Healthy runners, more high-quality fruit

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- High quality runners, free of disease, are key to a successful final product
- Underpinning the success and viability of the Australian strawberry industry is a certification and inspection program that supports production of high-quality high-health runners
- With increasing climatic variation, as well as the phasing out of methyl bromide for fumigation, the Australian strawberry industry may see the emergence of new serious pathogens
- Starting with certified planting stock will help the strawberry industry to limit the impact of emerging and re-emerging pests and disease

Strawberries (*Fragaria x ananassa*), known for their vibrant colour and sweet taste, are an important Australian berry fruit crop and a staple in most Australian households. In 2022, Australian growers produced approximately 68,000 tonnes of fruit valued at over $416 million (Hort Innovation 2023). While strawberry fruit are the face of the industry, behind the scenes, the runner growers propagate and supply the planting stock for fruit production.

High quality runners, that are free of disease, are key to a successful final product; they produce more high-quality fruit. Underpinning the success and viability of the Australian strawberry industry, from planting stock to fruit production, is a certification and inspection program that supports production of high-quality high-health runners. Certification of strawberry runners aims to guarantee the biosecurity of onshore strawberry planting stock through regular pathogen testing for virus, bacteria and fungi.

The Australian strawberry industry runner certification scheme has been in place for more than 60 years. Noticing increasing losses in fruit production fields due to virus infections, the Victorian Government introduced a certification scheme in the 1960s and it has resulted in a significant reduction in disease in both runner and fruit production. The Victorian Government managed the scheme until the 1990s when responsibility for the scheme shifted to industry.

Currently, both the Australian Strawberry Propagators Accreditation Authority (ASPAA) and the Victorian Strawberry Industry Certification Authority (VSICA) schemes offer growers assurance that the planting stock is of the highest health status.

Strawberry varieties that are certified within the schemes comprise of nucleus plants from which daughter runner plants are grown and increased in number through several generations before they are supplied to fruit growers (Figure 1).

According to the rules of the schemes, to be certified nucleus plants of varieties within schemes are tested annually for select viral, bacterial and fungal pathogens (Table 1). Annual pathogen testing not only serves to ensure that initial planting stock is of the highest quality available, but it also helps maintain the biosecurity of the nursery environment. Daughter plants, at every generation, are inspected by trained inspectors for evidence of disease throughout the growing season.

