INTERNATIONAL WORKSHOP ON EUROPEAN CANKER

The effect of nitrogen source and quantity on disease expression of *Neonectria ditissima* in apple[†]

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(Original submission received 23 September 2021; accepted in revised form 30 November 2021)

Abstract The effects of nitrogen on the interaction between apple trees and European canker caused by *Neonectria ditissima* are not well understood. Previous field and laboratory studies have shown that nitrogen affects *N. ditissima* disease development, germination and germ-tube growth in vitro but the type of nitrogen source has not been examined *in vivo*. Therefore, the aim of this study was to determine the effects of root-applied nitrogen from different sources on the development of European canker on inoculated potted trees. One-year-old 'Royal Gala' trees were planted in a low-nitrogen growth substrate and treated with a range of concentrations of calcium ammonium nitrate (CAN) or other nitrogen sources ($\text{Ca}(\text{NO}_3)_2$, KNO_3 , $(\text{NH}_4)_2\text{SO}_4$, NH_4NO_3 , urea, YaraMila™) at equivalent molar rates of nitrogen as the highest CAN treatment. Treatments were applied during the growing season (Nov to May). The control treatment received no applied nitrogen. Bud and leaf scar wounds were inoculated at leaf fall with *N. ditissima* conidia. Tree growth and health, disease progression and leaf nitrogen content were monitored. The rate of nitrogen application affected tree diameter and leaf nitrogen content while the nitrogen source mainly affected tree survival, powdery mildew incidence, leaf weights, leaf nitrogen and European canker symptom expression. Trees treated with $(\text{NH}_4)_2\text{SO}_4$ had the lowest survival rates and highest leaf nitrogen content. Disease expression was highest with NH_4NO_3 and lowest with KNO_3 applications. The control plants (which received no additional nitrogen), showed the least amount of both growth and disease expression. Applications of CAN, even at the lowest rate (20 g), increased disease susceptibility. Increasing rates of CAN applications did not significantly increase disease incidence. Nitrogen concentration is an important factor in the disease development of European canker of apple. Field evaluation is recommended to further validate these results.

Keywords *Nectria galligena*, calcium ammonium nitrate, urea, potassium nitrate, ammonium sulphate, ammonium nitrate, fertiliser

INTRODUCTION

Mineral fertilisers are applied to fruit trees to boost productivity and plant health and are an essential tool for improved production efficiency. However, application of organic and inorganic fertilisers can also negatively affect the environment, such as the damage caused by run-off or leaching.

Nitrogen (N) is the fourth most abundant element in plants, after carbon, hydrogen and oxygen. Nitrogen is a gas but is generally taken up by plant roots in ionic form either as an anion (e.g. nitrate; NO_3^-) or a cation (e.g. ammonium; NH_4^+). Ammonium ions are toxic at low concentrations so, nitrate is generally the preferred form of uptake by plant roots as it is reduced to nitrite ions (NO_2^-) in plant cells.

[†]Based on a presentation given at the Fourth International Workshop on European Fruit Tree Canker and Resilient Orchards, 2–6 November 2020 via an online forum using Zoom.

Nitrogen is an essential component of many molecules that occur in plants (e.g. proteins, enzymes and nucleic acids) so plays a role in nearly all plant processes (Rice 2007). The addition of soil-applied N fertiliser increases the vigour of apple trees but this N also increases the susceptibility of trees to European canker caused by the fungus *Neonectria ditissima* (Campbell et al. 2018). These authors determined that various nitrogen sources and concentrations could influence the germination as well as the germ tube growth of *N. ditissima*. However, that study was done *in vivo* but *N. ditissima* infection also occurs *in planta* and is particularly damaging to young trees as it often occurs on the main stem, killing the plant (McCracken et al. 2003).

