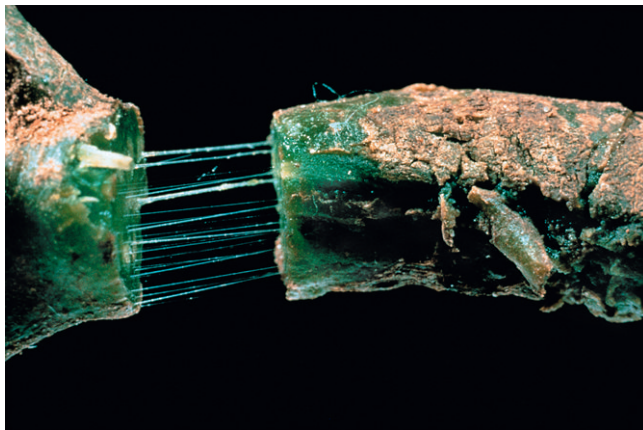

Fig. 2. Bacterial "slime" can sometimes be observed as strands that form during the gentle separation of cut stems of wilted vines infected by *Erwinia tracheiphila*. Photo courtesy M. P. Hoffmann, reprinted from: Zitter, T. A., Hopkins, D. L., and Thomas, C. E., eds. 1996. *Compendium of Cucurbit Diseases*. American Phytopathological Society, St. Paul, MN.

Fig. 3. Wilted leaves are a characteristic early symptom of cucurbit bacterial wilt, as shown on muskmelon.

Fig. 4. Infected plants often die before they yield fruit, as shown here for muskmelon.

availability, and other host and environmental factors may also contribute to variation in symptom expression and disease progression.

Pathogen Biology

Erwinia tracheiphila is a gram-negative, motile, xylem-limited, facultative anaerobic bacterium. Smith (1911) noted the difficulty of isolating and working with this pathogen, which grows slowly in culture and is easily overgrown by other microorganisms. Unlike most *Erwinia* species, *E. tracheiphila* can be challenging to isolate from macerated plant tissues. Nevertheless, consistent isolation can be accomplished by surface-sterilizing stem and petiole segments and directly plating the ooze exuded near the vascular elements (Hauben and Swings 2005; Smith 1911), or in the absence of recoverable ooze, placing the exposed vascular tissues directly onto solid medium such as nutrient agar (NA) (Saalau Rojas et al. 2013).

On NA medium, *E. tracheiphila* colonies are generally small, circular, viscous, and hyaline or white in color (Burkholder 1960). Colony growth on NA, often amended with peptone, becomes visible 3 to 4 days after streaking (de Mackiewicz et al. 1998; Hauben and Swings 2005) when incubated within the optimal range of 25 to 30°C (Hauben and Swings 2005). Identification of *E. tracheiphila* strains based on phenotypic techniques such as nutrient requirements and carbon utilization profiles has been unreliable due to variation among the few strains examined (Hauben and Swings 2005; Starr and Mandel 1950; Wells et al. 1994). However, molecular-based methods have provided consistent identification (Saalau Rojas et al. 2013).

Pathogenesis. In 1899, microscopic observations on artificially inoculated plants led Smith (1911) to believe that mechanical plugging of xylem vessels by *E. tracheiphila* cells interfered with water conductance and induced wilting. *E. tracheiphila* has not been found to produce hydrolytic or pectolytic enzymes (Main and Walker 1971), and in vivo assays suggested that impedance due to rapid bacterial multiplication and direct blockage of vessels (Fig. 5) is the main mechanism for leaf and vine wilting (Smith 1911; Watterson et al. 1972).

The ability to obstruct xylem flow suggests the presence of many *E. tracheiphila* cells in symptomatic tissues. Populations may reach 2.5×10^2 CFU/cm in stem sections dissected from disease-susceptible cucumber plants. In contrast, only 10 CFU/cm were found in stem sections of the resistant cucumber breeding line WR 18, suggesting that bacterial multiplication was limited in the resistant line (Watterson et al. 1972). Resistance was also associated with restriction of movement to only the first few internodes of inoculated cucumber plants (Watterson et al. 1972), but this conclusion contradicted an earlier study that found that *E. tracheiphila* moved to all stem internodes of inocu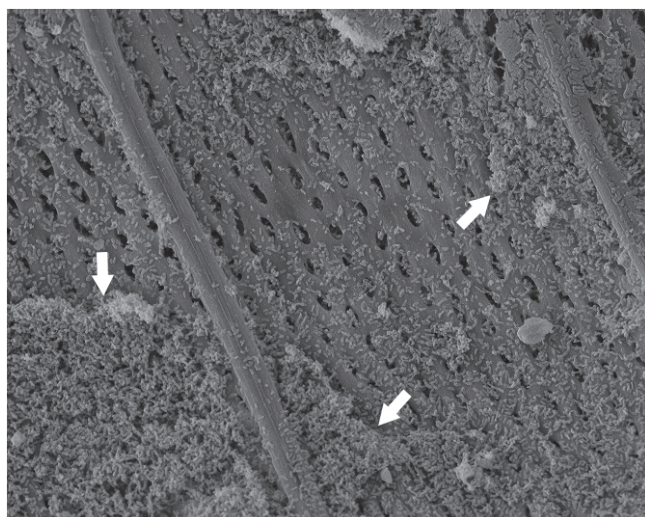lated WR 18, as well as watermelon and pumpkin cultivars, which either recovered from wilting or displayed partial wilt symptoms (Watterson et al. 1971).

Genetic diversity and virulence. *E. tracheiphila* has consistently been placed within the genus *Erwinia* since the initial description of the pathogen. The species shows 94% similarity in 16S rDNA sequence and 23% DNA relatedness by hybridization to the fire blight pathogen *E. amylovora*, with 16S rDNA sequence-based phylogenetic analysis indicating a strong relatedness to *Enterobacter* and *Pantoea* as well as other *Erwinia* species (Brenner et al.1974; Hauben et al. 1998). Comparison of 16S rDNA sequence data of six *E. tracheiphila* strains isolated from various cucurbit crop hosts and geographic regions revealed a difference of only one base pair among strains (E. Saalau Rojas, *unpublished data*).

