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A Systems-Based Approach to Safeguard the Strawberry Industry from Virus Diseases

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ABSTRACT
Strawberry is susceptible to several systemic pathogens and, in particular, viruses. There are several examples of disease epidemics that resulted from virus-infected propagation material and led to losses estimated in the tens of millions of dollars. This communication focuses on actions needed to minimize the risk of similar outbreaks by developing a systems-based approach from breeding to the commercial field. This includes the development of molecular biology and bioinformatic tools that allow for the detection of all known viruses infecting strawberry but most importantly for the discovery of viruses that circulate in the system undetected and may have contributed to past epidemics. Surveys in commercial fields have yielded a map of the most prevalent viruses in strawberry production areas across the United States, knowledge that will be used in the nascent National Certification Program for Strawberry.

KEYWORDS
Nursery certification; next generation diagnostics; bioinformatics; vectors

Introduction
Strawberry production is expanding around the globe. As is the case with any crop moved to new geographic areas, there are several issues that need to be addressed so as to develop profitable, sustainable production schemes for both the producer and the environment. Among the most significant concerns is pests and diseases. It is often the case that new pathogens are found in crops recently introduced to new areas (El-gaied et al., 2008). In addition, as chemistries used to control pathogens change to more environmentally-friendly alternatives, pathogen and diseases may re-emerge to cause significant losses (Koike, 2008).

These are largely inevitable problems. Even though the crop has largely moved towards annual plasticulture, a system that should eliminate many of the problems commonly associated with perennial crops, such as inoculum build-up, there are still significant disease problems that face strawberry. Those are often associated with infection in the nursery and are largely
detectable with today’s knowledge and technology. This communication aims to discuss the available tools and how they can be applied to safeguard the crop from viruses starting from the breeder’s field to the nursery and the production field.

**Step 1: From a breeding selection to a named cultivar**

The time between a crossing and release of a strawberry cultivar may exceed 7 years. In that period plants are grown in the field and there is high probability that they will become infected by one or more viruses. Genotypes that develop symptoms or do not grow well are usually eliminated from the breeding pipeline. Yet, there may be genotypes that do not show symptoms and do not have an obvious yield reduction because of infection. It is uncommon for modern genotypes to develop symptoms when infected by a single virus (Martin and Tzanetakis, 2006). Symptoms develop when plants are infected by multiple viruses, something that can take place during the multiplication process at a nursery or commercial field setting (Martin and Tzanetakis, 2006). For this reason it is important that breeding material is free of all pathogens of concern before they are named and move through the propagation pipeline.

New technologies, such as large scale or next generation sequencing (NGS) paired with custom-made bioinformatics pipelines, able to detect all known viruses and identify new viruses have been developed and used on breeding material (Ho et al., 2015; Ho and Tzanetakis, 2014). In addition to the universality of the new technologies, detecting old viruses and discovering new viruses, they also provide the advantage of rapid results normally obtained in a period of a few weeks versus the months needed with grafting indexing. Although the NGS appears superior to the grafting assays, they have not been tested side-by-side, nor have protocols been developed for the standardization of such methods, including sampling, depth of sequencing, etc. Standardization is currently underway within the National Clean Plant Network-Berries group. Even if the working hypothesis, NGS being superior to current techniques, is substantiated by the results of those studies, it is important to note that no system is absolute. The bioinformatics tools use sequence data and algorithms based on probability theories. A disease agent may have no obvious orthologs to previously annotated genes and may remain undetected, marked as unknown. On the other hand, there have been multiple examples of commonly used indicators among berry, fruit trees, and grapevine indicators that fail to identify new viruses or diverse isolates of previously characterized ones (Al Rwahnih et al., 2015; Gergerich et al., 2015). After the completion of the aforementioned studies there should be an open discussion on the future of the industry as it may be uneconomical to continue with both approaches, NGS and grafting.
Step 2: Nursery propagation

Strawberry nurseries take many precautions to avoid infection by pathogens that move with plants as a result of the propagation process. This includes planting in areas without strawberry production or virus vectors (e.g., plant virus vectoring nematodes) and mitigation to eliminate potential vectors. Still, a major issue remains: viruses that are yet to be discovered. Strawberry pallidosis presents an excellent example: The disease was only identified through graft transmission and was disregarded as a rarity until the discovery of whitefly-transmitted viruses associated with the disease (Tzanetakis et al., 2003, 2004). Those viruses remain asymptomatic in single infections; still, when found in mixed infections with aphid-borne or other virus groups, they cause a severe decline and even plant death (Martin and Tzanetakis, 2013). Before the discovery of the pallidosis viruses, the whitefly vectors were not considered pests of concern in strawberry, as their feeding had no noticeable effect in plant vigor and were not known as vectors of strawberry viruses. This led to the invasion of the pallidosis viruses and their dissemination within the system. When those plants grew in the field and accumulated additional viruses, symptoms developed and producers suffered significant losses (Martin and Tzanetakis, 2006).

