viticulture tech data UPDATE

XylPhi-PD™ Bactericide for use in grapevines.

2020 Field Trial Results: XYLPHI-PD™ for Control of Pierce's Disease (5 sites, 2-3 years of treatment)

While vineyard managers must continuously confront many obstacles to optimal productivity, Pierce's Disease (PD) poses a particularly insidious threat due to limited options for effective prevention and control. PD is a deadly disease of grapevines caused by *Xylella fastidiosa* bacteria which are easily spread by 2 main insect vectors, blue-green and glassy-winged sharpshooters. The *X. fastidiosa* bacteria block the xylem of grapevines, causing chlorosis and scorching of leaves that eventually kills entire vines in 1 to 5 years.[1] Thus, PD represents a major threat to major US wine regions, accounting for widespread economic damage (e.g., roguing and replanting of vines, low fruit production, etc.) and costly deployment of resources aimed at disease moderation.

Few methods for controlling and treating PD have been available, with efforts historically focused on controlling the sharpshooter vector (e.g., insecticides) or roguing seriously ill vines, both of which have demonstrated only limited success. However, an option that reduces PD in grapevines is now available, XYLPHI-PD.™

KEY POINTS

Two field studies assessed the year-to-year efficacy of XYLPHI-PD injectable bacteriophage for the treatment and/or prevention of Pierce's Disease (PD) at 5 major commercial vineyards in Sonoma County, CA.

- A 2-year, multi-location field study evaluated the curative efficacy of XYLPHI-PD against endemic PD across 4 sites and 2 production seasons.[2]
- PD incidence (PCR) fell 57% from 2019 to 2020 for vines consistently treated for 2 seasons with XYLPHI-PD, while untreated control vines had only a 14% seasonal reduction in year-over-year PD incidence.
- A 3-year field study evaluated XYLPHI-PD efficacy for preventing PD and preserving productivity of vines exposed to PD.[3]
- Healthy vines treated with XYLPHI-PD demonstrated up to 72% PD incidence reduction, less disease severity, no mortalities, and produced 1.34 lb more fruit/vine/year compared to buffer-treated control vines.

XYLPHI-PD™

XYLPHI-PD is a novel, OMRI Listed biological treatment for PD, a cost-effective break-through technology developed specifically for viticulture. XYLPHI-PD contains a cocktail of viral bacteriophages (bacteria-killing viruses) that enter and destroy *X. fastidiosa* bacteria.

XYLPHI-PD can be flexibly applied as a preventative to protect growing vines under disease pressure, or as a curative after disease symptoms become visible. XYLPHI-PD is available in 100-mL vials (treats up to 300 mature vines or 600 young vines), intended for injection into grapevines using the Pulse Xyleject™ pressurized injection device, and has no REI and minimal PPE when used in accordance with label Directions for Use.

A&P Inphatec

2019-2020, 4 CA Sites: Consistent Use, Endemic Infection Field Trial

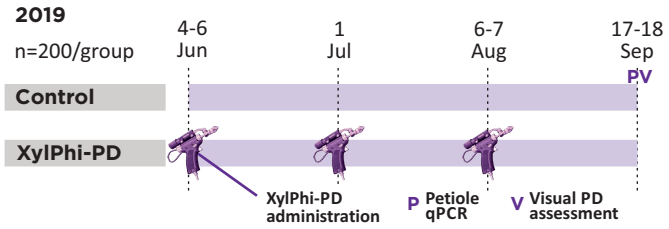
Experiment Design

A 2-year, multi-location, commercial (Wilbur-Ellis) field study evaluated the efficacy of XylPhi-PD against endemic PD across 4 sites and 2 production seasons.[2] The extensive research effort began in 2019 when a study was conducted that involved 400 vines (300 Chardonnay, 100 Pinot Noir) at 3 Sonoma County CA wineries with a history of PD (1 winery had 2 test fields).