Nevertheless, historic and recent data suggest the existence of at least two distinct *E. tracheiphila* subgroups. Smith's (1911) cross-inoculation assays on various cucurbit host species suggested that *E. tracheiphila* strains vary in their virulence in a host-dependent manner. For example, strains isolated from muskmelon were highly virulent on muskmelon and cucumber but caused much slower symptom development or no symptoms on squash (Smith 1911). Repetitive element palindromic PCR (rep-PCR) assays performed with ERIC1-2 and BOX A1R primers of 69 *E. tracheiphila* strains from eight U.S. states revealed distinct fingerprint profiles for strains isolated from *Cucumis* versus *Cucurbita* species (Saalau Rojas et al. 2013). Consistent with Smith's (1911) observations, pathogenicity assays using these strains indicated that *E. tracheiphila* induced wilt symptoms rapidly when inoculated onto hosts within the genus of isolation, but significantly slower when cross-inoculated onto hosts distinct from the genus of isolation (Fig. 6) (Saalau Rojas et al. 2013). These observations support a possible division of *E. tracheiphila* into subspecies that differ in host preference.

Vector and Transmission Biology

Vectors. Both striped and spotted cucumber beetles belong to the taxonomically diverse subtribe Diabroticina, which includes some of the world's most destructive insect pests of crop plants (Gould 1944). Diabroticite adults feed on stems, leaves, and flower parts whereas larvae feed primarily on roots (Tallamy and Krischik 1989). Although striped cucumber beetles (*Acalymma vittatum*) are cucurbit specialists, closely related *Diabrotica* species, including spotted cucumber beetles (Fig. 7), are polyphagous and occur commonly in cucurbit fields (Fisher et al. 1984; Metcalf and Lampman 1989). The role of spotted cucumber beetles and other *Diabrotica* species in transmission and spread of bacterial wilt has been largely unexplored. Preliminary data from a recent survey in Quebec, Canada, indicate that adult beetles of two additional species, the western (*Diabrotica virgifera* LeConte) and northern (*D. barberi* Smith & Lawrence) corn rootworm beetles, may also be vectors of *E. tracheiphila*, as suggested by PCR

Fig. 5. Cells of *Erwinia tracheiphila* multiply in xylem vessels, obstructing the flow of water. Cells are visible (arrows) in this scanning electron micrograph of the pit membrane of a xylem element. Photograph courtesy B. Bruton.

Fig. 6. Host preference of *Erwinia tracheiphila* strains is indicated by the slow rate of symptom development of a muskmelon (*Cucumis melo*) plant inoculated with an *E. tracheiphila* strain isolated from squash (*Cucurbita pepo*) (left) compared with the rapid rate of symptom development of a muskmelon plant inoculated with a strain originally isolated from muskmelon (right).

detection of *E. tracheiphila* in beetles collected in cucurbit fields (Toussaint et al. 2013). Insects outside the Diabroticina subtribe that are commonly found in cucurbit fields, including squash bugs (*Anasa tristis*), aphids (*Aphis gossypii*), squash lady beetles (*Epilachna borealis*), potato flea beetles (*Epitrix cucumeris*), and bees (*Apis mellifera*), have not been found to carry or transmit the pathogen (Rand and Enlows 1920).

Bacterial wilt epidemics are strongly associated with striped cucumber beetle population density and behavior (Fleischer et al. 1999), indicating the importance of this beetle as a vector of bacterial wilt. Striped cucumber beetles overwinter as adults, are distributed throughout the geographic range of bacterial wilt, and specialize on cucurbits (Gould 1944; Munroe and Smith 1980). They are present as potential vectors early in a growing season, when plants are more susceptible to the disease than at later crop stages (Liu et al. 2013). Several *Diabrotica* species overwinter as eggs or adults in the southern United States (Krysan 1986), which may explain why spotted cucumber beetles do not appear in northern states until after the spring emergence of striped cucumber beetles. To date, research on bacterial wilt epidemiology and vector interactions has focused primarily on striped cucumber beetles.

Vector-pathogen interactions. Transmission of *E tracheiphila* occurs when bacteria-infested frass and contaminated beetle mouthparts come into contact with plant wounds (Fig. 8) or floral nectaries (Fig. 9) (Mitchell and Hanks 2009; Rand and Enlows 1920; Sasu et al. 2010a). Rand and Enlows (1920) reproduced wilt symptoms on cucumber seedlings inoculated with extracts of mouthparts and intestinal tracts of infested striped cucumber beetles, as well as with spotted cucumber beetles that were initially allowed to feed on artificially inoculated plants and then transferred to healthy plants. Although *E. tracheiphila*-like cells were described within the digestive system of striped cucumber beetles several decades later (Garcia-Salazar et al. 2000a; Leach 1964), it was not until the turn of the 21st century that immunological methods enabled direct detection of *E. tracheiphila* within the insect's gut (Garcia-Salazar et al. 2000b). These studies employed immunolocalization and double antibody sandwich (DAS)-ELISA with laboratory-reared colonies that were fed on cucumber cotyledons smeared with *E. tracheiphila*—so-called "cotyledon sandwiches" (Garcia-Salazar et al. 2000a). Most bacterial cells were detected at the junction of the midgut and hindgut, but some were also found in the foregut. Although bacterial populations decreased significantly during the first 3 days after feeding, bacteria were detected for up to 35 days after acquisition of the pathogen.