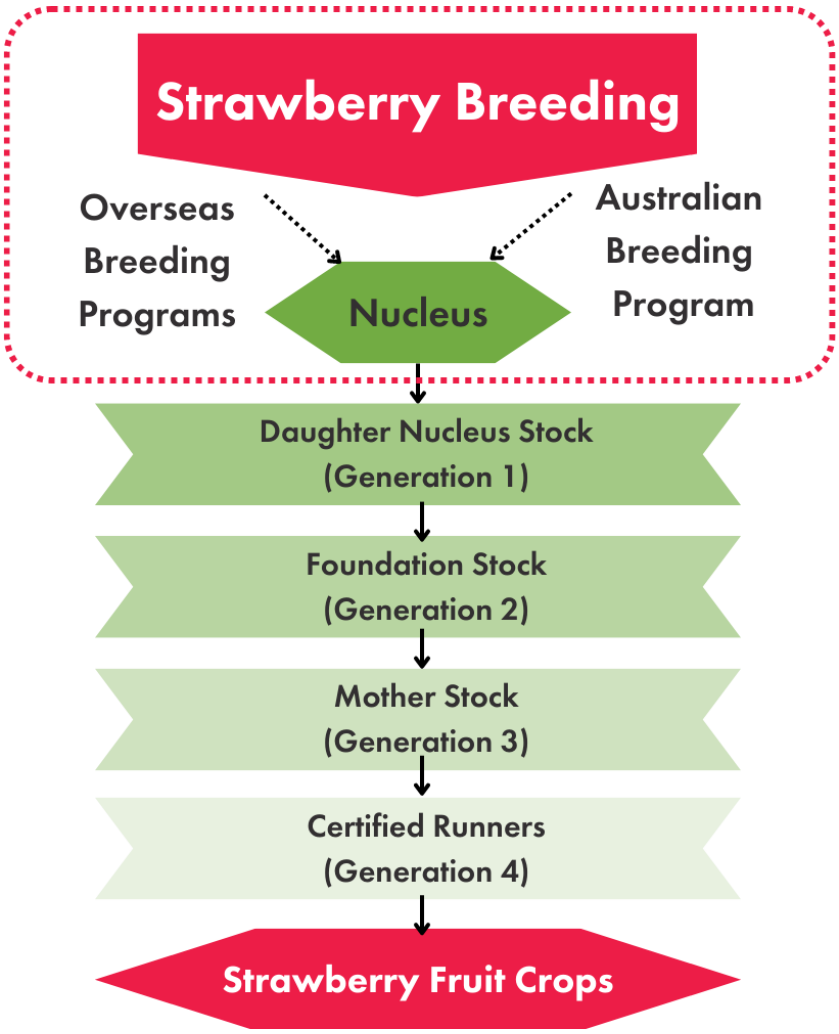

Figure 1. Strawberry certification pathway. Certified strawberry varieties are multiplied and inspected over several generations before going into fruit production.

Crop Hygiene Biosecurity Services (CHBS) is part of Agriculture Research Victoria (AVR) and located at AgriBio Centre for AgriBioscience. AgriBio is Australia's first integrated agricultural systems biology research centre and one of Australia's premier state-of-the-art agribioscience facilities, with a key emphasis on supporting and protecting Victoria's agricultural sector by focusing on advanced research to improve productivity, fight disease and reduce environmental impact.

CHBS operates as a fee-for-service commercial entity providing independent and confidential services to the Australian horticultural sector. CHBS has provided ongoing support for production of high-health runners since the ASPAA and VSICA took over accreditation schemes. The CHBS high health germplasm program provides pathogen testing services, germplasm management and daughter runner propagation.

The AVR screenhouse facilities at AgriBio and managed by CHBS are Nursery Industry Accreditation Scheme, Australia (NIASA) accredited and house nucleus plants and daughter nucleus runners on behalf of industry clients.

Strawberry varieties may also be maintained in an *in vitro* collection (Figure 2) for back up purposes, and micropropagation can also be used to increase the number of plants available for runner production.

CHBS provides equivalent pathogen testing services to clients who choose to maintain their own nucleus stock program. CHBS, via the '*BS19000 High health pre-commercial propagation material for Australian strawberry growers*' project, also works with the Australian Strawberry Breeding Program (ASBP) and Hort Innovation in the pathogen testing and distribution of promising new breeding selections for industry trials.

Table 1. A list of endemic and exotic pathogens of strawberries and methods used for detection of each pathogen. Not a complete list of significant pathogens affecting strawberries. Not all pathogens require active testing under runner certification schemes.

Pathogen	Detection method			
Virus	*Visual inspection*	*Culturing*	*Biological*	*Molecular*
Beet pseudo-yellows crinivirus (BPYV)	—	—	GI[2]	RT-PCR[4]
Strawberry crinkle cytorhabdovirus (SCV)	—	—	GI	RT-PCR
Strawberry mild yellow edge potexvirus (SMYEV)	—	—	GI	RT-PCR
Strawberry mottle sadwavirus (SMoV)	—	—	GI	RT-PCR
Strawberry necrotic shock ilarvirus (SNSV)	—	—	HI[3], GI	RT-PCR
Strawberry pallidosis associated crinivirus (SPaV)	—	—	GI	RT-PCR
Strawberry vein banding caulimovirus (SVBV)	—	—	GI	PCR[5]
Tobacco streak ilarvirus (TSV)	—	—	HI	RT-PCR
Bacteria	*Visual inspection*	*Culturing*	*Biological*	*Molecular*
Xanthomonas arboricola pv. *fragariae*[1]	Yes	Yes[7]	—	
Xanthomonas fragariae[1]	Yes	Yes[7]	—	PCR[7]
Rickettsia-like-organisim	Yes	—	—	PCR[7]
Phytoplasmas	Yes	—	—	PCR[7]
Fungi	*Visual inspection*	*Culturing*	*Biological*	*Molecular*
Colletotrichum species	Yes	Yes	—	—
Gnomoniopsis fructicola (syn. *Gnomonia comari)*	Yes	Yes	—	—
Verticillium dahliae	Yes	Yes	—	—
Phytophthora sp. including P. *fragariae* f.sp. *fragariae*[1]	Yes	Yes	B[6]	PCR[7]

[1] These are quarantine pathogens that are not known to occur in Australia or have been detected and eradicated - active surveillance by visual inspection will improve the biosecurity of the Australian strawberry industry.

