The effect of fertiliser on detection of *Apple stem grooving virus* and *Tobacco ringspot virus* by herbaceous bioassay

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Abstract Herbaceous indicator bioassays are used to screen for unwanted plant viruses on imported plant material. To optimise virus detection, the effect of plant nutrition was investigated to determine whether this plays a role in virus symptom expression and severity. *Apple stem grooving virus* and *Tobacco ringspot virus* were inoculated onto a range of herbaceous indicator species grown in potting mix supplemented with differing rates of slow-release fertiliser. Higher rates of fertiliser resulted in better plant nutrition and a greater incidence of virus expression and more severe expression of disease in the seven indicator plant species. Overall, disease assessments indicated that plant nutrition affects plant growth, virus infection rate, and virus symptom expression. As virus infection affects plant growth and leaf colour, good plant nutrition is important to avoid masking virus symptoms and to optimise the detection of viruses in post-entry quarantine facilities.

Keywords *Apple stem grooving virus*, *Tobacco ringspot virus*, fertilizer, infection rate, symptom expression.

INTRODUCTION

Herbaceous indicator bioassays are a traditional method for detecting viruses in plant material. This type of indexing remains a major part of the testing performed on plant material imported into post-entry quarantine in New Zealand, to screen plant material for unwanted, unknown or latent viruses that may present a biosecurity risk. It is required by New Zealand Ministry for Primary Industries' (MPI) commodity import health standards for most high value crops (e.g. pipfruit, berryfruit, *Actinidia*, *Citrus*, *Ipomea*, *Prunus*, *Solanum*, *Vitis*). Such material must either undergo MPI-accredited high-

health testing offshore prior to importation or undergo the required testing while in post-entry quarantine in New Zealand. The requirements apply to plants from budwood and tissue cultures, and to seedlings raised from imported seed and pollen.

Plant & Food Research operates an accredited facility for plant post-entry quarantine inspection and testing, with a focus on herbaceous indicator bioassay. The Plant & Food Research diagnostic laboratory currently tests significant numbers of imported *Actinidia* germplasm by herbaceous indicator assays whilst in post-entry quarantine.

The testing for this commodity requires the use of the indicator species *Nicotiana benthamiana*, *N. glutinosa*, *N. occidentalis* 37B, *Phaseolus vulgaris* ‘The Prince’ and *Chenopodium quinoa*. The indicators *N. tabacum* and *Cucumis sativus* were also included in this experiment as they are commonly used to screen for unwanted viruses in other commodities.

It is essential indicators are grown so that bioassays are consistently sensitive and robust. It has long been known that the nutritional status of the host plant has a marked effect on virus replication and the numbers of local lesions produced by viruses (Matthews 1991). The interactions between different nutrients are quite complex. However, in general, nutritional conditions that are most favourable for plant growth are those also giving the greatest virus susceptibility (Matthews 1991). Therefore, the aim of this study was to determine the optimal nutritional conditions for plant growth and check whether the sensitivities of bioassays for a specific unwanted virus (*Apple stem grooving capillovirus*, ASGV) and a reference virus (*Tobacco ringspot nepovirus*, TRSV) were affected when fertiliser dosage varied beyond the range normally used for growing indicators for post-entry quarantine testing.

MATERIALS AND METHODS

Production of indicator plants

Seventy-two plants of each herbaceous indicator species—*Cucumis sativus* ‘Slicer 637’, *N. benthamiana*, *N. tabacum*, *N. glutinosa*, *N. occidentalis* 37B, *P. vulgaris* ‘The Prince’ and *C. quinoa*—were grown in pasteurised 60:40 fine bark/pumice mix containing lime (4 kg/m³), dolomite (2 kg/m³) and gypsum (2 kg/m³) amended with either 1, 2, 3 or 5 g/litre of the commercial fertiliser Osmocote® Plus 3-4M (NPK 15-4.8-10.8+1.2MgO+TE, Everris Australia Pty Ltd). Plants were grown in a temperature-controlled greenhouse at 22°C/18°C (day/night) and 14 h photoperiod (daily peak of 200–400 µmol/m²/s¹ photosynthetically active radiation, extended with supplemental high pressure sodium lighting) until suitable for inoculation. *Phaseolus* seedlings were grown

to the fully-expanded primary leaves stage with the first trifoliate leaves no bigger than 2 mm, *Cucumis* was grown to the fully expanded cotyledon stage, while all other seedlings were grown to the 4–6 leaf stage of growth.