Mitchell and Hanks (2009) further explored transmissibility of *E. tracheiphila* via frass using PCR-based methods and frass-inoculation experiments. The authors allowed adult striped cucumber beetles to feed on *E. tracheiphila*-smeared "sandwiches" for 24 h and frass samples were collected at 24, 48, and 72 h after the feeding period. Frass from each sampling period was used to inoculate cucumber seedlings and assayed for the presence of *E. tracheiphila* DNA. Bacterial DNA was detectable for up to 72 h after ingestion of the

pathogen, and frass remained capable of transmitting *E. tracheiphila* for up to 48 h. In a second assay to determine whether longer feeding periods increased pathogen retention in striped cucumber beetle adults, beetles were allowed to feed on *E. tracheiphila* cotyledon sandwiches for a 7-day period and bacterial DNA was detected in frass for up to 4 days after pathogen ingestion (Mitchell and Hanks 2009). Taken together, these findings suggested that pathogen retention and transmission dynamics are heavily influenced by duration of the feeding period, but do not support the possibility that striped cucumber beetles serve as long-term vectors of *E. tracheiphila* within

Fig. 8. Transmission occurs mainly when diabroticite beetles, such as the striped cucumber beetle (*Acalymma vittatum*) shown here, deposit *Erwinia tracheiphila*-infested frass onto fresh feeding wounds. Photograph courtesy R. Durgy.

Fig. 7. Spotted cucumber beetles (*Diabrotica undecimpunctata howardi*) transmit *Erwinia tracheiphila*; however, their importance in the epidemiology of cucurbit bacterial wilt has not been elucidated. Photograph courtesy M. Asche.

Fig. 9. Cucumber beetles are highly attracted to cucurbit flowers. In at least some cucurbit hosts, *Erwinia tracheiphila* can enter the vascular system through floral nectaries. Photograph courtesy A. Boesch.

a growing season. Mitchell and Hanks (2009) also noticed that detection and transmission of the pathogen varied among the different *E. tracheiphila* strains they used, suggesting that strain diversity could play a role in vector competence and disease dynamics.

More recently, Shapiro et al. (2014) developed a real-time quantitative PCR assay to characterize pathogen acquisition and retention time by striped cucumber beetles. After 3- and 24-h acquisition access periods (AAP) on *E. tracheiphila*-infected plants, they estimated bacterial populations in whole beetles and frass. As in Mitchell and Hanks' work, longer AAP resulted in longer-term pathogen retention (Shapiro et al. 2014). Interestingly, quantification of *E. tracheiphila* populations at 5 and 28 days after AAP suggested that multiplication was occurring within the beetle gut. Although the mechanisms underlying persistent colonization of striped cucumber beetle remain undescribed, it is now clear that *A. vittatum* is a persistent vector and long-term reservoir of *E. tracheiphila* (Garcia-Salazar et al. 2000b; Shapiro 2012).

Although Erwin Smith (1911) recognized the mechanism and epidemiological importance of transmission through leaves, infection through floral nectaries was demonstrated only recently as a route of infection in a wild gourd (*Cucurbita pepo* ssp. *texana*) and muskmelon (Gautam et al. 2011; Sasu et al. 2010a). Striped cucumber beetles and other diabroticite beetles are strongly attracted to the yellow color of cucurbit flowers as well as to certain volatile compounds they emit (Lewis et al. 1990; Metcalf and Lampman 1989). In Pennsylvania field trials, 95% of *C. pepo* plants had *E. tracheiphila*-infested frass on the corolla or nectaries of flowers. This discovery led to the suggestion that beetle aggregation behavior and deposition of bacteria-infested frass in flowers may result in high rates of exposure to the bacterial wilt pathogen during the middle of the growing season, when each plant may produce up to several dozen flowers daily (Sasu et al. 2010b). In greenhouse experiments, nectar was present and found to have antimicrobial properties (Sasu et al. 2010b). However, after removal of nectar, artificial inoculation through floral nectaries resulted in a 36% increase in bacterial wilt incidence (Sasu et al. 2010b).

The extent to which epiphytic populations of *E. tracheiphila* function as reservoirs of inoculum is uncertain. Preliminary work by Brust (1997a) suggested that the pathogen could survive and infect muskmelon for up to 6 h after inoculum had been placed on leaf surfaces. A recent growth chamber study found that epiphytic populations of *E. tracheiphila* remained viable for up to 2 days on muskmelon leaves, even after exposure to alternating 12-h cycles of wet and dry conditions (Saalau Rojas and Gleason 2012). As with other epiphytic pathogens, however, survival was enhanced by leaf wetness, supporting previous anecdotal observations that disease development was favored by wet weather conditions (Rand and Enlows 1920; Sherf and MacNab 1986). These findings raise the possibility that epiphytic populations on leaf or floral surfaces serve as inoculum reservoirs that could potentially influence disease development.