- *Site A:* located along the Russian River, an area known for high PD pressure in adjacent fields; Chardonnay field added in last 5 years; plot not actively rogued for PD (none after study start).
- *Site B:* a plot of Pinot Noir bordering the Russian River; vines very actively rogued for PD (none after study start); site initially designed with treated areas close to the riparian area, but because significant portions of the field were replanted due to PD pressure, trial vines were selected further away from the edge of the field.
- *Site C:* a plot of Chardonnay at the same Russian River location/conditions as site B, and managed by the same winery/personnel; vines very actively rogued for PD (none after study start).
- *Site D:* located in the Dry Creek appellation, Chardonnay site mostly left to succumb to PD (vines not actively rogued) and displayed the highest PD pressure; neonicotanoid pesticides sprayed biweekly and large sticky tape deployed along riparian border in attempts to reduce sharpshooter numbers.

Vines were randomly selected in treatment blocks at each site and assigned to either of 2 treatment groups as follows:

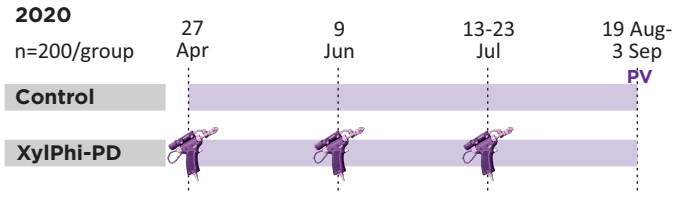
- Control (untreated): n=200 (50/site);
- XylPhi-PD: 3 treatments (Jun/Jul/Aug); 80 µL of XylPhi-PD injected twice in the trunk and once in each cordon (4-6 injections = 1 treatment); n=200 (50/site).



Six petioles from each vine were collected in September for analysis by quantitative polymerase chain reaction (qPCR) and confirmation of *X. fastidiosa* infection. Petioles were selected from the lower part of the vine and evenly distributed across the vine, but skewed towards the leaves displaying the most marginal chlorosis. Vines with a quantification cycle (C_q) ≤ 37.5 were considered affected by PD.

All study vines were also visually assessed by trained observers for PD development and symptoms using a scoring system that described the scope of marginal chlorosis. (Visual PD assessments can help provide a gauge of the time associated with observation of XylPhi-PD efficacy based on typical symptoms of PD.) In addition, insect traps were placed at each study site in an attempt to monitor vector pressure (blue-green, green, and red-headed sharpshooters).

A continuation of the same study protocol was followed in 2020, allowing another season of treatments and observations for the same vines/blocks at the same sites/wineries. The only differences from 2019 were the treatment dates; all injections, procedures, and data collections were similar to 2019.



Results

Year-to-year comparative outcomes for PD incidence, based on vines qPCR-positive for *X. fastidiosa*, are summarized in Figure 1. Under the conditions of only mild to moderate PD pressure and low vector populations, sequential

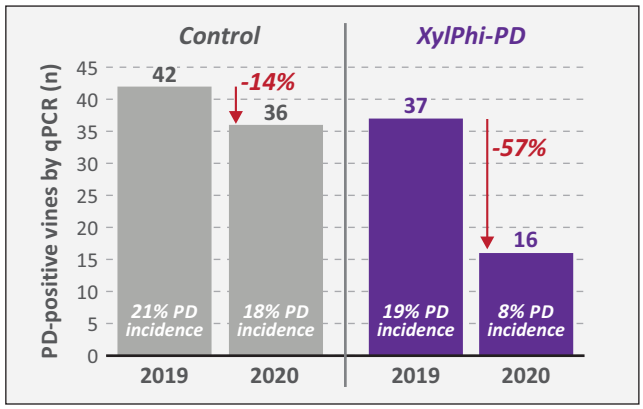


FIGURE 1: Vines qPCR-positive for *X. fastidiosa*; summary of 4 sites in Sonoma county, CA.