Datnoff et al. (2007) reviewed the benefits of mineral nutrition and their effect on plant diseases. This comprehensive review drew largely on results from annual crops, because most studies published on nutrient–disease interactions have focused on such systems. Generally, too much or too little nitrogen increases disease susceptibility (Fagard et al. 2014). However, any given form of nitrogen may favour one disease while suppressing another (Huber & Watson 1974). Disease potential also is influenced more by inorganic mineral nutrients than by inoculum potential, and the rate of nitrogen application is less important than the nitrogen source (Huber & Watson 1974). Biotrophs and necrotrophs also may respond differently to nitrogen forms and concentrations in plant tissues (Fagard et al. 2014). In the case of *N. ditissima*, foliar-applied nitrogen at early leaf fall increased disease expression six-fold compared with that in an unsprayed control (Dryden et al. 2016). In these field experiments, the timing of application was more important than the form or concentration of the nitrogen source (Dryden et al. 2016). The practice of foliar-applied nitrogen at early leaf fall into the canopy for control of the fungus *Venturia inaequalis* (the causal agent of apple scab) (Beresford et al. 2000; Wood et al. 2008) has since been suspended by New Zealand growers and now nitrogen is applied onto the leaves on the ground instead.

In apples, leaves and fruit (30–50% and 18% of plant N, respectively) are the biggest sinks for nitrogen (Batjer et al. 1952). Apple trees remove 0.6 to 1.1 kg nitrogen per tonne of fruit, with yields in New Zealand ranging between 52 and 130 t/ha depending on cultivar and production conditions (Journeaux et al. 2019). This translates into 31–143 kg nitrogen removal per hectare from soil. Nitrogen is needed in spring for bud burst, bloom and spring growth. These nitrogen reserves are derived largely from leaf nitrogen withdrawn before leaf fall and stored in the woody framework (Batjer et al. 1952). Excessive nitrogen present at harvest is highly undesirable as it adversely affects fruit quality, such as skin colour and storage (Guinto 2016). This knowledge means most of the nitrogen in mature orchards is applied postharvest, whereas a monthly side dressing is recommended for new plantings with an annual application of up to 240 kg nitrogen/ha/year (Journeaux et al. 2019).

To understand the relationship between nitrogen and disease susceptibility of young apple trees, the effect of soil-applied nitrogen, form and concentration, on *N. ditissima* infection and symptom development were studied in one-year-old potted ‘Royal Gala’ plants.

MATERIALS AND METHODS

In October 2018, ‘Royal Gala’ scions obtained from the Plant & Food Research repository, Motueka, were grafted onto ‘M9’ rootstocks obtained from Waimea Nurseries, Tasman. A total of 514 plants were potted (singly) into 12-L bags in potting mix produced by Wholesale Landscapes, Tasman NZ formulated to contain the lowest level of nitrogen possible. The growth substrate consisted of 20% pumice and 80% neutralised bark. The growth substrate was designed such that, other than nitrogen, none of the other macro or micronutrients was limited. A cubic metre of substrate was fortified with 3 kg magnesium, 2.5 kg calcium, 0.6 kg trace elements and 1.5 kg wetting agent (Wholesale Landscapes).

The plants were placed outside, in a sheltered tar-sealed location, onto wooden pallets to ensure sufficient drainage and prevent cross-contamination between treatments due to possible leaching. Plants were watered as required using individual drip irrigation. Thirteen fertiliser treatments were used (Table 1). The fertilisers comprised calcium ammonium nitrate (CAN, Ravensdown); potassium nitrate (KNO₃, Campbell’s Fertilisers Australasia); calcium nitrate (Ca(NO₃)₂, NitroCal, Campbell’s Fertilisers Australasia); ammonium nitrate (NH₄NO₃, Redox); ammonium sulphate ((NH₄)₂SO₄, NRich SOA, Ballance Agri-Nutrients); urea (CO(NH₂)₂, NRich Urea, Ballance Agri-Nutrients); and YaraMila™ Complex which contains nitrogen, phosphorus and potassium (YM, Ballance Agri-Nutrients).

Seven replicate plots of the thirteen treatments were used, in multiple plant-plots. Each ‘plot’ consisted of five or six adjacent trees (grouped, as opposed to a single line), which received the same treatment. The treatments plots were laid out according to a doubly-resolvable row-column design, with each replicate comprising two adjacent treatment plots by seven plots, with a single plot per replicate at the bottom of the trial (design generated using ‘RowCol.exe’, pers. comm. David B. Baird, VSN International¹; see Additional File Table A1 and Fig. A1). Trees were placed onto the pallets on 23/24 October 2019. The control treatment received no nitrogen. CAN was applied at six different rates (treatments 2–7) and with application frequencies as shown in Table 1. Six other nitrogen sources, at the equivalent molar nitrogen rates and frequency as those of the highest CAN application, were applied for treatments 8–12. Nitrogen was added to the growth substrate surface in 2, 4, 6, 8 or 10 doses (at roughly three-week intervals), starting on 13 November 2018 and ending on 30 April 2019 (Table 1).