Seasonal dynamics of vectors and disease. Adult striped cucumber beetles overwinter in or near cucurbit fields, in the top 2 to 3 cm of soil or under plant debris (Gould 1944; Radin and Drummond 1994b). Beetles become active in spring when air temperatures exceed 12°C. In the Midwest United States, striped cucumber beetles move into cucurbit fields from overwintering sites during late April through June (Fig. 10) (Brust 1997c; Radin and Drummond 1994b).
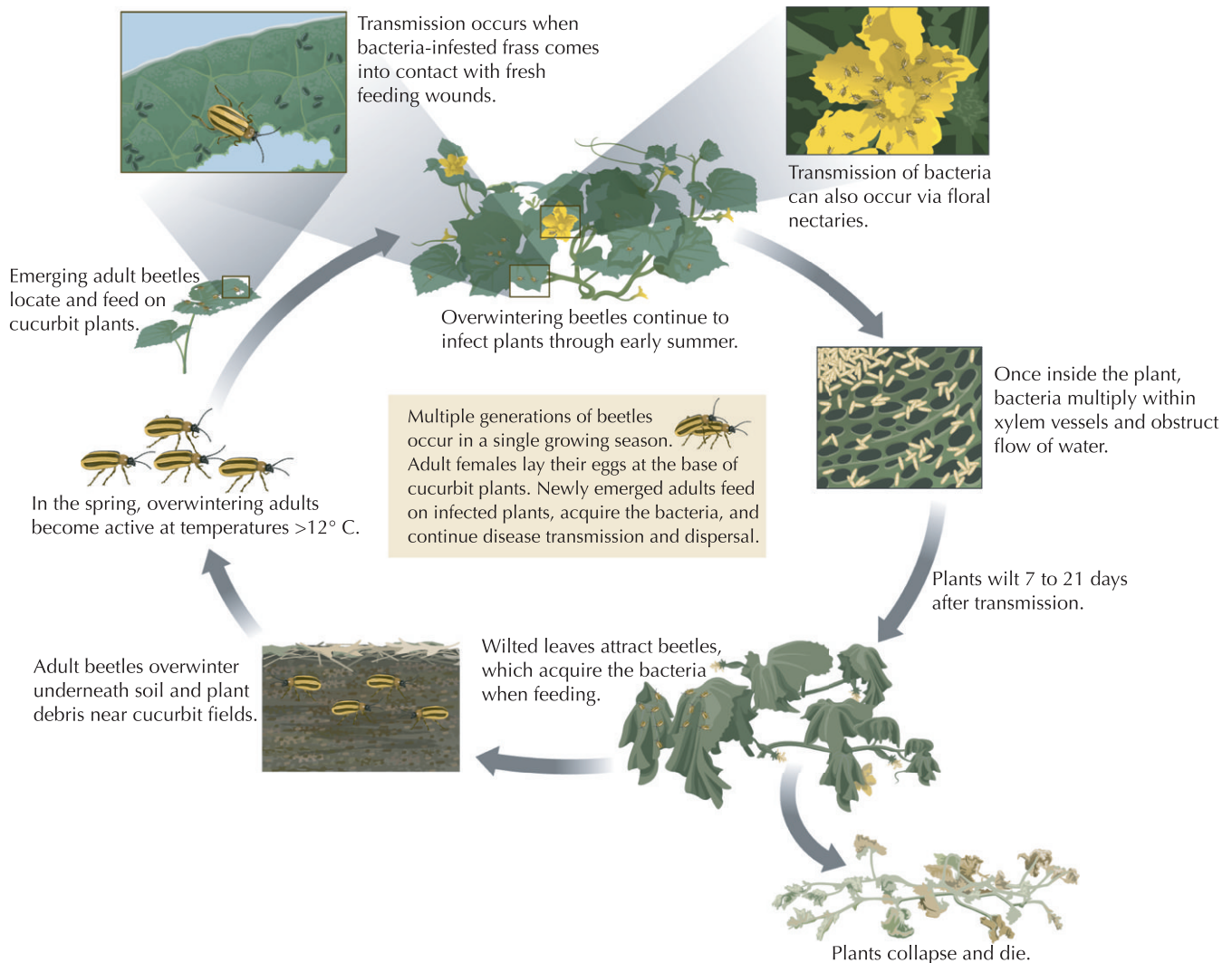

Transmission occurs when bacteria-infested frass comes into contact with fresh feeding wounds.

Transmission of bacteria can also occur via floral nectaries.

Emerging adult beetles locate and feed on cucurbit plants.

Overwintering beetles continue to infect plants through early summer.

Once inside the plant, bacteria multiply within xylem vessels and obstruct flow of water.

Multiple generations of beetles occur in a single growing season. Adult females lay their eggs at the base of cucurbit plants. Newly emerged adults feed on infected plants, acquire the bacteria, and continue disease transmission and dispersal.

In the spring, overwintering adults become active at temperatures >12° C.

Plants wilt 7 to 21 days after transmission.

Adult beetles overwinter underneath soil and plant debris near cucurbit fields.

Wilted leaves attract beetles, which acquire the bacteria when feeding.

Plants collapse and die.

Fig. 10. The cucurbit bacterial wilt disease cycle.

Field and serological assays suggested that a small proportion (1 to 10%) of overwintering striped cucumber beetles harbor and are able to transmit *E. tracheiphila* (Brust 1997c; Doolittle 1921; Fleischer et al. 1999). In Pennsylvania fields that had been planted to cucurbit crops the previous season, 7 to 10% of overwintering striped cucumber beetles that emerged within cages tested positive for *E. tracheiphila* by DAS-ELISA (Fleischer et al. 1999). In a 4-year study in Indiana, only approximately 1% of overwintering beetles collected in May or June transmitted the disease to muskmelon seedlings (Brust 1997c). When the experiment was repeated with beetles collected in July and September, however, disease transmission to seedlings rose to approximately 10%, suggesting that an increasing percentage of beetles carried and transmitted the pathogen as the season progressed. In a Pennsylvania study, the proportion of beetles testing positive for *E. tracheiphila* rose from 39 to 78% as the season progressed, but this increase did not necessarily lead to increased disease transmission when individual beetles were caged in muskmelon seedling bioassays (Fleischer et al. 1999). Despite a higher proportion of beetles carrying *E. tracheiphila*, a decrease in disease transmission rate that often occurs in cucurbit fields later in the season may be due to phenological changes in vector behavior or developmental changes in the host or host-pathogen interactions. For example, mature cucurbit plants may be less attractive to beetles because they may have lower levels of cucurbitacin (Tallamy and Krischik 1989). Additionally, as previously stated, older plants may become more tolerant of infection, as reflected in a delay in the onset of wilt symptoms, possibly due to increased ontogenic resistance, plant size, or both (Liu et al. 2013; Lukezic et al. 1996).

During the mid to late growing season, *E. tracheiphila* can be transmitted by subsequent generations of beetles. Overwintering females lay their eggs at the base of cucurbit plants, allowing the larvae to feed on the roots and develop into first-generation adults (Ellers-Kirk and Fleischer 2006; Ellers-Kirk et al. 2000). Depending on climatic conditions and geographic region, two or more generations of striped cucumber beetle may develop within a growing season. There is no evidence of transovarial transmission of *E. tracheiphila*; consequently, it is assumed that newly emerged adults must feed on *E. tracheiphila*-infected plants in order to acquire and further disseminate the pathogen. In-field monitoring of striped cucumber beetle in Iowa, Pennsylvania, and Kentucky showed significant generation overlap (Bachmann 2013), limiting ability to accurately predict timing of pest activity or extent of pathogen carryover to the following growing season.