Inoculation of indicator plants

Indicator plants were shaded for 16 h before inoculation to increase susceptibility. Each plant was dusted lightly with an abrasive (500-mesh Celite®). Inoculum was prepared in a 1:10 (w/v) ratio of inoculation buffer (100 mM phosphate-buffer solution, pH 7.5 containing 5% PVP-40 and 10 mM sodium sulphite) by macerating leaf material from *N. glutinosa* plants that were either virus free for mock inoculation, infected with *Apple stem grooving virus* (ASGV) or infected with *Tobacco ringspot virus* (TRSV). For each of the three inoculum types and each of the five fertiliser treatments, six replicate plants of each indicator species were inoculated by gently rubbing the suspension on to two young leaves of each of the plants or the cotyledons of the *Cucumis*. Inoculum was rinsed off the leaves 3–5 min after inoculation with distilled water. Plants were grown for 4 weeks under the same conditions as described above.

Plant assessments

Plant nutritional status was determined by measuring plant height and leaf hue. Plant height, symptoms of virus infection and leaf colour were assessed twice-weekly for 4 weeks post inoculation. Typical ASGV symptoms recorded included purple local lesions (bean), chlorotic or necrotic local lesions, systemic yellow mosaic, epinasty, veinal necrosis and systemic foliar chlorotic mottling. Typical TRSV symptoms included chlorotic or necrotic local lesions, systemic yellow mosaic, leaf distortions, veinal necrosis, systemic foliar chlorotic or necrotic mottling and tip die back. Leaf colour was measured with a Minolta ChromaMeter (Konica Minolta, Ramsey, NJ, USA), and hue angle derived from recorded CIE L, a and b values. Data were averaged across the seven indicator species. Significance testing was completed using the General Linear Model (Minitab V16).

RESULTS AND DISCUSSION

The mean plant height of the combined indicator species increased significantly ($P < 0.001$) as Osmocote® dosage rose from 1 g/litre to 3 g/litre. Beyond 3 g/litre the plant height did not increase (Figure 1). There was no significant difference in the mean plant height of the combined indicator species in response to the virus inoculation ($P = 0.182$) (Figure 1). This result was surprising as it is generally considered that virus infection reduces plant vigour. However, there was a significant reduction in plant height ($P < 0.001$; data not shown) between ASGV-infected and mock inoculated *C. quinoa* plants when grown in potting media containing a low dose of Osmocote® (1 g/litre). This stunting response to ASGV infection was only observed when the availability of nutrients was severely limited. The reallocation of nutritional resources away from the plant growth towards ASGV replication had a significant impact on the plant height only when nutrition was limited. Plant height was not reduced when enough nutrients were available for both the plant and virus to co-exist. Virus infection may have an effect on the capacity of roots to take up mineral nutrients from the soil and transport them to other parts of the plant. However, very little experimental work has investigated this aspect of virus infection (Hull 2001).

No virus symptoms were noted in any of the mock inoculated control plants. Following

inoculation with TRSV or ASGV, the number of indicator plants showing symptoms typical of their respective viruses was recorded for each fertiliser dosage. For both viruses, the number of symptomatic plants increased with increasing fertiliser dosage (Figure 2). The amount of fertiliser had a significant effect on the symptom expression in all individual plant species tested ($P < 0.001$). Averaged across all indicator species, increasing the amount of fertiliser from 1 g/litre to 5 g/litre resulted in a 100% increase in the number of indicator plants developing ASGV symptoms and 127% increase in the number of indicator plants developing TRSV symptoms. This result is similar to research by Lee & Singh (1972) who found that increasing manganese concentrations in sand from 0 to 9 mg/litre dramatically increased the characteristic veinal necrosis symptoms induced by *Potato spindle tuber 'virus'* in tomatoes. At 9 mg/litre manganese concentration, tomato cultivars that had previously been scored as symptomless carriers of this virus developed veinal necrosis symptoms. Likewise, Zafar & Athar (2013) found that increased concentrations of 3.88–62 mg/litre phosphorous in growth media of susceptible cotton resulted in greater symptom expression of *Cotton leaf curl virus*. Osmocote® contains 0.06% elemental manganese and 4.8% elemental phosphorous.

