UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON D.C., 20460

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

MEMORANDUM

SUBJECT: Review of the Resistance Monitoring Bioassay Final Report for Innate® Potatoes Varieties X17 and Y9
EPA Reg. Nos. 8917-1; 8917-2; 8917-3
MRID# 508560-01
Decision# 552787
DP Barcodes: 453084

FROM: Kara Welch, Biologist
Emerging Technologies Branch
Biopesticides and Pollution Prevention Division (7511P)

Digitally signed by KARA WELCH
Date: 2020.01.06 08:50:08 -05'00'

TO: Matt Weiner, Risk Manager
Emerging Technologies Branch
Biopesticides and Pollution Prevention Division (7511P)

PEER REVIEW: Alan Reynolds, Team Leader
Emerging Technologies Branch
Biopesticides and Pollution Prevention Division (7511P)

Digitally signed by ALAN REYNOLDS
Date: 2020.01.13 15:29:36 -05'00'

Summary

BPPD[1] has reviewed the final bioassay report (MRID# 508560-01) submitted by J.R. Simplot Company as part of the terms of registration for Innate® potatoes (EPA Reg. Nos. 8917-2; 8917-3) for resistance monitoring of this plant-incorporated protectant (PIP) against late blight disease (LB, *Phytophthora infestans*). Herein, BPPD assesses Simplot's proposed bioassay technique for resistance monitoring.

BPPD concludes that the bioassay methodology described in the final report is adequate to conduct a post-registration resistance monitoring program and sensitive to detecting resistance development.

Background

The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) requires that registered pesticide uses do not pose unreasonable adverse effects to the environment and humans. Resistance is considered an "unreasonable adverse effect" under FIFRA Sect. 2 (bb). To delay late blight pathogen resistance evolution against the VNT1 trait, the registrant conducts a post-

[1] BPPD refers to the Resistance Management (RM) team consisting of K. Welch and A. Reynolds

registration resistance monitoring program. A sensitive bioassay system is paramount to recognizing incremental steps of resistance development to a pesticide.

Late blight disease is a fungal pathogen causing economic loss to potato growers. Late blight infests potato foliage and tubers throughout the growing season of the crop. Symptoms of late blight include dark lesions on potato foliage and defoliation ultimately leading to plant death within days of infection. In addition, potato tubers infected with late blight are discolored, irregularly shaped, and rotted. Disease is exacerbated by weather conditions. Historically, late blight control was provided by conventional fungicide oversprays as well as cultural control measures (e.g., seed hygiene, crop destruction) because once this pathogen establishes in a field, there is no rescue fungicide treatment available.

There are two potato varieties currently marketed by Simplot to United States farmers with the late blight resistant Rpi-vnt1 gene (VNT1), including the X19 Ranger Russet potato and the Y9 Atlantic potato (EPA Reg. Nos. 8917-2; 8917-3). Another variety containing the VNT1 trait, the W8 potato, is registered with EPA (8917-1) but Simplot does not currently commercialize this product; thus, it is not discussed further herein. However, BPPD expects Simplot to use the final resistance management plan developed for the X19 and Y9 varieties should commercialization of W8 occur in the future

The VNT1 trait triggers a hyperactive immune response by the potato plant that kills infected plant cells locally and prevents spread of the pathogen to uninfected cells. The VNT1 trait is derived from wild potato species that are naturally resistant to late blight infection. Event VNT1 has been tested against several strains of late blight currently present in the United States and has been shown to be efficacious against at least US-8, US-22, US-23, and US-24. However, in areas with late blight pressure, a reduced but consistent fungicide overspray program is still necessitated to prevent disease in conjunction with the VNT1 trait.

The VNT1 trait may be overcome through changes in expression of, mutation in, or disruption of the transgene. Thus, the bioassay methodology herein was developed to monitor changes to the protection provided by VNT1 potatoes as part of the stewardship and management program.

BPPD has previously reviewed the resistance management proposal and integrated pest management (IPM) plan for Simplot potatoes expressing the VNT1 trait (Docket number EPA-HQ-OPP-2016-0036-0008). As a part of the terms of registration for Innate® potatoes, Simplot is required to conduct ongoing bioassays to monitor for resistance evolution to VNT1 by *P. infestans* isolates collected from fields with unexpected injury (UXI) reports.