Beetle behavior and population density strongly influence the severity of bacterial wilt epidemics. Even though available evidence indicates that few overwintering beetles carry *E. tracheiphila*, early-season infections often lead to more devastating outbreaks than late-season infections. Mass immigration into cucurbit fields is promoted by attraction to volatile biochemicals emitted by seedlings (Lewis et al. 1990). As with beetle aggregation behavior, this immigration likely increases the probability of bacterial wilt infection due to both increased beetle numbers and concentrated feeding damage. The importance of feeding damage was demonstrated in greenhouse studies by the finding that muskmelon plants were 50% more likely to develop wilt when *E. tracheiphila* inoculum was placed on leaves with large wounds than on leaves with small wounds (Brust 1997a). In field data from Alabama and Indiana, regression models revealed a positive, significant relationship between beetle populations and bacterial wilt severity and incidence (Brust and Foster 1999; Yao et al. 1996). A similar effect was observed in greenhouse experiments on muskmelon: preferential feeding of beetles on certain cultivars (i.e., 'Makdimon,' 'Rocky Sweet,' 'Legend,' and 'Cordele'), perhaps due to visual or gustatory cues, resulted in greater feeding damage and higher incidence of bacterial wilt than in other cultivars (Brust and Rane 1995).

Chemical ecology of beetles and cucurbits. One way to gain insight into the epidemiology of bacterial wilt is to look into the coevolutionary associations of cucumber beetles and the Cucurbitaceae family. Researchers conjecture that specialization of diabroticite species to cucurbit host plants may have originated from ancestral beetles that served as pollinators of cucurbits (Metcalf and Lampman 1989). Cucurbits produce a variety of chemical compounds, such as cucurbitacin and volatiles, which are not directly tied to primary metabolism but play major roles in host-plant identification, beetle feeding, and mating behavior (Lewis et al. 1990; Metcalf and Lampman 1989). Cucurbitacins are nonvolatile, extremely bitter-tasting plant compounds that are ubiquitous in cotyledons, leaves, roots, and fruit of most ancestral cucurbit species. They generally serve as feeding deterrents to protect plants from insect herbivory. With diabroticites, however, cucurbitacins stimulate locomotive arrest and compulsive feeding (Ferguson and Metcalf 1985). Molecular phylogenetic analyses support the hypothesis that cucurbitacin feeding by polyphagous diabroticites is a result of convergent evolution associated with pollen forage and cucurbitacin consumption benefits rather than an ancestral host association to cucurbit plants (Gillespie et al. 2003). For example, cucurbitacin protects cucumber beetles from birds, predaceous insects, and entomopathogenic fungi (Metcalf and Lampman 1989; Tallamy et al. 1998). Moreover, cucurbitacin consumption by diabroticite males can influence mating behavior; cucurbitacins are used as nuptial gifts that are transferred to females during mating, and influence female mating probabilities (Tallamy et al. 2000).

Male striped cucumber beetles are the first to locate cucurbit plants (Fig. 11), and these "pioneer males" have been shown to attract additional males through production of an aggregation pheromone, volatiles associated with their frass, and volatiles associated with herbivory on plants (Smyth and Hoffmann 2003). This behavior is seemingly unaffected by cucurbitacin consumption and more likely associated with male feeding rates or other chemical signals (Smyth and Hoffmann 2002). The evolutionary reason behind the production of an aggregation pheromone remains unclear; however, this behavior may serve to maximize early-season colonization of host plants (Smyth and Hoffmann 2003) and locate mates.

Whereas cucurbitacins and volatiles in young plants promote rapid beetle localization to a host and aggregated beetle feeding, cucurbit floral volatiles may help cucumber beetles to find host plants over long distances later in the season (Lewis et al. 1990). Olfactory attraction to cucurbit blossoms may lead diabroticite adults to pollen, an important component of their diets (Metcalf and Lampman 1989; Siegfried and Mullin 1990), and possibly explains the primary association of diabroticites with cucurbit plants (Gould 1944). The recent discovery that bacterial wilt infection can also occur via floral nectaries suggests that attraction to floral volatiles may be a more important factor in bacterial wilt epidemics than previously considered (Sasu et al. 2010a). The relative significance of nectar-removing pollinators versus disease-transmitting beetles and floral versus foliar routes of infection in the development of bacterial wilt epidemics in commercial cucurbit

Fig. 11. Striped cucumber beetles become active in the spring and can quickly locate and colonize young cucurbit plants. Photo courtesy E. Burkness.

fields is not clear. However, field experiments with wild gourd showed that genotypes producing the most flowers also had the highest incidence of bacterial wilt, suggesting that floral display attracts more vectors and results in significant disease exposure during the bloom period (Ferrari et al. 2006; Sasu et al. 2009).

Pathogen-induced volatiles. Factors influencing host selection and vector feeding preferences can have a direct impact on plant disease epidemiology, but these interactions have been largely overlooked in phytobacterial pathosystems. Striped cucumber beetle aggregation on wilting leaves was originally observed by Smith (1911) and noted in more recent studies (Moran 2001; Yao et al. 1996). Such behavior may be associated with higher levels of cucurbitacins in symptomatic tissue. For example, a cage assay showed that wilting of cucumber plants that was induced by *E. tracheiphila* inoculation or water stress significantly increased beetle aggregation and cucurbitacin concentrations when compared with nonwilted plants (Haynes and Jones 1975). This observation raises the question of whether *E. tracheiphila* induces host-plant changes that modulate vector behavior. Abundant evidence indicates that insect herbivory and pathogen infection can modify volatile plant emissions that influence disease dynamics (Mann et al. 2012). In both field and laboratory plant volatile collections, Shapiro et al. (2012) demonstrated that *E. tracheiphila* infection induced the release of a characteristic bouquet of volatile organic compounds in wilting *C. pepo* ssp. *texana* leaves. In field trials and feeding choice assays, striped cucumber beetles aggregated on and showed feeding preference toward symptomatic leaves over healthy leaves, and healthy flowers were significantly more attractive than flowers collected from bacterial wilt-infected plants. Collectively, these results support a model in which *E. tracheiphila* promotes its acquisition and dissemination by altering plant volatiles to attract beetles to symptomatic leaves and, moreover, exploits the attractiveness of beetles to healthy flowers by using floral nectaries as a site of infection (Sasu et al. 2010a; Shapiro et al. 2012).