[2] GI = Biological indexing by graft inoculation onto the susceptible *Fragaria vesca* cvs UC-4 and UC-6 and *F. virginiana* cv UC-10 indicators

[3] HI = Biological indexing by rub inoculation onto *Chenopodium quinoa* (herbaceous indexing)

[4] RT-PCR = Detection of pathogen RNA by reverse transcription (RT) PCR

[5] PCR = Detection of pathogen DNA by polymerase chain reaction (PCR)

[6] B = Detection of pathogen by baiting susceptible *F. vesca* subsp. vesca forma *sempeflorens* 'Alpine'

[7] Culturing and /or PCR for X. *arboricola pv fragariae*, X. *Fragariae*, Rickettsia-like-organisim and phytoplasmas and PCR for *P. fragariae* f.sp. *fragariae* is not mandatory but may be requested by the client for pathogen detection or used by the diagnostic laboratory for confirmation of infection.

Supporting the AVR plant holding facilities used by CHBS are state-of-the-art laboratories and controlled environment growth rooms, utilised for *in vitro* micropropagation. Expert plant pathology diagnostic capabilities provided through Crop Health Services (CHS), which is AVR's commercial plant pest and disease diagnostics service, also support CHBS strawberry pathogen testing services.

Figure 2. Tissue culture (in vitro) strawberry plantlets ready for transplant and acclimatisation.
Photo credit: Geoff Kelly, AVR

Pathogens and biosecurity

When it comes to strawberry pathogens, Australia is fortunate to have area freedom from several devastating pathogens affecting overseas strawberry production, such as angular leaf spot (*Xanthomonas fragariae*) and red stele disease (*Phytophthora fragariae* f.sp. *fragariae*). Maintaining area freedom from such pathogens is not only critical for protecting the Australian industry, but also for retaining market access when exporting planting stock and fresh fruit. Importations of strawberry growing stock undergo post-entry quarantine pathogen testing on arrival to manage the risk of exotic pathogens entering Australia. Once released from quarantine, ensuring the continued biosecurity of the material by testing for endemic pathogens is critical.

Both the runner nursery production and the fruit industry are susceptible to losses caused by viral, fungal, and bacterial pathogens. Undetected infections and accumulation of pathogen inoculum in the runner multiplication phase can potentially have serious future economic impacts when plants (along with pathogens!) are distributed to fruit growers.

Prior to fruiting fields, strawberry varieties will undergo several generations of vegetative multiplication, which gives ample opportunity for exposure to and cross-contamination with pathogens, potentially resulting in reduced fruit quality or yield losses, or even death of planting stock. Visual screening of plants can be useful for the detection of harmful pathogens as some, such as powdery mildew (*Podosphaera aphanis*), common leaf spot (*Mycosphaerella fragariae*) or fruit rot (*Botrytis cinerea*) may have readily observed symptoms and can be controlled through cultural and chemical means. Whereas infection by other pathogens, such as some systemic fungi, viruses and phytoplasmas, may not always be apparent and rely on diagnostic testing for detection.

Screening for viral pathogens has been a major aspect of the strawberry runner certification program since its inception. Viral infection in strawberries may be insidious, with impact varying depending on a combination of factors including the susceptibility of the host variety, the virus species, and the virus strain. Common viruses, such as strawberry mild-yellow edge virus (SMYEV), strawberry crinkle virus (SCV) and strawberry mottle virus (SMoV) are generally considered to be mild, or latent infections in most modern strawberry varieties (Martin and Tzanetakis 2006). Once infected, planting stock may remain asymptomatic, or may exhibit subtle effects such as mild leaf symptoms, reduced fruit yield, or reduced runner production – these may be slight effects, difficult to parse out from nutritional disorders or other environmental factors.

Significant impacts, however, may be induced by some virus species or strains: especially in susceptible strawberry varieties. Some of the more damaging strains of SMoV may result severely stunted plants and a yield reduction of 30 per cent (Thompson 2003). Generally, serious economic losses are most frequently observed when a combination, or complex, of viral infections occur in the strawberry plant. When SCV occurs in a complex with SMYEV, SMoV or SVBV, strawberry plants may develop severe symptoms of decline, including

stunting, reddening and crinkling of leaves, and reduced fruit size (Martin and Tzanetakis 2006). As such, having an undetected or asymptomatic infection with a "low impact" virus present may open the grower up to potentially devastating losses if another virus enters the property.