How many ‘pallidosis-like’ agents circulate in the system? The discovery of strawberry polerovirus-1 (Xiang et al., 2015) indicate that there are more strawberry viruses to be discovered. We have taken a first step by mapping the virus distribution in the major production areas across the United States (Martin and Tzanetakis, 2013). This study can be used as a guide for the vectors of concern in the nursery system. As an example, we know that all major aphid-and whitefly-borne viruses circulate in California. All nurseries in the state should take extra precautions to mitigate those vectors. On the other hand, the Upper Midwest does not have significant virus problems. In those cases, a program to intercept vectors should be in place but does not need to be as stringent as the ones implemented in areas with heavy virus loads.

The draft guidelines of the National Strawberry Certification Scheme (NSCS; available at www.ncpnberries.org) include the best management practices for pest and disease control. In addition, the draft NSCS includes new requirements for testing. It does not only call for indexing of the top tier or generation 1 (G1) material but also in subsequent generations during the propagation process (G2–G4) allowing for virus presence at different threshold levels. For example, it calls for testing for all major systemic pathogens of the crops that are indigenous in the nursery locale at the G4 level (plants to be distributed to producers) so as to detect a 5% infection with 95% confidence. Testing is not excessive, minimizing the cost for the nurseries, but also minimizing the possibility of disseminating viruses at high enough levels that could initiate an epidemic (Gergerich et al., 2015). Those guidelines are
being tested in pilot studies and will be formulated to bring the best possible flexibility to the industry without risking the repeat of the epidemics of the past.

**Step 3: Commercial fruiting fields**

The majority of strawberry production is on an annual system. This minimizes the risk of disease epidemics caused by viruses as there is not enough time for viruses to build titers and cause severe losses. If all appropriate measures are taken before planting, the average producer should not have major problems due to viruses, because by the time plants accumulate enough of a load they will be replaced with clean material. The exception comes when there are multi-year strawberry fields in the area. Those fields can serve as the primary source of inoculum. New fields are being fertigated providing optimal conditions to the young transplants; also providing the optimal environment for the vectors that identify a new, better source of feeding. In such a case there may be a major influx of viruses that infect plants at an early stage affecting their growth and therefore their fruiting potential. Growers that use the annual system may opt to agree that no multi-year fields are allowed in their area, in similar ways that producers in seed production areas do not allow for material that cross pollinate with the crops of interest (Wright, 1980).

In areas where multi-year fields are the norm, and there are viruses circulating in the system, there should be a comprehensive control strategy for the major vectors. If there are multiple vector species present then the producers need to identify the ones that are easier to control. If, for example, there are both aphid and whitefly-borne viruses circulating in the area, then priority should be given to aphid control. This is not only because aphid-borne viruses can cause epidemics in mixed infections by themselves (unlike the whitefly-transmitted viruses) but also because aphids are easier to control. They have one or two major flight times during the growing period and the major vector, the strawberry aphid, tends to feed only on strawberry. On the other hand, the greenhouse whitefly, the sole whitefly vector of strawberry viruses identified to date, is a strong flyer and has a wide host range. Therefore, control would require multiple applications and could still not affect the major vector reservoir that may be feeding in another crop either close or far away.

**Discussion**

Technological advances provide precise, all-inclusive, detection of plant and animal pathogens. Those technologies are currently applied in strawberry so as to obtain the cleanest ‘mother’ or G1 material possible, free of all
pathogens of concern. This allows for the development of a systems-based approach that follows plants as they move through the propagation pipeline and reach commercial fields. The drafted NSCS provides state-of-the-art guidelines, based on solid scientific knowledge, on how to propagate material and grow it under conditions that minimize infection. As a result, producers obtain elite plants, free of pathogens of concern and with high yielding potential. The epidemiological knowledge on strawberry viruses and the detailed mapping of the virus presence in the United States allows producers to make educated decisions on vector management, so as to eliminate the possibility of virus-caused decline with minimal interventions that aim to break the weakest link in the disease cycle, the vectors that are easier to control.

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