As discussed in BPPD's (2016; 2018) prior bioassay reviews, Simplot developed a bioassay methodology to evaluate changes in late blight protection of the VNT1 varieties. The initially proposed bioassay used a 'dead or alive' approach for scoring potential resistance of the late blight pathogen to VNT1 potatoes from UXI fields (BPPD 2016). BPPD requested that the registrant consider an intermediate scale instead as well as other resistance monitoring methods (e.g., molecular techniques or UV light methods).

Simplot developed an intermediate 1-7 scale in a follow up report and validated the bioassay methodology over two years of data collection (BPPD 2018). Bioassay results from 2016-2017 confirmed that X17 and Y9 potatoes were protected against US-8, US-22, US-23, or US-24 but not protected against the positive control, EC-1. Additionally, the control potato varieties without VNT1 protection failed against all five *P. infestans* strains. BPPD (2018) requested the registrant consider of a molecular method for resistance monitoring, use of sentinel plots if applicable, increased sample collection education, and rationale for threshold choice for bioassay resistance determination on the intermediate scale.

In the final bioassay report, Simplot validated the bioassay methodology against field collected *P. infestans* samples. Below, BPPD will describe the final bioassay methodology and Simplot's response to BPPD (2018) queries.

Summary of Simplot (2019) Final Bioassay Report for VNT1 Potatoes
Simplot's final bioassay report for VNT1 potatoes is contained in one volume listed below:

MRID# 508560-01: Monitoring Late Blight Protection in Innate® Potatoes – Bioassay Final Report

A bioassay of VNT1 potatoes, X17 and Y9, is triggered after grower reported unexpected injury (UXI). UXI is defined as "sporulating foliar lesions or plant defoliation characteristic of late blight". Following UXI, there are two stages of Simplot's proposed bioassay method: sample collection and the bioassay itself. Growers are required to send samples from the UXI field consisting of damaged leaves as well as healthy leaves, preferably from the same plant, to a third-party laboratory using a kit provided to them by Simplot. The sampling kit includes materials to aid in proper moisture retention of the sample to ensure the late blight pathogen can be cultured. Submitted samples are then inspected by the third-party laboratories for disease confirmation, late blight strain identification, and VNT1 variety confirmation. For stewardship purposes, the bioassay will only be performed if the UXI field has confirmed late blight infection from a labeled strain (US-8, US-22, US-23, or US-24) and the affected potato variety is X17 or Y9. If the above criteria are met, a laboratory bioassay is conducted to ascertain if VNT1 resistance has developed to the *P. infestans* isolate.

Simplot assessed their bioassay methodology in 2016 and 2017 using laboratory *P. infestans* isolates. The results of the 2016 and 2017 bioassays conducted by Simplot were described again in the 2019 submission (MRID# 508560-01) but BPPD herein will discuss only the new 2018 bioassays (see background section for general overview).

In the Simplot (2019) final bioassay report reviewed herein, four potato varieties (X17, Y9, alongside their conventional counterparts, Ranger Russet and Atlantic potatoes) were assayed against four late blight isolates including field collected samples. The strains included three US-23 isolates derived from a lab sample as well as two field collected strains from Florida and New York, as well as EC-1, a positive control late blight strain to which X17 and Y9 are not resistant. A sample of 20 plantlets per treatment from all four potato varieties were propagated for 24-days before exposure to one-million spores/plant of the four isolates of *P. infestans*. This spore

exposure rate is meant to simulate field conditions. Ten-days later, results were scored for protection phenotype using a seven-point scale. The scale is defined below:

- 1: sporulation occurs, plants dead
- 2: sporulation occurs, meristem dead, plant dies
- 3: sporulation occurs, meristem alive, plant survives
- 4: lesions detected, more than 50% defoliation
- 5: lesions detected, less than 50% defoliation:
- 6 no lesions, more than 50% defoliation
- 7 no lesions, less than 50% defoliation

Varieties with mean protection scores greater than 3.5 were considered protected, scores ranging from 2.4-3.5 were considered intermediate protection, and scores less than 2.4 are considered unprotected.

Simplot reported that in 2018, the X17 and Y9 strains were protected against all three isolates of US-23 strains but not EC-1, the positive control strain (see Fig. 1). The scoring for the VNT1-traited varieties ranged from 5-7, while the conventional varieties scored from 1-2 on the scale described above. Thus, the 2018 final bioassay validated the 2016-2017 assays with field collected isolates of US-23.

