

## FAQ

**General**

Who is A&P Inphatec?

- We specialize in developing and manufacturing bacteriophages to kill bacteria that cause plant diseases at the source of the problem. Our first product is XylPhi-PD, a bacteriophage cocktail to reduce symptoms of Pierce's Disease in grapevines.

What are bacteriophages (phages)?

- Bacteriophages (or phages) were discovered over 100 years ago and are viruses that specifically target bacteria. Phages can kill only their targeted bacteria, and do not infect plants and people.

**Pierce's Disease (PD)?**

What is Pierce's Disease?

- PD is a lethal disease of grapevines and is caused by the xylem-limited bacterium, *Xylella fastidiosa* (*Xf*). Once infected, the bacteria will eventually block the xylem, which conducts water throughout the plant. PD symptoms include chlorosis, leaf scorching, grape wilting, and overall stunted growth. PD is spread by xylem-feeding leafhoppers known as sharpshooters.

How do we know which vines have PD?

- Visual symptoms in the fall can be used to correlate to the severity of disease. Testing the presence of *Xf* DNA by polymerase chain reaction (PCR) of petioles can definitively identify the disease in the fall.

Can PD be confused for other diseases?

- Yes, in particular Grape measles (Esca), Eutypa dieback, Grapevine leafroll disease

How can we predict the level of vector/sharpshooter presence?

- Sharpshooters can be monitored using yellow sticky traps around your field.

Is PD usually found in a vineyard every year after the first appearance?

- *Xf* is harbored in asymptomatic host plant reservoirs as well as infected grapevines and is easily transmitted year after year by sharpshooters who feed on infected grapevines or other plants if they cannot be completely removed.

Are Sharpshooters present in a given vineyard every year once initially found in that vineyard?

- In the North Coast of California, blue-green sharpshooters (BGSS) feed, reproduce, and are often abundant on cultivated grape. They are commonly found in riparian vegetation. Adults become active in late winter and early spring, and a small percentage begin moving into nearby vineyards for feeding and egg laying starting just after budbreak. Their movement into vineyards increases as natural vegetation dries up. Eggs hatch from May through July. Some of the nymphs become adults by mid-June, and the number of young adults continues to increase through July and August. In August when grape foliage is less succulent, BGSS begin to move back to nearby

natural habitats. The proportion of infectious BGSS increases throughout the season as they pick up *Xf* from symptomless host plants. Once an adult acquires *Xf*, they remain infectious for the remainder of their lives.

### **XylPhi-PD (“product”)**

What is the active ingredient?

- The active ingredient in XylPhi-PD is bacteriophage active against *Xylella fastidiosa*.

On what crops can XylPhi-PD be used?

- XylPhi-PD is registered for use on grapevines in California.

Can XylPhi-PD be used in organic programs?

- Yes, XylPhi-PD is OMRI-listed for organic use.

Is XylPhi-PD preventative or curative for PD?

- Both! As stated in the label, XylPhi-PD can be used both to prevent and treat PD.

### **XylPhi-PD Application**

How is XylPhi-PD applied?

- XylPhi-PD is injected directly into the xylem using a specialized injector system called “Xyleject”.

What part of the grapevine needs to be treated?

- XylPhi-PD is injected into active xylem vascular tissue and injected above ground.
- Please refer to our product insert for detailed instructions on injection locations based on individual vine training type.

What is the rate/vine for different vine ages?

For smaller replants or new vines, refer to the product label insert to determine the correct rate/vine.

- For mature vines of any size, refer to our product label insert to determine the correct rate/vine, as this depends on the vine training type.

Does turgor pressure affect treatment of the grapevine?

- Inject XylPhi-PD™ when uptake of water or product into the xylem vessels, the plant’s vascular system, is optimal. Do not inject XylPhi-PD™ when plants’ xylem is saturated or shortly after heavy rainfall or irrigation.

How long does it take to inject a vine?

- For young vines or replants, injection is as fast as 15 seconds/vine
- For mature vines, injection is as fast as 30 seconds/vine

What is the frequency of application?

- The first injection is made 6-8 weeks after first flush from dormancy (bud break). Up to two more injections follow every 4-6 weeks for a total of 3 injections/season. This provides seasonal coverage as the vector remains active.

Are three applications per growing season really necessary? Will XylPhi-PD still work if we only inject once per season?

- Follow the label and apply XylPhi-PD two to three times across the growing season. Academic field trial data has shown a reduction in efficacy when XylPhi-PD was applied only once vs. three applications across the growing season<sup>1,2</sup>.

Do you use the same injection site each time?

- New sites on the vine are preferred, but not required. However, application locations must be consistent with the product label.

Does the injection needle transfer other diseases between vines?

- To date, we have not seen evidence of the Xyleject injection device transferring disease between vines. However, needles can be sterilized in accordance with standards for pruning as an extra precaution.

Are there restrictions on use in certain temperatures? Does injection time of day have any impact on efficacy of the product?

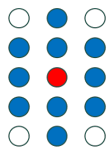
- For best results, the product should not be injected when the sun is down due to lack of transpiration in the plant. We also don't recommend injections when temperatures above 107.6°F (42°C)

In the North Coast of California, how far away from the edge of the riparian area should vines be treated to prevent infection by BGSS?

- In the North Coast of California, we recommend applying as far as BGSS can travel into the vineyard. It has been shown that the majority of BGSS only venture 50 m from riparian edge<sup>3</sup>.

Should vines around infected plants be treated even if not in the riparian treatment area?