Figure 1 Mean height (cm) of indicator plants when uninfected (control) or infected with *Tobacco ringspot virus* (TRSV) or *Apple stem grooving virus* (ASGV) and given varying amounts of Osmocote® fertiliser in growing media. Data are means for all seven species, *Cucumis sativus*, *Nicotiana benthamiana*, *N. glutinosa*, *N. occidentalis*, *N. tabacum*, *Phaseolus vulgaris* and *Chenopodium quinoa*. Bars indicate standard error.

Figure 2 Percentage of indicator plants expressing virus symptoms when grown in media with different amounts of Osmocote® fertiliser, assessed 14 and 28 days post-inoculation (DPI). Data are means for all seven species, *Cucumis sativus*, *Nicotiana benthamiana*, *N. glutinosa*, *N. occidentalis*, *N. tabacum*, *Phaseolus vulgaris* and *Chenopodium quinoa*, infected with Tobacco ringspot virus (TRSV) or Apple stem grooving virus (ASGV).

Figure 3 Average leaf hue of the seven indicator species, *Cucumis sativus*, *Nicotiana benthamiana*, *N. glutinosa*, *N. occidentalis*, *N. tabacum*, *Phaseolus vulgaris* and *Chenopodium quinoa*, when uninfected (control) or infected with Tobacco ringspot virus (TRSV) or Apple stem grooving virus (ASGV) were grown in media with different amounts of Osmocote® fertiliser. Leaf colour is measured as hue angle in degrees.

Leaf colour is a good visual indicator of plant nutritional health status, so this was used as an indication of plant health throughout this experiment. One would expect to observe greener leaves at higher fertiliser dosage because there is more available nutrient for photosynthetic pigment development. As expected, leaf colour,

expressed as hue angle, increased from a more-yellowish (116°–117°) to a greener hue (123°–125°) with higher fertiliser dosage, whether or not plants were infected with viruses (Figure 3). Plants in the present experiment infected with ASGV had slightly lower average hue angle than mock inoculated plants, but the difference was

not statistically significant. The average leaf hue of plants infected with TRSV was not statistically different from mock inoculated plants.

Minor elemental nutrition (zinc, iron, boron and copper) is known to have a variable effect on the capacity of plants to support virus replication, and effects of virus accumulation often parallel effects on plant growth (Hull 2001). When nutrients such as manganese are limited, deficiency symptoms such as interveinal chlorosis develop. Similarly, virus infection in plants frequently results in a phenotypic response of leaf chlorosis. Therefore the ability to distinguish virus infection in plants from those that are underfed can be problematic, as the symptoms may be the same or the incidence of interveinal chlorosis may not be apparent against a light coloured leaf. If plants are grown under nutritionally challenged conditions, virus infection symptoms may go unnoticed and result in a false negative result when screening for virus infections. Therefore average leaf hue angle, whilst a good indicator of the plant's nutritional status, is not a good indicator of virus infection.

Overall, disease assessments indicated that plant nutrition affected plant growth and virus symptom expression in the herbaceous indicators used for indexing imported plants. Higher

fertiliser dosage resulted in better plant nutrition and consistently increased virus symptom expression in the various indicator plants using these virus examples. The higher 'optimal' dosage is in the upper range or above those normally used for growing similar plants commercially. On the other hand, low nutrient supply affects leaf colour and may mask virus symptom expression. Therefore, the liberal use of a suitable fertiliser is important for growing indicator plants to maximise the chance of detecting viruses during testing of plants in post-entry quarantine facilities.

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