News on Ooze, the Fire Blight Spreader

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Fire blight, a bacterial disease of pome fruits caused by the pathogen *Erwinia amylovora*, should be constantly on the minds of growers. Under the right conditions, this disease is known for spreading quickly and devastating an orchard with reverberating effects for years. Two of the reasons that this pathogen is so successful in seemingly being everywhere at once are the abilities of the organism to grow very quickly and to disseminate readily within and between trees. Once fire blight bacteria find their way onto apple flower stigmas, populations can undergo as many as ten cell doublings a day when temperatures are in the upper 70s to 80s F, leading to upwards of 1,000,000 bacterial cells on one flower. From there, pollinators and weather events can spread bacteria quickly to new flowers. Once those large populations build up on stigmas, as little as 0.01" of rain or even a heavy dew can provide the moisture needed for the fire blight pathogen to swim from the stigma tip down the style to the nectaries, where the bacteria infect flowers through natural openings.

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Once the bacteria have moved into the flower nectary and initiated blossom blight infection, they will begin to spread systemically through the tree. Similarly, when fire blight bacteria initiate shoot blight infections at shoot tips, the bacteria also will begin spreading systemically. It is during this systemic spread inside trees that the fire blight pathogen also initiates a second method of dispersal, dissemination via the emergence of ooze droplets onto the plant surface. Though flower to flower dissemination is important, ooze droplets are the major dispersal factor for fire blight. Ooze droplets emerge onto the surfaces of cankers in the spring and represent the source of primary disease inoculum that can be disseminated to flowers by insects including flies (Norelli et al. 2003). Ooze emergence and pathogen dissemination from blossom blight and shoot blight infections drive the occurrence of fire blight into the summer. In addition, severe storms that both spread pathogen cells from ooze droplets and provide entry wounds in trees result in major epidemics that can ultimately cause significant tree losses in affected orchards.

Physical and biological characteristics of fire blight ooze droplets

Ooze droplets are colorful, ranging in color from white to dark red hues, and are mostly comprised of exopolysaccharide sugars and bacterial cells (Figure 1). Darker colored ooze tends to harbor larger populations of bacterial cells (Slack et al. 2017). Ooze droplets can emerge from any infected tissue, including immature fruit (Figure 1A), flower pedicels (Figure 1B), shoots (Figure 1C), and leaf petioles (Figure 1D). There is seemingly no pattern to the colors, as ooze droplets of several different colors can appear close together on the same stem or the same fruit. We've done some preliminary chemical analyses of different-colored ooze droplets, and darker-colored ooze contained higher levels of flavonoids, which are plant-derived compounds that could be released in response to pathogen activity inside the host (Slack and Sundin, unpublished data).