Management

Management of bacterial wilt relies primarily on insecticide applications against cucumber beetles. Although feeding damage alone can significantly impact plant stand and yield in commercial cucurbit plantings (Hoffmann et al. 2000), the primary motivation for using insecticides against cucumber beetles in the high-risk geographic regions is the threat of bacterial wilt transmission (Burkness and Hutchison 1998). Because commercially acceptable disease-resistant cultivars are nearly nonexistent, cucumber beetle management is the mainstay of bacterial wilt management programs. The first few weeks
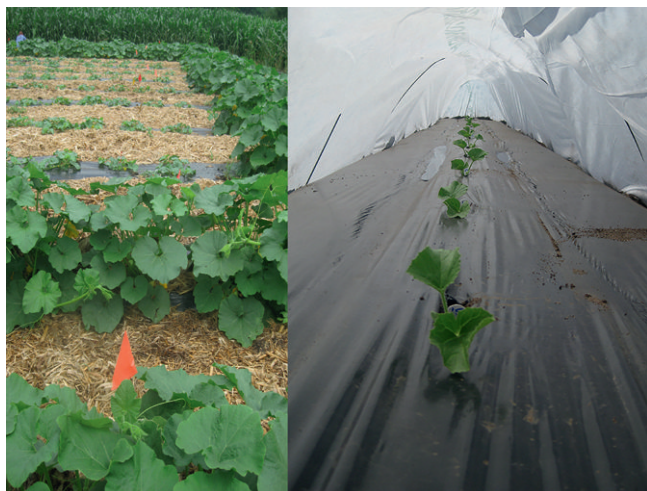

Fig. 12. Perimeter trap crops can be an effective strategy to manage bacterial wilt with fewer insecticide applications than dictated by conventional management practices. The image on the left shows two rows of buttercup squash (*Cucurbita maxima*) planted around a muskmelon (*Cucumis melo*) main crop; in this cropping pattern, the squash, which is highly attractive to striped cucumber beetle, can serve as a barrier against beetle entry into the main crop. The image on the right shows row covers protecting muskmelon seedlings from beetle damage. Photograph courtesy J. Batzer.

after transplant or seedling emergence are critical for bacterial wilt suppression. Conservative economic thresholds for striped cucumber beetles often lead to either preventative or calendar-based insecticide applications at the first sight of beetles in the field (Burkness and Hutchison 1998; Fleischer et al. 1999).

Insecticides and kairomonal baits. In the United States, cucumber beetles are managed using systemic insecticides on transplants before planting, or in the furrow during planting (Dively and Kamel 2012). Neonicotinoid insecticides are widely used as seed treatments. Neonicotinoids largely replaced earlier materials, such as carbofuran, which were much more toxic to humans and birds, were a concern for ground water contamination, and exhibited reduced effectiveness in soils with a history of use (EPA 2006). Neonicotinoids, however, have been shown to be toxic to bees and other pollinators, and many recent studies have suggested that they play a role in declining honeybee populations (Whitehorn et al. 2012).

In cucurbit production, however, neonicotinoid insecticides are effective against cucumber beetles and suppress bacterial wilt epidemics. In Pennsylvania for example, application of the neonicotinoid imidacloprid on seedlings resulted in reduced striped cucumber beetle colonization and bacterial wilt transmission (Fleischer et al. 1998). Although bacterial wilt symptoms were observed in the field, low doses of systemic insecticides applied at planting suppressed colonization by striped cucumber beetles while reducing the need for weekly foliar applications of insecticides (Fleischer et al. 1998; Mac Intyre Allen et al. 2001). If contact insecticides are used, numerous foliar applications may be required throughout the growing season to adequately control beetle immigration, and bacterial wilt transmission may occur even when sprays are applied weekly (Brust and Foster 1995). When facing large striped cucumber beetle populations, some growers deploy these insecticides at 5-day intervals, which can sum to 8 to 10 applications in a single season (Brust and Foster 1995; Lam and Foster 2006).

Whether systemic or contact insecticides are used to manage cucumber beetle populations, periodic scouting is recommended to reduce unnecessary pesticide applications. In the Midwest, Brust and Foster (1999) established an action threshold of one beetle per plant for adequate protection against cucumber beetle damage and bacterial wilt. Implementation of action thresholds on curcurbits along with direct scouting and/or the use of yellow sticky traps for monitoring can effectively control bacterial wilt while avoiding several insecticide sprays (Burkness and Hutchison 1998; Lam 2007).

Toxic kairomonal baits have been used as an alternative to foliar insecticide applications. Baits containing cucurbitacins and floral volatiles in combination with the insecticide carbaryl (1-naphtyl methylcarbamate) reduced beetle damage in the field (Brust and Foster 1995, Fleischer and Kirk 1994). In Indiana, baits significantly reduced beetle feeding damage when compared with nontreated plants (Brust and Foster 1995). However, toxic baits did not provide rapid knockdown of beetle populations. Because bacterial wilt transmission may occur even at low beetle densities, rapid knockdown is essential for adequate disease management (Brust and Foster 1995). Additionally, effectiveness of kairomonal baiting methods and traps varies among diabroticite species and between genders (Fleischer and Kirk 1994).

Perimeter trap cropping. Over the past 20 years, research efforts have emphasized alternative approaches to manage cucumber beetles with fewer insecticide applications (Burkness and Hutchison 1998; Pair 1997; Radin and Drummond 1994a; Saalau Rojas et al. 2011). A perimeter trap crop (PTC) strategy consists of planting a border that can intercept incoming pests and thereby protect the main crop from damage (Fig. 12) (Boucher and Durgy 2004). In a cucurbit cropping system, a PTC strategy exploits cucumber beetle feeding preferences and aggregated feeding patterns by deploying highly attractive border plants (Adler and Hazzard 2009). In New England, a PTC strategy reduced insecticide applications by >90% on a butternut squash main crop, and acceptable bacterial wilt control was achieved by monitoring beetle populations and focusing most insecticide applications on the 'Hubbard' squash trap crop (Cavanagh et al. 2009). Field trials in Iowa and Ohio, using buttercup squash (*Cucurbita maxima*) as a perimeter trap crop, suppressed bacterial wilt incidence on muskmelon while eliminating three to four insecticide applications per season (Bartel 2012).