Inoculations

Frozen *N. ditissima* inoculum was used (Orchard et al. 2018). This was prepared from wild-type spores on 4 September 2018 and obtained from ‘Scilate’ apple trees. Four leaf scars and two bud scars (made below the leaf scars) on each tree were brush inoculated (Walter et al. 2015) with spores (20–30 µL) on 22 May 2019 at a concentration of 3.3×10^4 spores/mL and with a germination rate of 95% after 16 hours.

¹ Incorporated into the AFRRESOLVABLE procedure, (VSN International 2020).

Table 1 Nitrogen (N) form, concentrations and application timings used for topical application on 12-litre potted 'Royal Gala' trees during the 2018/19 season.

Treatment ^a		Product Rate (g)	Nitrogen (g) per application	Number and date of applications 2018-----2019-----											
				Number	13/11	04/12	25/12	15/01	05/02	26/02	19/03	09/04	30/04	21/05	
1	Control	0	0	0											
2	CAN	20	4.66	2	x	x									
3	CAN	40	9.32	2	x	x									
4	CAN	20	4.66	4	x	x		x				x			
5	CAN	20	4.66	6	x	x		x		x		x		x	
6	CAN	20	4.66	8	x	x		x	x	x		x	x	x	
7	CAN	20	4.66	10	x	x	x	x	x	x	x	x	x	x	
8	KNO ₃	34	4.66	10	x	x	x	x	x	x	x	x	x	x	
9	Ca(NO ₃) ₂	27	4.66	10	x	x	x	x	x	x	x	x	x	x	
10	NH ₄ NO ₃	13	4.66	10	x	x	x	x	x	x	x	x	x	x	
11	(NH ₄) ₂ SO ₄	38	4.66	10	x	x	x	x	x	x	x	x	x	x	
12	YM	39	4.66	10	x	x	x	x	x	x	x	x	x	x	
13	Urea	10	4.66	10	x	x	x	x	x	x	x	x	x	x	

^a CAN = calcium ammonium nitrate; KNO₃ = potassium nitrate; Ca(NO₃)₂ = calcium nitrate; NH₄NO₃ = ammonium nitrate; (NH₄)₂SO₄ = ammonium sulphate; urea = CO(NH₂)₂; YM = YaraMila™ Complex (contains nitrogen, phosphorus and potassium).

Assessments

The number of **surviving trees** in each plot was assessed on five dates in 2019 (7 January, 31 January, 20 February, 11 April, 14 October). Any dead trees were removed at these assessments.

Leaf weights and leaf N were determined by taking leaf samples in February and October 2019. One leaf per plant per plot was harvested using the "youngest mature leaf principle" (Hill Laboratories Crop Guide – Apple). Leaves were weighed, dried at 60°C, reweighed, ground up and about 0.7 g from each treatment replication combined to give an approximately 5 g sample for each treatment. Subsamples were sent for nitrogen analysis to Hill Laboratories (RJ Hill Laboratories Ltd, Private Bag 3205, Hamilton 3240, New Zealand).

Tree height and diameter were measured in 2019 on 31 January, and again on 28 February, 28 March, 13 June, 26 August, and 25 September. The diameter was measured 10 cm above the graft union using a digital calliper. The site of measurement was marked with a paint pen at the first assessment.

The incidence of **powdery mildew** (*Podosphaera leucotricha*) was assessed on 27 February 2019, using a four-point scale: 0 = no or negligible mildew evident; 1 = some mildew but only on the older leaves; 2 = some mildew on young leaves and some older leaves; and 3 = severe mildew causing stunting and distortion of the young leaves.

Neonectria ditissima disease expression on the inoculated leaf and bud scars showing European canker symptoms was assessed in 2019 on 29 July, 12 August, 26 August, 9 September, 23 September and 14 October. Scars with visible

symptoms were painted with pruning paint (PruneTec®) to prevent conidia dispersion (Walter et al. 2017).