Infected strawberry material also provides a virus reservoir for acquisition by vectors. Viruses in strawberry, generally, are not mechanically transmitted, and transmission requires the presence of the associated vector (aphids, white fly, nematodes or pollen). Once infected, vegetative propagation of subsequent runner generations is a highly effective method for the propagation and distribution of viruses. One virus infected mother plant may result in over 100,000 infected runners within three generations, through vegetative spread alone. Each of these daughters also serves as an inoculum source for vector acquisition resulting in the further spread of virus, highlighting the importance of ensuring the cleanliness of the initial strawberry stock plants.

Annual pathogen testing

The annual pathogen testing program at AgriBio commences in spring and involves graft indexing candidate nucleus mother plants onto indicator plants; sensitive cultivars of *Fragaria vesca* and F. *virginiana*. If the graft union is successful, sap transfer from the virus infected candidate leaflet will carry virus particles to the indicator, where they will infect and replicate in the host plant, resulting in symptom expression, such as stunting, vein banding, leaf crinkle or mottle.

Each of the indicators used in the testing are sensitive to different viruses and will display diagnostic symptoms associated with a particular virus (Figure 3). To prevent false positives due to physiological symptoms, suspect plants are generally confirmed using molecular methods.

Molecular diagnostic techniques (polymerase chain reaction, PCR) may be employed to target specific viral pathogens (Table 1) for either confirmation of suspect positives, or at the request of a grower to rapidly screen material to provide added assurance of the health status. Graft indexing is labour intensive and can take up to 12 weeks to observe for the indicators, whereas the advantages of PCR testing are that it requires less labour and can return a result within a week.

However, each specific PCR test needs a significant investment of time and money to validate, as well as specialised equipment and consumables. Another concern often raised when discussing PCR testing is the greater specificity resulting in the potential for false negatives against novel strains of a particular virus. Therefore, it is important that tests are verified and updated as information about new genetic variants become available.

More advanced molecular diagnostic capabilities such as High-Throughput Sequencing (HTS), also referred to as Next Gen Sequencing (NGS), may also be utilised at AgriBio. This technology can be used to indiscriminately detect any pathogen in a strawberry sample or can be targeted to specific pests.

While this currently is not routinely employed for strawberry diagnostic samples by CHBS and CHS, application of this technology may play an important complementary role with biological techniques and can be useful for the identification of the potential causal agents where a disease of unknown etiology is observed in the plants.

In future, HTS may also enable a comprehensive rapid report of the complete virus status of a strawberry variety. HTS has been adopted by the Department of Agriculture, Fisheries and Forestry at its Mickleham post entry quarantine facility to screen imported strawberry stock for exotic plant viruses and viroids.

Pathogen testing of nucleus mother stock is not just concerned with viral pathogens, but under the accreditation schemes, consideration is also given to the impact that certain fungal pathogens can have on the viability of strawberry production systems. Nucleus plants are sampled during the growing season and cultured on agar media to isolate any systemic fungal pathogens that may be present.

Root sample culturing and baiting with Alpine strawberries (F. *vesca*) is also conducted to screen for *Phytophthora fragariae f.sp. fragariae*. CHS mycologists examine the cultures for the presence of specified fungi. Suspect fungal isolates will undergo further identification for confirmation to species level using genetic sequencing and comparison to the genetic sequence of known species.

Figure 3. Grafted *Fragaria vesca* "UC-4" indicator infected with a combination of SPaV, SMoV and SMYEV
Photo credit: M. D. Jones, AVR

Future challenges

With increasing climatic variation, as well as the phasing out of methyl bromide for fumigation, it could be expected that the Australian strawberry industry may see the emergence of new serious pathogens from the ranks of those previously considered minor or secondary pathogens.

Pestalotia leaf spot and fruit rot (*Neopestalotiopsis spp.*) and Charcoal Rot (*Macrophomina phaseolina*) have emerged in US strawberry fields as increasingly significant pathogens since the reduction in methyl bromide soil fumigation. Additionally, changing seasonal conditions can favour the development of fungal pathogens and may also favour the spread of the insect vectors that transmit viruses.

Increased insect vector pressure may be seen in strawberry fields as well as the potential for movement of vectors and associated viruses into previously protected areas due to changing climate. Warmer temperatures may favour both virus acquisition by insect vectors and transmission rate of some viruses such as SCV (Martin and Tzanetakis 2006).

Vector control can be effective in the management of viral pathogens; however, the development of chemical resistance can reduce the effectiveness of control measures. Changes to vector and pathogen behaviour may present new challenges for the industry to overcome, starting with certified planting stock will help the strawberry industry to limit the impact of emerging and re-emerging pests and disease.

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