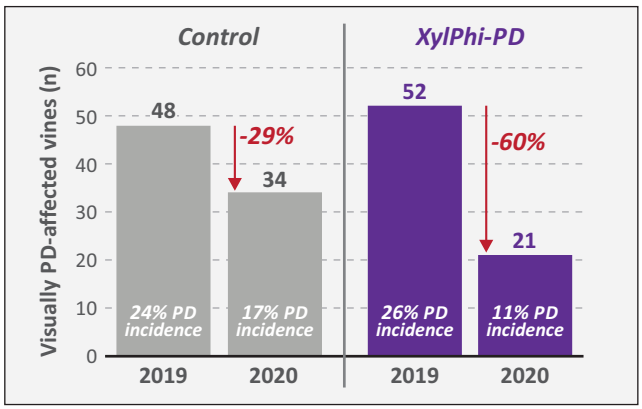


FIGURE 2: Vines showing visual signs of PD; summary of 4 sites in Sonoma county, CA.

year-to-year use of XylPhi-PD generated impressive results. PD incidence fell ***57%*** from 2019 to 2020 for vines consistently treated for both seasons with XylPhi-PD (21 fewer vines with PD in 2020 vs 2019). In contrast to this large reduction, untreated control vines demonstrated only a 14% seasonal reduction in year-over-year PD incidence, reflecting natural fluctuation in PD incidence.

The visual assessment of vines for signs of PD was another important study parameter, and outcomes (Figure 2) were similar to those using qPCR confirmation of infection. Vines treated with XylPhi-PD for 2 years (2020 data) generated a ***60%*** year-over-year reduction in visual PD incidence, compared to only a 29% year-over-year reduction in controls. Notably, at every site, vines treated for 2 years showed fewer PD symptoms than the previous year. The similarity of these data with qPCR results suggests that visual assessments can help vineyard managers tangibly gauge the efficacy of XylPhi-PD.

Implications

This study conducted at major commercial vineyards successfully demonstrated the ability of XylPhi-PD to both prevent and treat endemic PD infections in riparian areas, including a site with insecticide use. Though the study sites originally had high PD pressure, lower incoming numbers of new PD cases/disease pressure in 2020 were experienced at all 4 sites, likely due to a combination of insecticide use and the naturally cyclical nature of PD outbreaks.

While an overall recovery from PD was noted across both treated and control groups, a much greater change was observed in groups treated with XylPhi-PD. As with most disease situations, better efficacy was achieved for prevention and the treatment of early disease than for the treatment of later-stage infections.

Conclusions

The benefits and value of continued year-to-year use of XylPhi-PD were confirmed by this 2-year study conducted under commercial conditions in the presence of endemic PD pressure. Two sequential seasons of XylPhi-PD administration reduced overall PD pressure and severity, especially for vines harboring early infections.

2018-2020, CA: Preserving Productivity in Healthy Vines

Experiment Design

A 3-year field study was conducted at a major commercial winery in Sonoma County CA (Ridge, Lytton Springs; Dry Creek) to evaluate the efficacy of XylPhi-PD for preventing PD and thus preserving the productivity of healthy vines naturally exposed to PD.[3] The winery employs organic production practices and does not use conventional chemical insecticides or other vector control measures such as netting or habitat management (though sticky traps are monitored for sharpshooters). Research was initiated in 2017 when 242 vines in a common