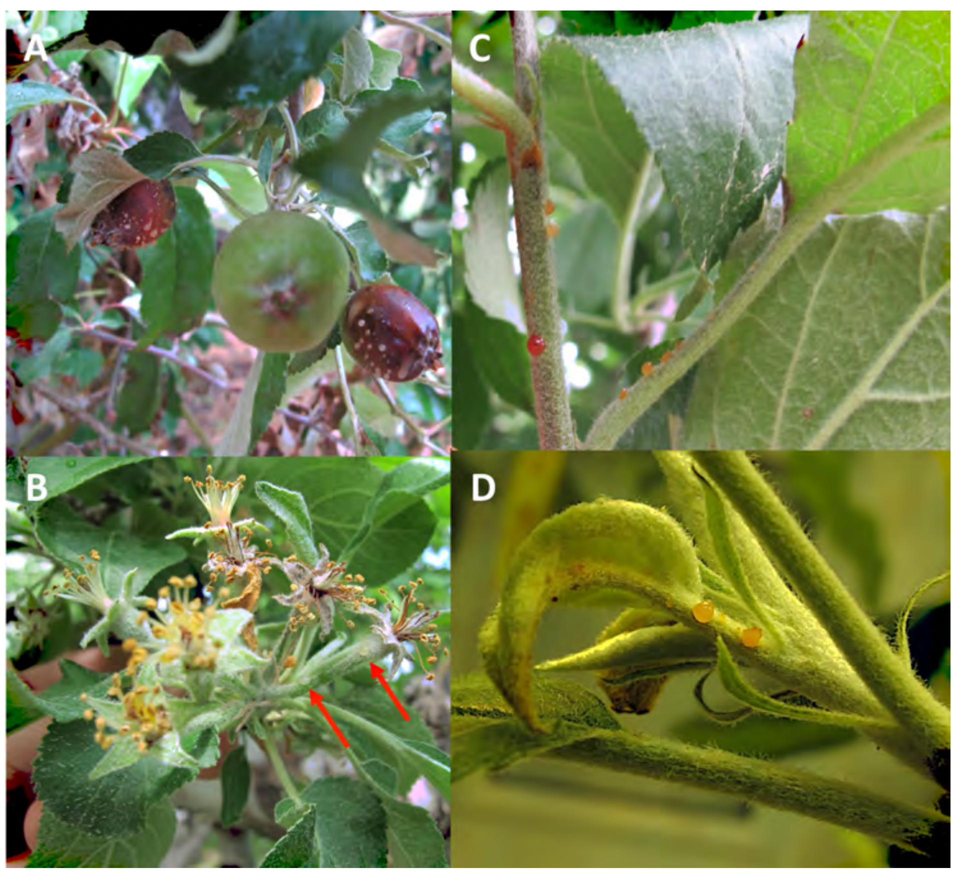

Figure 1. The range of ooze droplet colors and sizes on various host organs. (A) Ooze forming on immature apple fruit. (B) Ooze emerging on a flower pedicel (arrows) in a flower cluster exhibiting blossom blight symptoms. (C) Display of various-colored ooze droplets on apple shoots ahead of shoot blight symptoms. (D) Ooze droplets forming along the mid-vein of a leaf as well as on the petiole.

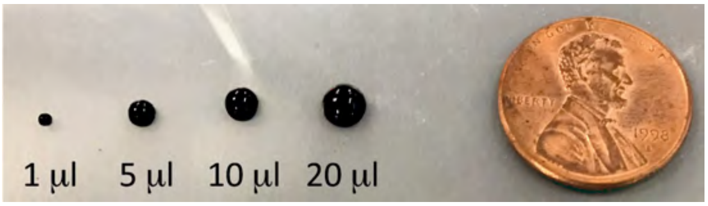

Figure 2. Comparison of representative ooze droplet volumes to a standard United States penny. Droplets of dye in the image range from 1µl (0.001 ml) to 20 µl (0.02 ml). Ooze droplet volumes emerging on apple shoots in orchards can range from 1 – 20 µl, with an average volume of close to 5 µl.

Ooze droplets are small, with a typical volume between 1 to 20 µl (0.001 to 0.02 ml). To put this into perspective, Figure 2 shows drops of colored dye of various volumes compared to a penny (Figure 2). Though they are quite tiny, these colorful ooze droplets are perfectly packaged to spread *E. amylovora* bacteria that will infect new apple trees. The exopolysaccharide sugars protect the bacterial cells inside the ooze droplet; these cells can survive within a dried ooze droplet for over one year (Hildebrand 1939). When ooze droplets first emerge from a tree, they are highly viscous and sticky. When disturbed, ooze droplets can produce bacterial strands, due to the droplet's viscous nature, that can stick to insects or to other animals. These strands, with or without insect aide, can enable further spread through an orchard (Keil and van der Zwet 1972). If ooze droplets get wet, such as through rain events or from spraying activities, they can dissolve and the bacteria within the ooze can spread. We equate this method of spreading as wetting events releasing a "cloud" of bacteria from the ooze droplet that will then spread with water and wind. If any of these bacteria are deposited on shoot tips and can invade through microscopic wounds, a new shoot infection will occur. The "cloud" concept also explains how significant and widespread fire blight outbreaks can occur following strong storms and strong wind events. The storm and wind activity generates wounds and simultaneously moves very large populations of bacteria that can ultimately settle onto shoot tips and cause new infections.