Statistical analyses

All the analyses were carried out with Genstat (Payne et al. 2019). For ease of interpretation, the results for the CAN treatments and the treatments with other nitrogen sources are shown separately throughout. Leaf nitrogen data were summarised graphically only, because the samples were pooled across replicates before nitrogen assessment. Tree height and diameter, mildew score, and leaf weight data were analysed with analyses of variance. The percentage of scars expressing European canker was calculated as the total number of scars with canker across all assessments showing expression for leaves and for buds, out of the total number of scars. These, and the percentage tree survival, were analysed using a binomial generalized linear model with a logit link (McCullagh & Nelder 1989) with a binomial total of the total number of scars inoculated and total trees, respectively. The dispersion was estimated for scars. Treatments and contrasts between treatments were assessed with F-tests for scars and χ^2 tests for tree survival within the analysis of deviance carried out as part of the analysis. The extent of disease expression for each treatment and associated 95% confidence limits were obtained on the linear prediction (i.e. logit link) scale, and converted back to the percentage scale for presentation.

The rate of disease expression was also examined by calculating the area under the disease curve (AUDPC). For this, the cumulative number of leaf or bud scars was

calculated, and converted into the cumulative percentage scars infected at each date for each tree. The area under the resulting curve was calculated in the standard way. The AUDPC values were then analysed with restricted maximum likelihood (REML), with replicate and plot as random effects, and treatment (or treatment contrasts) included as the fixed effects. The various treatment comparisons were assessed with F-tests, with denominator degrees of freedom estimated using the method of Kenward & Roger (1997).

RESULTS

Leaf N at the first sampling date (February 2019) was lowest in the control treatment (1.4%). All treatments had similar leaf N values but these were twice that of the control (mean of 3.0%). Leaf N in November 2019 was higher in all treatments compared with the February values, and was highest for $(\text{NH}_4)_2\text{SO}_4$ (5.4%) followed by CAN (4.4%), NH_4NO_3 (4.4%), YaraMila™ (4.1%), urea (4.0%), $\text{Ca}(\text{NO}_3)_2$ (3.9%), and KNO_3 (3.7%). Increasing CAN concentrations also increased leaf N in the November 2019 assessments. The lowest leaf N in trees receiving the lowest CAN application was 3.5% (Fig. 1; Additional File Table A2). The effect of nitrogen form on leaf N was also reflected in leaf weight (Fig. 2).

Overall, there was variation among treatments in **leaf weight** ($p < 0.001$ for both fresh and dry weights). The primary difference was between the control (with the lowest weights per leaf) and the other treatments ($p < 0.001$ comparing the control with the mean of the other treatments). For the CAN treatments, changing the application rate did not substantially affect leaf weight ($p = 0.276$ and 0.178 for fresh and dry weights respectively). However, amongst the non-CAN treatments, leaf weights did vary ($p = 0.006$ and 0.010 for fresh and dry). Leaf weights for trees in each of the non-CAN compound treatments were all higher than those in the control. The weights for these compounds varied from 1.8 g fresh/1.0 g dry for KNO_3 to 2.5 g/1.4 g for $(\text{NH}_4)_2\text{SO}_4$ treatment (Fig. 2; Additional File Table A3).

Tree survival varied substantially among treatments ($p = 0.002$). The primary difference was between the low

survival rate (76%) for the $(\text{NH}_4)_2\text{SO}_4$ treatment, followed by 93% for the KNO_3 treatment and the control with 95%; all other treatments resulted in survival rates of 97–100% (Fig. 3; Additional File Table A4).

Tree diameter varied between the treatments at both assessments ($p = 0.001$ and $p < 0.001$ for January and September respectively). Tree diameter was smallest on average for the control trees, at 5.3 and 5.6 mm for the two dates. Diameter varied among the six CAN treatments ($p = 0.041$ and 0.021 for the two dates), primarily because the diameters were smaller for the 80 g and 200 g treatments (treatments 3, 4 and 7; Fig. 4). The mean diameters for the CAN treatments were 6.5 and 8.2 mm in January and September respectively. However, for the non-CAN nitrogen treatments, diameter was very similar ($p = 0.829$ and 0.901 for the two dates), with mean diameters of 6.4 and 8.2 mm in January and September respectively (Fig. 4; Additional File Table A5).

Tree height varied substantially among the treatments at both assessments ($p < 0.001$ for both January and September). At both assessment dates, the height of the control trees was the smallest on average, at 56 and 58 cm (Fig. 5). Tree height increased considerably more but relatively similarly in all other treatments (CAN treatments: $p = 0.337$ and 0.124 for the two dates; non-CAN nitrogen treatments, $p = 0.513$ and 0.694 for the two dates), with mean heights of 80 and 109 cm in January and September respectively (Fig. 5; Additional File Table A6).