Growers with successive plantings within a season can benefit from the beetles' propensity to colonize specific areas. By aggressively controlling beetles on early-planted crops and deploying late plantings, growers in Pennsylvania achieved a management result similar to that with PTC (S. Fleischer, *unpublished data*).

The efficacy of a PTC strategy to control bacterial wilt depends greatly on perimeter crop host selection. Because cucumber beetle behavior and field aggregation patterns are heavily influenced by chemical and visual cues (Lewis et al. 1990, Metcalf and Lampman 1989, Radin and Drummond 1994a), an ideal perimeter crop should be equally or more attractive to beetles than the main crop. Moreover, the perimeter crop should be moderately resistant to bacterial wilt to prevent disease from spreading into the main crop.

Row covers. Row covers are commonly used in vegetable crops to enhance earliness of yield and protect plants from harsh weather and insect pests (Orozco-S. et al. 1994; Vaissiere and Froissart 1996). In Iowa, deployment of spunbond polypropylene row covers after transplant protected muskmelon against bacterial wilt (Mueller et al. 2006; Saalau Rojas et al. 2011). Traditionally, row covers are removed at anthesis (first appearance of perfect flowers) on cucurbit crops to enable pollination and avoid harvest delays (Gaye et al. 1991). Interestingly, delaying removal of row covers until 10 days after anthesis reduced bacterial wilt incidence in Iowa field trials by 33 or 50% compared with row cover removal at anthesis or noncovered controls, respectively (Saalau Rojas et al. 2011). However, there are tradeoffs associated with this strategy. Extending the row-covered period beyond the start of bloom delays not only cucumber beetle access but also pollinator access, which can in turn delay pollination and harvest. This delay could be a disadvantage for growers in markets that award premium prices to early-season produce (Brust and Foster 1995). Despite its potential as an alternative to insecticide use, delayed harvest, high material costs, and intensive labor requirements currently constrain large-scale implementation of row covers (Saalau Rojas et al. 2011).

Biological and cultural control. Few studies have focused on biological control methods against cucumber beetles. Tachinid flies (*Celatoria setosa*) and braconid wasps (*Centistes diabroticae*) have been reported to parasitize striped cucumber beetles; however, the impact of these parasitoids on cucumber beetle management in field conditions has not been quantified (Smyth and Hoffmann 2010).

Soil management practices and deployment of plastic mulches can reduce cucumber beetle populations in muskmelon, cucumber, and squash (Andino and Motsenbocker 2004; Necibi et al. 1992). Aluminum-coated or reflective mulches repel cucumber beetles with variable results, whereas black plastic mulch can reduce the survival of striped cucumber beetle larvae (Caldwell and Clarke 1999; Necibi et al. 1992). Introducing entomopathogenic nematodes through drip irrigation of cucumbers grown in black plastic mulch can also control cucumber beetle larvae (Ellers-Kirk et al. 2000). Integration of this method into a cucurbit production system could decrease larval populations but would not control early-season or overwintering beetle populations.

Plant growth-promoting rhizobacteria (PGPR) applied to cucumber seeds can significantly reduce bacterial wilt incidence in greenhouse and field experiments (Zehnder et al. 1997), but has not been consistently effective (Hernández Heredia 2008). Field experiments comparing PGPR seed treatments on cucumber have demonstrated that the use of PGPR can result in significantly lower bacterial wilt incidence and cumulative cucumber beetle populations at the end of the season (Zehnder et al. 1997). The mechanism behind the beneficial effect of PGPR on cucurbits is not fully understood, but PGPR may induce systemic resistance against *E. tracheiphila* and other cucumber pathogens, and may also deter feeding by cucumber beetles by reducing cucurbitacin levels in plant tissues (Raupach and Kloepper 1998; Zehnder et al. 2001).

Outlook: Piecing Together the Puzzle

An increased awareness of pesticide hazards to human health and nontarget organisms, including pollinators and beneficial insects, has propelled arguments for reducing pesticide reliance and substituting or supplementing with nonchemical pest management strategies. In the case of cucurbit bacterial wilt, our rudimentary understanding of the genetic and ecological components of the pathosystem has been a major barrier

to developing effective ecologically based disease management strategies. In this section, we highlight progress in understanding ecological, genetic, and epidemiological aspects of the bacterial wilt pathosystem, and pinpoint important areas that remain largely unexplored.

Pathogenicity and host specificity. At present, we have an incomplete understanding of traits or genes contributing to the virulence and host specificity of *E. tracheiphila*. As with other xylem-invading pathogens, the ability of *E. tracheiphila* to induce wilt may be due to bacterial multiplication within the xylem as well as to the production of extracellular polysaccharides (EPS) (Denny 1995). A role for EPS in the pathogenesis of *E. tracheiphila* has not been shown and preliminary data indicate variable rates and quantities of EPS production among *E. tracheiphila* strains (Mason 2012). Knowledge of the chemical and physical properties of EPS and the factors influencing EPS production by *E. tracheiphila* may provide insights into the process of host colonization and symptom induction at distinct phenological stages and among different host species (Denny 1995).

The recent discovery of genetic and virulence differences among *E. tracheiphila* strains isolated from different cucurbit genera raises the possibility that host selection pressure has driven pathogen specialization (Saalau Rojas et al. 2013). Preliminary genome sequencing data for *E. tracheiphila* provided some clues to genes associated with pathogenicity by indicating the presence of genes encoding components of a type III secretion system (Shapiro 2012). A deeper understanding of the genetics involved in host specificity could ultimately hasten progress in developing cultivars that are resistant or tolerant to bacterial wilt.