Ooze droplets are the main dissemination factor for fire blight. This means that without ooze droplets, there would be less spread of fire blight from tree to tree. Figure 3 shows a modified disease cycle of fire blight highlighting the effects of ooze droplets. Ooze droplets enable the bacteria to spread and infect multiple plant organs as well as multiple trees throughout the growing season. In the spring, cankers that were missed during winter pruning can start oozing. Bacteria from these ooze droplets on cankers are thought to be disseminated to flowers and young shoots by various insects, including flies, as well as by weather events. If blossom blight infection occurs, ooze droplets can emerge on flower pedicels (Figure 1B) which can then enable pathogen cells to be disseminated to the young, growing shoots. When the shoots become blighted, ooze droplets can emerge, enabling inoculum spread for further infection of new shoots. Thus, the earlier in the season that shoots become blighted, the more significant are disease issues, because bacterial inoculum from ooze drives further secondary cycles of shoot blight. In many cases, ooze emergence from shoot blight infection occurs

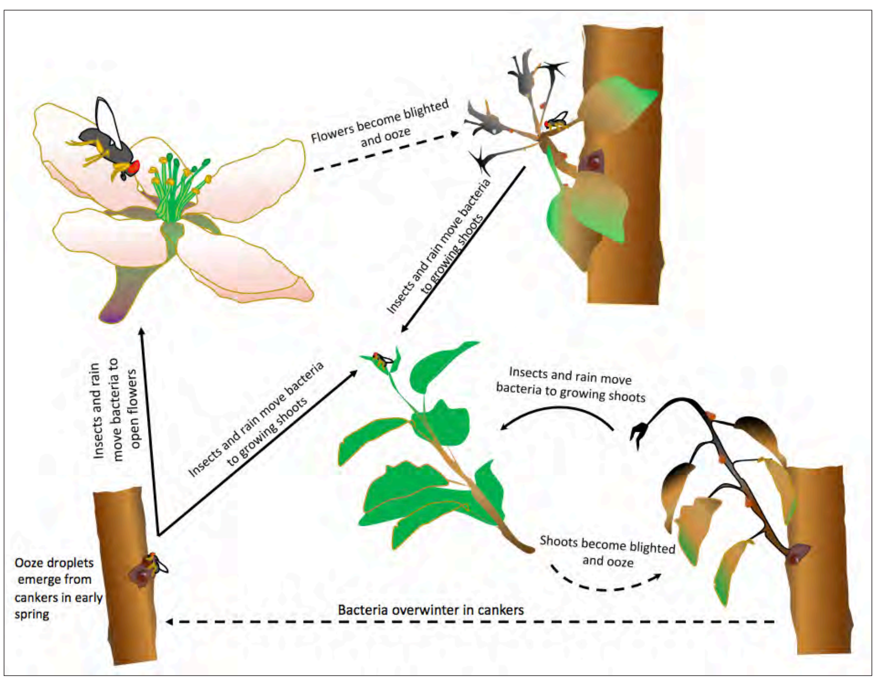

Figure 3. Fire blight disease cycle depicting when ooze droplets are important for disease spread. Solid lines indicate the spread of bacteria from ooze droplets via insects or weather events; dashed lines indicate disease progression. Starting from the bottom left, *Erwinia amylovora* bacterial cells emerge from overwintering cankers within ooze droplets; these bacteria can be disseminated to open flowers via insects and weather events. When the flowers start to succumb to blossom blight, ooze again emerges facilitating pathogen dispersal to shoots. The cankers can also ooze post bloom, providing inoculum to infect young shoots. When the bacteria move into the trunk, they can form a canker and overwinter, resulting in a continuation of the cycle the following year.

Figure 4. Emergence of white ooze droplets on an apple shoot well below the appearance of fire blight disease symptoms, which are just showing up at the shoot tip.