The mean **powdery mildew** score varied among treatments ($p = 0.002$), with the score for the control being the lowest, at 0.5. Scores for the other CAN treatments did not vary strongly with CAN rate ($p = 0.367$), with a mean of 1.2. However, there was some variation in scores among the non-CAN treatments ($p = 0.051$), with the mean scores varying from 0.7 for $\text{Ca}(\text{NO}_3)_2$ to 1.5 for NH_4NO_3 (Fig. 6; Additional File Table A7).

For both leaf and bud lesions, the percentage ***N. ditissima* leaf and bud scar expression**, varied substantially between the treatments ($p = 0.037$, $p < 0.001$ for the overall treatment effect for leaf and bud inoculations respectively, Fig. 7;

Figure 1 Percentage Leaf N for each treatment at two assessments, for (left) CAN treatments, vs total rate; (right): non CAN treatments and the control, with treatments in increasing order of the value for Nov. Feb: dark blue; Nov: Cyan (with red/ pink or the 2 x 40 g treatment). Note that the %N for the control, November, is guessed: it was set to be the same as the Feb value.

Figure 2 Leaf weight per ‘Royal Gala’ plant for each fertiliser treatment for Fresh Weight (left) and Dry Weight (right). Top: CAN treatments 2–7 (Table 1); bottom: highest CAN (treatment 7, Table 1) and other equivalent molar nitrogen fertiliser treatments 8–13 (Table 1) and the control (treatment 1, Table 1), with treatments in increasing order of the mean. Error bars are 95% confidence limits.

Additional File Table A8). For both variables, the lowest percentage expression was in the untreated control, at 15 and 37%, with trees in all other treatments having expression of above 70%. This was the primary treatment effect ($p < 0.001$ for leaf and bud scar comparing the control

with the mean of the other treatments). Amongst the six CAN treatments, there was little difference between the two 80-g total CAN treatments (treatments 3 and 4; $p = 0.393$, $p = 0.931$ for leaf and bud scar inoculations), with around 75% and 85% expression for leaf and bud scars. Furthermore, there

Figure 3 Percentage tree survival expression for each treatment. left: CAN treatments, vs total rate; right: non-CAN treatments and the control, with treatments in increasing order of the mean. Error bars are 95% confidence limits. A lower limit for 100% is difficult to obtain and so is not shown.

Figure 6 Powdery mildew score (*Podosphaera leucotricha*) assessed on 27 February 2019 on 'Royal Gala' plants under different fertiliser treatments. Left: increasing CAN treatments 2–7 (Table 1). Right: other nitrogen equimolar fertiliser treatments 8–13 (Table 1) and the control (treatment 1, Table 1), with treatments in increasing order of the mean. Error bars are 95% confidence limits.

was also little variation in response between trees in the six CAN treatments ($p=0.743$ and 0.295 for leaf and bud scars respectively), with expression in the CAN treatments varying from 71 to 82% for leaf and from 85 to 97% for bud scar inoculations. Responses also varied little amongst the six non-CAN treatments ($p=0.466$ and 0.259 for leaf and bud scar inoculations).

Similarly to the overall disease expression (total percentage data), there was substantial variation in the rate of disease expression with the AUDPC values between the treatments ($p=0.001$, $p<0.001$ for the overall treatment effect for leaf and bud scar inoculations respectively). For both variables, the lowest AUDPC was for the untreated control, with an area of around 480% infected scar-days for

Figure 7 Percentage *Neovectria ditissima* disease expression in 'Royal Gala' plants for each fertiliser treatment for Leaf (left) and Bud scars (right). Top: CAN treatments 2–7 (Table 1); bottom: other nitrogen equimolar fertiliser treatments 8–13 (Table 1) and the control (treatment 1, Table 1), with treatments in increasing order of the mean. Error bars are 95% confidence limits.

both leaf and bud scar inoculations. All other treatments resulted in AUDPC values above 920 and 1045. This was the primary treatment effect ($p < 0.001$ for leaf and bud scar inoculation comparing the control with the mean of the other treatments). Amongst the six CAN treatments, there was little difference between the two 80-g total CAN treatments (treatments 3 and 4; $p = 0.582$, $p = 0.563$ for leaf and bud scar inoculations), with areas of around 1100% infected scar-days for both leaf and bud scars. However, there was also little variation in response between the six CAN treatments ($p = 0.335$ and 0.926 for leaf and bud scar respectively). Responses varied somewhat amongst the six non-CAN treatments ($p = 0.095$ and 0.002 for leaf and bud scar), with AUDPC lowest in both cases for trees in the KNO_3 treatment and highest in the YaraMila™ treatment (Fig. 8; Additional File Table A9).