- Yes, we recommend treating known PD hotspots, including the surrounding vines. We recommend treating the 10 surrounding vines once a PD infected vine is identified.



● : Identified PD vine

● : Recommended vines to treat

What are the treatment recommendations for different PD symptom ratings?

Likely healthy vines	Early Stage Infections	Chronic Infections
no visible symptoms	only a small portion (<5%) of the vine is showing symptoms, such as marginal chlorosis	a significant portion (>5%) of the vine is showing symptoms
Healthy appearing vines should be treated if they are in a known hotspot or riparian area to prevent PD.	Vines that are starting to show visible symptoms of infection should be treated.	<p>If the entire vine appears to be dead or dying, rogue the vine and treat the re-planted vine to prevent PD.</p> <p>If only a portion of the vine is very sick, prune back the dead or dying portions and treat the living tissue.</p>

What are the personal protective equipment (PPE) requirements for applying XylPhi-PD?

- Applicators and other handlers must wear a long-sleeved shirt, long pants, and shoes plus socks. Follow the manufacturer’s instructions for cleaning and maintaining PPE. If there are no such instructions for washing, use detergent and hot water. Keep and wash PPE separately from other laundry.

**XylPhi-PD Storage and Disposal**

What temperature should the product be stored at upon receipt?

- The product should be stored according to the label, “in a dry area at either room temperature or under refrigerated conditions (36 – 80°F), away from direct sunlight and extreme heat.”

If all ordered product is not used for one injection cycle, can it be saved for the next injection cycle?

- Unopened bottles may be stored in the appropriate conditions for future injections. Unused product may be disposed through a waste disposal facility or pesticide disposal program (often such programs are run by state or local governments or by industry)

Are there restrictions on the disposal of needles and empty phage bottles?

- Broken needles should be disposed in a sharps container. Empty bottles cannot be reused or refilled and should be disposed either in a sanitary landfill or by offering for recycling. Other procedures from local authorities may apply.

**XylPhi-PD Efficacy**

Do you see reduced levels of *Xf* in grapevines after treatment with XylPhi-PD?

- Yes. Our previous research and field trial results document if grapevines are positive or negative for *Xf* and we see fewer vines with *Xf* after treatment with XylPhi-PD in accordance with the label.

How long will phages remain in the vine after injection?

- Phage remained detectable in the vine for up to 12 weeks<sup>4</sup> in a greenhouse study, but XylPhi-PD is injected every 6 – 8 weeks because academic field trial data has shown a reduction in efficacy when XylPhi-PD was applied only once vs. three applications across the growing season<sup>1,2</sup>.

How long after application do the phages start working?

- Phage can move quickly within the vine after application. Once the phage contacts the bacteria, it can infect and kill the bacteria.

Do vectors like the sharpshooter transfer phage to the other vines?

- Data have shown that glassy-winged sharpshooters can transfer phage in lab settings<sup>5</sup>.
- Phages have been detected in sharpshooters, weeds, and untreated vines in previous field studies. It is still being studied whether effective amounts of phage are transferred by sharpshooters.

Do you need to treat for multiple years?

- Yes, new generations of sharpshooter populations acquire and transmit *Xf* to plants every year, necessitating treatment in years of high sharpshooter activity.

How and when can efficacy be recognized by the grower?

- Visual disease rating guidelines are provided by your WE-CO PCA and UCCE. Reduction or absence of visual symptoms is an indication of efficacy.
- Note that PD can cause significant damage to the vine before treatment begins, so it may take multiple years of treatment for vines to recover visually. The rate of improvement in visual symptoms will also depend on the amount of PD pressure at each individual vineyard.
- Efficacy can also be confirmed by PCR (polymerase chain reaction), a molecular diagnostic method used to detect the bacteria.

Is the phage equally effective on all grapevine varieties?

- XylPhi-PD has been demonstrated to be effective in all tested varieties (i.e. chardonnay, cabernet sauvignon) when applied in accordance with the product label. However, different wine grape varieties have been shown to have differing susceptibilities to PD (UC ANR publication 21600 Pierce's Disease)

Does administration of the phage have any impact on fruit yield?

- PD decreases fruit yield. Therefore, prevention and treatment of PD will result in a better fruit yield, overall.

Does administration of the phage have any impact on fruit quality?

- pH and brix did not change in grapes treated with XylPhi-PD in field trials where XylPhi-PD was applied in accordance with the label.<sup>6</sup>

### **Technical support**

Who is the technical resource for injector issues? What is the contact information?

- Pulse Biotech, 913-599-1590

From where do we obtain injector parts and needles?

- Pulse Biotech, 913-599-1590

Who is the technical resource for product issues? What is the contact information?

- Please contact your Wilbur-Ellis PCA directly with any issues.

### **References**

<sup>1</sup> Technical Bulletin

<sup>2</sup> California field trial progress report, 2017

<sup>3</sup> Matt Daugherty, UCCE Sonoma County Grape Day presentation, Feb 7, 2019

<sup>4</sup> Das M, Bhowmick TS, Ahern SJ, Young R, Gonzalez CF (2015) Control of Pierce's Disease by Phage. PLoS ONE 10(6): e0128902. <https://doi.org/10.1371/journal.pone.0128902>

<sup>5</sup> Bhowmick TS, Das M, Heinz KM, Krauter PC, Gonzalez CF. Transmission of phage by glassy-winged sharpshooters, a vector of *Xylella fastidiosa*. *Bacteriophage*. 2016;6(3):e1218411. Published 2016 Aug 2. doi:10.1080/21597081.2016.1218411

<sup>6</sup> LEW 32 Trial: 2019 wine presentation