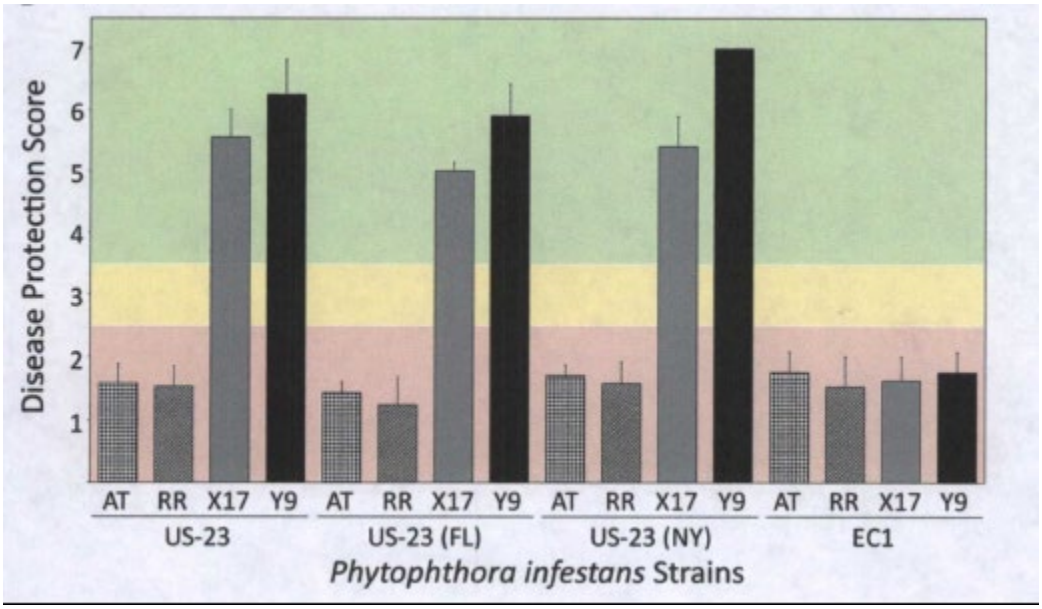

Figure 1. Bioassay conducted in 2018 using two different US-23 isolates (FL and NY). Background coloring represents the three categories of late blight protection: protected (green), intermediate (yellow), and not protected (beige). EC-1 was used in the 2018 bioassay as a positive control. AT and RR stand for Atlantic and Ranger Russet potatoes, the negative control for the transgenic varieties, and are depicted with patterned bars. X17 is denoted in the solid grey bar, and Y9 is denoted with solid black. Results are the mean of 20 independent observations. This figure is reproduced from Figure 5B in Simplot (2019).

Simplot Answers to BPPD 2018 Queries and Recommendations

Below are the recommendations BPPD (2018) made to Simplot followed by Simplot (2019)'s response.

1. **BPPD (2018):** Simplot did not provide a rationale for why the recommendation made in BPPD (2016) to investigate molecular methods or UV based methods for resistance detection was not addressed, as described in Chapman et al. (2014) and Engelhardt et al. (2012).

Simplot (2019) Response: The molecular method of resistance detection described in Engelhardt et al. (2012) may not adequately predict how pathogens will evolve resistance in the field and additionally Simplot has been unable to detect the VNT1 gene in planta. For the UV based detection method described in Chapman et al. (2014), Simplot provided side-by-side white-light and UV-B examples, demonstrating that either visualization method provides the same amount of information.

BPPD Review: BPPD is satisfied by the answer provided above and has determined the bioassay methodology described herein is robust for resistance monitoring.

2. **BPPD (2018):** BPPD recommends Simplot discuss the feasibility/ usefulness of sentinel plots to monitor resistance evolution of *P. infestans* to VNT1 and whether that could complement UXD field investigations.

Simplot (2019) Response: Simplot described that sentinel plots have not previously been deployed by potato growers for blight detection and weather forecasting has proven to be an effective means of predicting late blight outbreaks. Additionally, sentinel plots may likely provide an unmanaged source of *P. infestans* inoculum, thus increasing disease pressure and resistance pressure against the VNT1 trait.

BPPD Review: BPPD concludes sentinel plots are not an advisable method for resistance monitoring for the VNT1 trait.