DISCUSSION

In this experiment, adding nitrogen fertiliser to potted apple trees had the biggest effect on the various parameters tested. A potting mix with a low level of nitrogen was used for the control but even this small amount supported a slight increase in tree height between the two assessment dates of 2 cm. In contrast, application of additional nitrogen led to much larger increases in tree height and diameter. Even a

single 20-g application of CAN in spring supported maximum tree growth under the conditions tested, with no additional growth in tree height and diameter observed by increasing the CAN rates or by changing nitrogen form. Therefore, lower nitrogen rates than those tested here will be required to determine the dose-response curve. The main trends in disease expression were also between presence or absence of fertiliser, rather than the quantity of fertiliser supplied so lower amounts will also be required here to determine the dose-response curve. The data indicate that application of even low amounts of nitrogen fertiliser increases the risk of young apple trees to *N. ditissima* infection and symptom expression. Young orchards receive up to 240 kg N/ha/year (Journeaux et al. 2019) or in the order of 120 g N/plant in high-density orchard plantings. CAN applied in this trial at 20 g/plant is only 4.2–5.4 g N/plant.

We have not conducted a total mass-balance analysis of nitrogen in our experiments. Disease severity and plant resistance to disease are often more affected by the form of N rather than the amount of N (Huber & Watson 1974) so the main aim of this study was to understand the effects of nitrogen form on *N. ditissima* plant susceptibility. Different concentrations of two nitrogen source were applied but the results indicate that the lower amounts were still not growth limiting although a total mass-balance analysis of nitrogen was not undertaken. For *N. ditissima*, the form of N

Figure 8 Area (% infected scar-days) under the *Neonectria ditissima* disease progression curve (AUDPC) for 'Royal Gala' plants under each fertiliser treatment for (left) Leaf and (right) Bud scars. Top: CAN treatments 2–7 (Table 1); bottom: other nitrogen equimolar fertiliser treatments 8–13 (Table 1) and the control (treatment 1, Table 1), with treatments in increasing order of the mean. Error bars are 95% confidence limits.

applied in the current study also made very little difference and there is little published literature to support or refute this finding. For autumn infections, the foliar-applied forms of N did affect tree susceptibility to pathogen infection and disease expression (Dryden et al. 2016). *In vitro* studies have shown that the form of N applied affected rate of pathogen germination, it did not affect growth of germ tubes (Campbell et al. 2018). However, very few spores are required for successful *N. ditissima* infection (Walter et al. 2016).

The different nitrogen sources used here were adjusted to have equal applied nitrogen contents. While there were no statistical significant differences amongst the fertilisers, trees treated with KNO₃ had the lowest powdery mildew and European canker disease scores. European canker in the KNO₃ treatments also peaked latest in the season (Fig. 8). Potassium has been found to decrease plant disease caused by powdery mildew pathogens on other crops, as well as fusaria diseases (Datnoff et al. 2007). Potassium phosphites were also found to be more effective than other phosphite salts in controlling European canker by Boneti et al. (2015). The role of potassium in decreasing the intensity of many diseases has been described in Datnoff et al. (2007), and its role in *N. ditissima* disease development warrants further investigation.

The effects of nitrogen on potted plants might be difficult to assess and compare owing to arbitrary rates of nitrogen application and the limited volume of soil available (Huber & Watson 1974) in different trials. In our experiments, the planter bag sizes were large, with room for root growth at the end of the experiment (data not shown). Further work is required to determine the interaction of nitrogen concentration and form on young tree establishment, susceptibility to *N. ditissima* infection, as well as transcriptomics and metabolomic responses in the host-pathogen interaction.