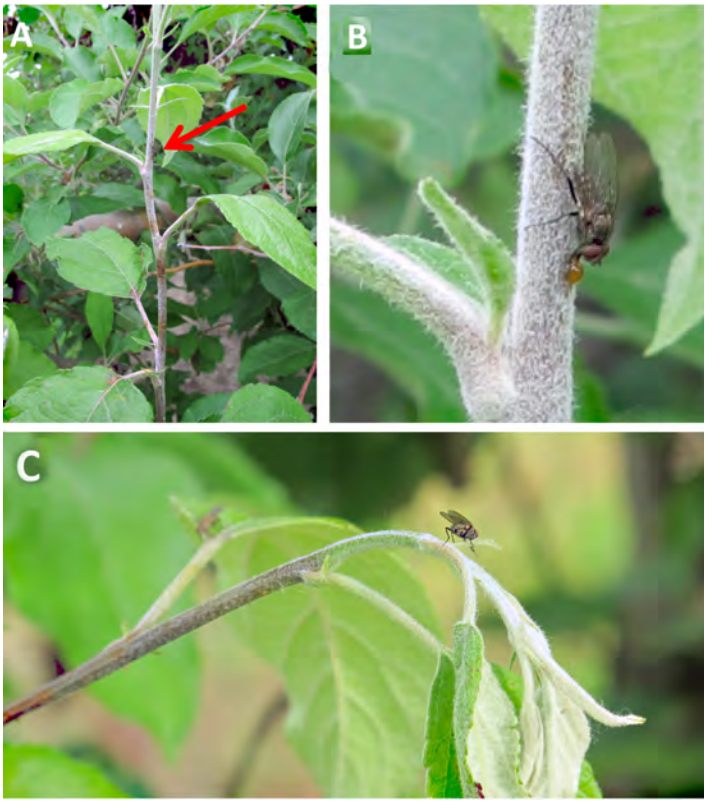

Figure 5. (A) A fly (denoted by arrow) visiting an ooze droplet on an infected apple stem. (B) Close-up view of fly touching the ooze droplet with its front legs. (C) Flies walking around tips of actively-growing shoots could deposit *Erwinia amylovora* bacterial cells capable of initiating a shoot infection.

well ahead of the actual symptoms of shoot blight (Figure 4). These droplets provide visual evidence of the *E. amylovora* pathogen moving systemically in the host ahead of symptoms, demonstrating the reasoning behind pruning recommendations to make cuts at least 18–24" below visible symptoms.

Ooze, the bacterial numbers game, and role in disease epidemics and insect transmission

Why is ooze so important to the fire blight disease cycle? The main reason is that ooze harbors very large populations of the fire blight pathogen, *E. amylovora*. In our recent studies, we determined that individual ooze droplets harbored on average between 100 million to over 1 billion bacterial cells. In contrast, only a small number of pathogen cells, as few as 35–100 cells, can cause shoot blight infections in inoculation experiments. Thus, a single ooze droplet may hold enough bacteria to infect 1–10 million shoots! The incredible infection capacity of the bacterial numbers in ooze droplets can help to explain how significant fire blight epidemics can appear in such sudden, widespread, and devastating fashion after storm events when, prior to the storm, little fire blight infection was noticed.

Within ooze, the bacterial cells are also encased within an exopolysaccharide sugar matrix that protects them from environmental stresses such as desiccation. This sugar matrix, and also possibly the color of the ooze droplet, is attractive to insects such as flies that can be observed landing on ooze droplets (Figure 5A). By touching the ooze droplet with their legs (Figure 5B), the flies may remove a small fraction of the volume of the ooze droplet, but still a significant number of bacterial cells. For example, even if the fly only removes 1/1000th of the ooze droplet, it could still be removing as many as 1 million cells. If it then flies off and lands on a nearby shoot (Figure 5C), it is possible that enough cells could be deposited to cause a new shoot infection. We hypothesize that the concentrated cell numbers and their protective sugar matrix facilitate insect transmission of fire blight by providing the numbers component necessary for infection and an ability to survive the time component needed for the fly to land on a new shoot tip.