Vector-pathogen interactions. Despite seemingly close evolutionary associations between striped cucumber beetle and *E. tracheiphila*, key details of this interaction remain unknown. Although *E. tracheiphila* overwinters in the digestive tract of striped cucumber beetle, Mitchell and Hanks (2009) observed different levels of transmission efficiency among bacterial strains in frass assays. The extent to which *E. tracheiphila* strains differ in their acquisition, retention, and transmission by cucumber beetles and other diabroticites has not been rigorously examined. In other vector-transmitted bacterial pathosystems, strain differences can significantly impact pathogen acquisition, retention, and transmission, all of which may have direct implications for disease incidence and distribution. For example, the efficiency of vector acquisition and transmission of *Xylella fastidiosa*, another xylem-limited pathogen vectored by insects, varies among strains, potentially affecting disease incidence and spread (Lopes et al. 2009). Interestingly, *Pantoea stewartii*, a close relative of *E. tracheiphila* and the causal agent of Stewart's wilt of corn, employs a type III secretion system (T3SS) for retention and transmission by flea beetles, and this is distinct from the T3SS that it uses for interacting with plants (Correa et al. 2012). Investigating these interactions between *E. tracheiphila* and cucumber beetles, including differences among strains, could provide insights into new avenues for suppressing bacterial wilt. A key epidemiological gap is in understanding the bacteria-beetle relationship during insect diapause and determining rates of overwinter survival of *E. tracheiphila* in cucumber beetles. Answers to these questions will help to explain how the disease propagates among growing seasons.

Geographic range. Given the widespread distribution of the vectors and bacterial wilt-susceptible crops throughout North America, why is cucurbit bacterial wilt apparently limited primarily to the northeastern quarter of North America? Possibilities include limited distribution of competent vectors of the pathogen and geographic differences in the environmental conditions conducive to plant-pathogen or vector-pathogen interactions. Given the critical role of striped cucumber beetle to the disease, additional possibilities include geographical factors influencing beetle behavior, their feeding preferences and modes of plant colonization, or differences in these factors among diabroticites, such as spotted cucumber beetles, which can also transmit *E. tracheiphila* but have not been examined for their impact in bacterial wilt epidemics.

Ecology-based management approaches. The near-absence of commercial disease-resistant cucurbit cultivars, combined with the critical role of beetles in pathogen dissemination, emphasizes the critical need for effective management of the vectors. Employing

phenological models to predict beetle arrival and emergence, thus enabling growers to move beyond calendar-based applications and reduce the number of insecticide sprays per season, may reduce the use of insecticides. Non- and reduced-pesticidal strategies such as row covers and PTC can be effective, but would benefit from strategies to reduce the cost and labor involved in row cover deployment and broader evaluation of PTC systems across host species and cucurbit production areas, respectively. Field trials aimed at optimizing row cover and PTC strategies, including approaches to mechanize row cover deployment and removal, are underway by several authors of this review.

Summing up. After slumbering for nearly 75 years since the pioneering discoveries of Erwin, Rand, and Enlows, research on cucurbit bacterial wilt has reawakened over the past two decades. The modern era of bacterial wilt research has made substantial progress toward understanding this complex pathosystem thanks to collaborations among plant pathologists, entomologists, horticulturists, chemical ecologists, and molecular geneticists. Many puzzle pieces are still missing, but efforts are underway to validate less insecticide-dependent management methods, pinpoint pathogen genes responsible for pathogenicity and host preference, and clarify the complex interplay among *E. tracheiphila* strains, cucurbit hosts, and beetle vectors. A major advancement would be the discovery of host genes that can facilitate resistance breeding in the many vulnerable cucurbit crops.

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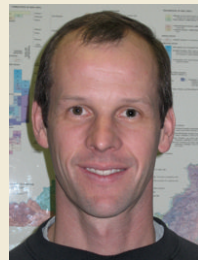
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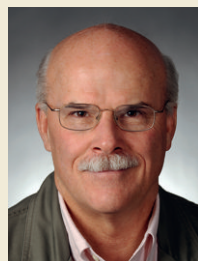
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Dr. Bessin has worked at the University of Kentucky since 1991 and his appointment as Professor of Entomology is 100% extension with responsibilities including insect management on field and specialty crops, and greenhouses. He has worked to develop and evaluate IPM decision guidelines and management strategies for specialty and field crop insect pests and some of his recent research includes evaluation and implementation of reduced-risk and organic controls for cucumber beetle, bacterial wilt, squash bug, and yellow vine decline in cucurbits. He serves as the Kentucky Integrated Pest Management Coordinator and Kentucky liaison to the IR-4 Project.

Dr. Bruton was employed by the USDA-ARS for 33 years at Weslaco, Texas and Lane, Oklahoma as a Research Plant Pathologist. He received an M.S. at Oklahoma State University and a Ph.D at Texas A&M University in plant pathology. He is adjunct professor at East Central University and Oklahoma State University. His research focused on postharvest and soilborne diseases of cucurbits with an emphasis on watermelon and melon. He did extensive research in Spain and Central America during his employment.

Dr. Boucher has served the vegetable growers of Connecticut and the Northeast as UConn's Commercial Vegetable Crops IPM/Sustainable Ag Extension Educator for the past 29 years. He maintains an active applied research program, provides weekly on-farm IPM training, works with UConn's Beginning Farmers Program, and has helped introduce a form of reduced-tillage known as deep zone tillage to growers throughout New England. He is the current General Chairman of both the CT Vegetable & Small Fruit Conference and the New England Vegetable & Fruit Conference and Trade Show, and conducts annual special-topic workshops, twilight meetings, and farm tours.

Dr. Jesse has directed the multidisciplinary Iowa State University Plant & Insect Diagnostic Clinic since 2008. Laura received her Ph.D. in Entomology and Ecology and Evolutionary Biology from Iowa State University. She works in extension and outreach to provide education on IPM, insect pests, beneficial insects, and pollinator health to a wide variety of audiences.

Dr. Gleason has been on the faculty of the Department of Plant Pathology and Microbiology at Iowa State University for 30 years. His research, teaching, and outreach focus on diseases of horticultural crops. Current research involves bacterial wilt (cucurbits), sooty blotch and flyspeck (apple), and anthracnose fruit rot (strawberry). He is the current Editor-in-Chief of *Plant Disease* and a Fellow of The American Phytopathological Society (APS).

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