CONCLUSIONS

Nitrogen concentration is an important factor in the disease development of European canker of apple. Even small amounts of nitrogen increased susceptibility of 'Royal Gala' to *N. ditissima*. Therefore control of timing, amount and form of nitrogen application is important for European canker management at the nursery and orchard scales. Long-term field studies, from planting to mature tree management, are recommended to further validate these results, particularly the effect of applying nitrogen to the ground around older trees in autumn.

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ADDITIONAL FILE: Layout of Trial Design and Tabulated Data**Table A1** Nitrogen (N) form, concentrations and application timings used for topical application on 12-litre potted 'Royal Gala' trees during the 2018/19 season.

Treatment ^a		Product Rate (g)	Nitrogen (g) per application	Colour
1	Control	0	0	White
2	CAN	20	4.66	Yellow
3	CAN	40	9.32	Orange/Black
4	CAN	20	4.66	Pink/Black
5	CAN	20	4.66	Neon Orange
6	CAN	20	4.66	Blue/White
7	CAN	20	4.66	Blue
8	KNO ₃	34	4.66	Black Dot
9	Ca(NO ₃) ₂	27	4.66	Yellow/Black
10	NH ₄ NO ₃	13	4.66	Blue/Black
11	(NH ₄) ₂ SO ₄	38	4.66	Orange/White
12	YaraMila™	39	4.66	Red
13	Urea	10	4.66	Green

^a CAN = calcium ammonium nitrate; KNO₃ = potassium nitrate; Ca(NO₃)₂ = calcium nitrate; NH₄NO₃ = ammonium nitrate; (NH₄)₂SO₄ = ammonium sulphate; urea = CO(NH₂)₂; YM = YaraMila™ Complex (contains nitrogen, phosphorus and potassium).

Figure A1: Schematic diagram of trial layout. Colours indicate the treatment applied to five or six trees per plot.**Table A2** Percentage Leaf N for each treatment at two assessments, for leaf samples pooled over replicates.

	Treat	CAN rate	Colour	Feb	Nov	Nov/Feb
1	Control	0	White	1.4	-	-
2	CAN	2 x 20g	Yellow	3.0	3.5	1.17
3		2 x 40g	Orange/Black	3.1	3.8	1.23
4		4 x 20g	Pink/Black	2.8	4.1	1.46
5		6 x 20g	Neon Orange	3.0	4.4	1.47
6		8 x 20g	Blue/White	3.1	4.8	1.55
7		10 x 20g	Blue	3.1	4.4	1.42
8	KNO ₃		Black Dot	3.0	3.7	1.23
9	Ca(NO ₃) ₂		Yellow/Black	3.1	3.9	1.26
10	NH ₄ NO ₃		Blue/Black	3.0	4.4	1.47
11	(NH ₄) ₂ SO ₄		Orange/White	3.0	5.4	1.80
12	YaraMila™		Red	3.1	4.1	1.32
13	Urea CO(NH ₂) ₂		Green	3.0	4.0	1.33

Table A3 Leaf fresh and dry weights weight per plant for each treatment (95% confidence limits.)

	Treat	CAN	Colour	FWt	DWt
1	Control	0	White	1.0 (0.7,1.3)	0.7 (0.4,0.9)
2	CAN	2 x 20g	Yellow	1.8 (1.5,2.1)	0.9 (0.7,1.1)
3		2 x 40g	Orange/Black	2.2 (1.9,2.5)	1.2 (1.0,1.4)
4		4 x 20g	Pink/Black	1.9 (1.6,2.2)	1.0 (0.8,1.2)
5		6 x 20g	Neon Orange	1.8 (1.4,2.1)	0.9 (0.7,1.1)
6		8 x 20g	Blue/White	1.8 (1.5,2.1)	0.9 (0.7,1.1)
7		10 x 20g	Blue	1.8 (1.5,2.1)	0.9 (0.7,1.1)
8	KNO ₃		Black Dot	1.8 (1.4,2.1)	0.9 (0.7,1.1)
9	Ca(NO ₃) ₂		Yellow/Black	1.8 (1.5,2.1)	1.0 (0.8,1.2)
10	NH ₄ NO ₃		Blue/Black	2.2 (1.9,2.5)	1.2 (1.0,1.4)
11	(NH ₄) ₂ SO ₄		Orange/White	2.5 (2.2,2.8)	1.4 (1.2,1.6)
12	YaraMila™		Red	2.0 (1.7,2.3)	1.0 (0.8,1.2)
13	Urea CO(NH ₂) ₂		Green	2.1 (1.8,2.4)	1.2 (1.0,1.4)