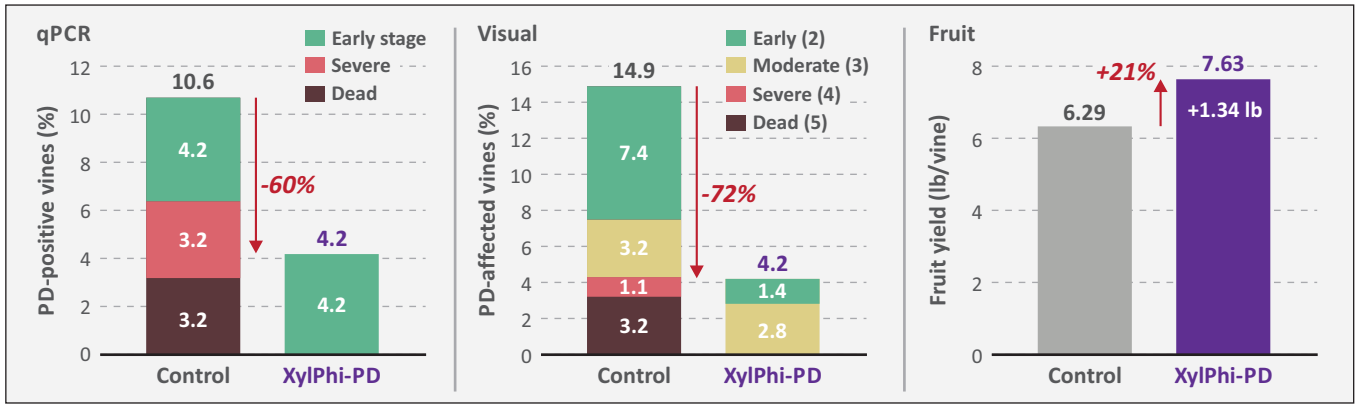


FIGURE 3: Results of 2020 production season for vines negative for *X. fastidiosa* by PCR in 2017; percent of vines qPCR-positive for PD (*X. fastidiosa*), percent of vines showing visual signs of PD, and average fruit yield (lb).

plot were enrolled in the study and small groups of vines were randomly assigned to either of 2 treatment groups. Of these, 165 vines were confirmed negative for *X. fastidiosa* by PCR (healthy) and consistently managed in 2018, 2019, and 2020 as follows:

- Control (buffer-injected): 3 treatments, n=94;
- XylPhi-PD: 3 treatments, n=71.

The study protocol thus provided 3 years of continuous data regarding infection status and productivity. Treatments were administered in May/Jun/Jul, Jun/Jul/Aug, or May/Jun/Aug (varied by year), with 1 treatment consisting of injections twice into each trunk and into all 1-year spurs on 3 of the cordons. Other neighboring vines in the plot were PCR positive or displayed signs of PD, so study vines were continuously exposed to PD over the 3 seasons.

Petiole collections (for qPCR analysis) and visual PD assessments were conducted as in the previous study (late summer or early autumn). Disease severity was quantified for qPCR by categorizing a vine with C_q >33.5 to ≤37.5 as an 'early stage' infection, but one with C_q ≤33.5 as a 'severe' infection. A disease rating score based on the percent of leaves with marginal chlorosis was used to quantify the visual assessments (2/early: >1% to ≤5%; 3/moderate: >5% to ≤30%; 4/severe: >30%). In addition, fruit yield (weight) at harvest was measured for every enrolled vine.

Results and Conclusions

Results for qPCR testing, visual PD assessments, and fruit yield for 2020 (the most recent year of data collection) are summarized in Figure 3. Healthy vines (2017) treated with XylPhi-PD demonstrated overall PD incidence reductions ranging from ***60% to 72%*** (qPCR and visual detection, respectively) compared to control vines. XylPhi-PD was clearly useful as a preventative to help protect growing vines.

Disease *severity* was also moderated in vines treated with XylPhi-PD. The few instances of PD in treated vines were relatively mild, in contrast to the severe infections or mortalities that were observed in the control group. Notably, no vines in the XylPhi-PD group succumbed to PD over the 3 years of treatment and observation ***(0% mortality)***, but 3 vines (3.2%) in the control group died due to PD.

Perhaps most important, vines in the group treated with XylPhi-PD produced ***1.34 lb (+21%)*** more fruit per vine than controls.

Study outcomes clearly support use of XylPhi-PD for prevention of PD and helping maintain fruit yield when used in accordance with label Directions for Use, even under organic production conditions in the presence of substantial PD pressure.

REFERENCES

1. Pierce's disease research updates. CDFA. http://piercesdisease.cdfa.ca.gov (accessed April 2021).
2. Field trial report, 2020. Wilbur-Ellis/A&P Inphatec. Data on file.
3. Field trial report, 2020. Ridge Lytton Springs/A&P Inphatec. Data on file.

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