Ooze emergence from infected apple tissue

We were interested in determining the mechanism of egress from plant tissue of fire blight bacteria emerging as ooze. In some situations, for example in pears, ooze emerges from the host through natural openings such as lenticels (Zamski et al. 2006). We examined apple tissue under ooze droplets using scanning electron microscopy to determine how the *E. amylovora* pathogen was escaping the host. In these studies, we never observed *E. amylovora* escaping through natural openings. Instead, we observed bacteria emerging from the host through wounds or tears in tissue, and in some cases, we observed erumpent mounds of tissue underneath ooze droplets (Figure 6A and 6B, and additional figures in Slack et al. 2017). We interpreted these results as indicating that internal proliferation of fire blight pathogen cells in the confined spaces inside apple stems, fruit, petioles, and pedicels, results in a buildup of pressure that ultimately causes a wound, opening a microscopic hole that enables the bacteria to spill out onto the surface. At this point, the viscosity of the exopolysaccharide sugars holds the cells within a droplet (Figure 6C).

Controlling ooze in orchards

Since ooze emerges after fire blight infection, the best method to control the amount of ooze present in orchards is to control fire blight infections. The best materials available for blossom blight control are streptomycin and kasumin. Use of fire blight prediction models to time spray applications, maintaining excellent spray coverage, and maintaining focus and diligence in fire blight control during bloom are all keys to success. The shoot blight phase of fire blight is best managed using prohexadione calcium (Apogee; BASF Corp.), a shoot growth inhibitor that also provides excellent control of shoot blight (Sundin 2014).

A second method of managing ooze emergence is through pruning. Winter pruning of cankers is critical for the removal of cankers and removal of the primary inoculum that oozing cankers provide for flower infection in the spring. In-season removal of active shoot blight infections is possible, but must be done very carefully. As stated above, when pruning infected shoots (strikes), it is always suggested to remove the shoot at least 18–24 inches below the active infection. In high-density trees, if this is not possible due to shorter branch lengths, branches should be pruned back to the central leader. As we determined from sampling studies, when ooze is present, even higher populations of bacteria are present in the infected shoot (Slack et al. 2017), and these bacteria are capable of moving very quickly, especially through branches of young trees of highly susceptible cultivars. Pruned cuttings should be dropped into orchard row middles and allowed to dry thoroughly over a period of weeks before removal. This will reduce the potential for further spreading of fire blight bacteria within an affected block. Always be careful to not touch any ooze droplets during pruning activities, as this could also result in unintentional spreading of fire blight.

Figure 6. **(A) Scanning electron micrograph (SEM) of the surface of an immature apple fruit surrounding two ooze droplets that had emerged on the surface (30X). (B) Close-up SEM image of the two ooze droplets showing surface tear wounds where the droplets (denoted by arrows) have emerged (200X). (C) Emergence of a yellow ooze droplet on an apple shoot from a small wound site (denoted by arrow). As the ooze emerges onto the stem surface, it forms a droplet through the actions of gravity and viscosity.**

Conclusions

Ooze droplets represent the most important mechanism enabling the spread of fire blight through an orchard and between orchards. Incredibly large bacterial populations, densely packed into ooze droplets, spread as a "cloud" with wetting and wind. Intense weather events that result in tree injury, including hail storms or storms with high winds, can effectively move these "bacterial clouds" through orchards. Because of the large population sizes in even one ooze droplet, storm events can trigger fire blight epidemics, even when the amount of existing infection prior to the storm was relatively low. The microbiological characteristics of ooze, large bacterial populations environmentally protected in a viscous sugar matrix, along with inevitable storm events, enable the fire blight pathogen to seemingly be everywhere at once. Effective management of fire blight infections, and in particular blossom blight and early shoot blight infections, will give the best prevention of devastating fire blight outbreaks.

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