3. **BPPD (2018) Query:** BPPD has concerns over the 1-7 scale used by Simplot to score resistance development and whether such action levels are sufficiently protective. Simplot defines UXI triggers as, “foliar lesions or late blight-caused defoliation on otherwise healthy plants”. BPPD asks Simplot to clarify why 3.5 was selected as the trigger for resistance confirmation if foliar lesions or defoliation are accounted for in scores ranging from 4-6. BPPD understands that a trigger of 3.5 may reduce false positives for resistance declaration, but given the Innate® potato varieties are a single trait system, not a pyramid, it may be beneficial to have a more protective parameter.

Simplot (2019) Response: During a February 13, 2019 meeting with Simplot company, representatives explained that a 3.5 resistance trigger is protective because it is hard to differentiate the hypersensitive response of the VNT1 trait from late blight symptoms. Thus, scoring between 2.5-3.4 has room for subjectivity and is classified as “intermediate”. This is further expounded on in the narrative of Simplot (2019). Note, Simplot redefined UXI to be, “sporulating foliar lesions or plant defoliation characteristic of late blight” in the 2019 submission.

BPPD Review: BPPD accepts the Simplot (2019) response and has determined the bioassay methodology described herein is robust for resistance monitoring.

4. **BPPD (2018) Query:** BPPD is concerned about the potential for grower error in the collection of samples. 1.) Two samples do not provide enough room for error in sample collections, and 2.) samples may not be preserved properly for moisture retention even with grower education. BPPD recommends that Simplot consider increased grower sample collection education or sending trained company personnel, university, or extension representatives to UXI fields to collect samples on behalf of growers.

Simplot (2019) Response: In the case of UXI, company personnel will work directly with growers and extension representatives to ensure samples are collected and handled correctly for successful identification of the *P. infestans* strains and potato variety.

BPPD Review: BPPD is satisfied with the Simplot (2019) response.

5. **BPPD (2018) Query:** BPPD suggests Simplot report the confirmed strain in UXD fields using Innate® potatoes regardless of whether it is a labelled strain (US-8, US-22, US-23, or US-24). This may serve as an early warning system for Simplot and BPPD to understand if sexual reproduction produced a new strain of late blight.

BPPD Review: BPPD determined that Simplot's bioassay approach is sufficient to provide a proactive resistance monitoring plan for VNT1 which addresses the RM team's previous concerns. It is outside the scope of the terms of the registration to monitor for production of new strains of late blight.

BPPD Review and Recommendations

BPPD has the following conclusions regarding the final bioassay report and requests Simplot address the below in future annual resistance monitoring submissions to EPA:

1. Simplot must clarify the exact number of days after inoculation with *P. infestans* the bioassay is scored. The narrative of the text of the MRID states this is done 10-days after inoculation, however, in Fig. 3 it is stated to be "approx. one week" (see Simplot 2019).

2. BPPD notes Simplot has registered another variety of VNT1-traited potato, the W8 Russet Burbank. While the W8 potato is not currently commercialized, BPPD expects Simplot to use the final resistance management plan developed for the other two varieties should commercialization of W8 occur in the future.

3. In the 2018 bioassay, Simplot tested US-23 alone of the four labeled *P. infestans* strains. If UXI occurs in the field, BPPD recommends confirming the injury is from a labelled strain but then assaying the VNT1 plantlets against all four labelled strains and not only the one that caused the UXI. This will provide valuable information to determine if the resistance mechanism is global for all late blight strains.

References

BPPD. 2016. BPPD's review of resistance management proposal and integrated pest management (IPM) proposal for Simplot potato expressing RPi-VNT1. US Environmental Protection Agency Biopesticide and Pollution Prevention Division. Available online at regulations.gov, Docket number: EPA-HQ-OPP-2016-0036-0008).

BPPD. 2018. Review of the Resistance Monitoring Bioassay Interim Report for Innate® Potatoes Varieties X17 and Y9. US Environmental Protection Agency Biopesticide and Pollution Prevention Division. Available online at regulations.gov, Docket number: EPA-HQ-OPP-2016-0036-0008).

Chapman, S., L. J. Stephens, P. C. Boevink. S. Engelhardt, C. J. Alexander, B. Harrower, N. Champouret, K. McGeachy, and P. S. M. Van Weymers. 2014. Detection of the virulent form of AVR3a from *Phytopfthora infestans* following artificial evolution of potato resistance gene R3a. PLoS ONE, Vol. 9, e110158.

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