Table A4 Percent trees surviving (95% confidence limits)

	Treat	CAN	Colour	%
1	Control	0	White	95.1 (82.5,98.8)
2	CAN	2 x 20g	Yellow	100.0 (*,100.0)
3		2 x 40g	Orange/Black	100.0 (*,100.0)
4		4 x 20g	Pink/Black	96.7 (79.8,99.5)
5		6 x 20g	Neon Orange	96.9 (80.9,99.6)
6		8 x 20g	Blue/White	100.0 (*,100.0)
7		10 x 20g	Blue	100.0 (*,100.0)
8	KNO ₃		Black Dot	92.9 (75.5,98.2)
9	Ca(NO ₃) ₂		Yellow/Black	96.6 (79.2,99.5)
10	NH ₄ NO ₃		Blue/Black	100.0 (*,100.0)
11	(NH ₄) ₂ SO ₄		Orange/White	76.0 (55.8,88.8)
12	YaraMila™		Red	100.0 (*,100.0)
13	Urea CO(NH ₂) ₂		Green	100.0 (*,100.0)

*: a lower limit for 100% is difficult to obtain and so is not shown

Table A5 Mean tree diameter (mm) at two assessments (95% confidence limits).

	Treat	CAN	Colour	31 st Jan	25 th Sept
1	Control	0	White	5.3 (4.6,6.0)	5.6 (4.7,6.4)
2	CAN	2 x 20g	Yellow	7.1 (6.4,7.8)	9.1 (8.3,9.9)
3		2 x 40g	Orange/Black	6.1 (5.4,6.8)	7.7 (6.9,8.6)
4		4 x 20g	Pink/Black	6.6 (5.9,7.3)	8.6 (7.7,9.4)
5		6 x 20g	Neon Orange	7.3 (6.7,8.0)	8.9 (8.1,9.7)
6		8 x 20g	Blue/White	7.2 (6.6,7.9)	9.4 (8.5,10.2)
7		10 x 20g	Blue	6.2 (5.5,6.8)	8.0 (7.1,8.8)
8	KNO ₃		Black Dot	6.3 (5.6,7.0)	7.9 (7.0,8.7)
9	Ca(NO ₃) ₂		Yellow/Black	6.0 (5.4,6.7)	8.0 (7.2,8.9)
10	NH ₄ NO ₃		Blue/Black	6.6 (5.9,7.3)	8.4 (7.5,9.2)
11	(NH ₄) ₂ SO ₄		Orange/White	6.6 (5.9,7.2)	8.1 (7.3,8.9)
12	YaraMila™		Red	6.6 (5.9,7.2)	8.5 (7.7,9.3)
13	Urea CO(NH ₂) ₂		Green	6.4 (5.7,7.1)	8.1 (7.3,9.0)

Table A6 Mean tree height (cm) at two assessments (95% confidence limits).

	Treat	CAN	Colour	31 st Jan	25 th Sept
1	Control	0	White	56.4 (48.3,64.5)	58.3 (47.2,69.3)
2	CAN	2 x 20g	Yellow	85.0 (76.9,93.1)	118.7 (107.6,129.7)
3		2 x 40g	Orange/Black	75.0 (66.9,83.1)	102.8 (91.7,113.8)
4		4 x 20g	Pink/Black	76.9 (68.8,84.9)	102.2 (91.1,113.2)
5		6 x 20g	Neon Orange	79.9 (71.8,87.9)	107.1 (96.0,118.1)
6		8 x 20g	Blue/White	83.2 (75.1,91.2)	114.8 (103.7,125.9)
7		10 x 20g	Blue	76.6 (68.5,84.7)	105.0 (93.9,116.0)
8	KNO ₃		Black Dot	74.6 (66.6,82.7)	102.5 (91.5,113.6)
9	Ca(NO ₃) ₂		Yellow/Black	77.5 (69.4,85.5)	110.4 (99.4,121.5)
10	NH ₄ NO ₃		Blue/Black	86.0 (77.9,94.0)	112.9 (101.8,123.9)
11	(NH ₄) ₂ SO ₄		Orange/White	78.6 (70.5,86.7)	102.7 (91.7,113.8)
12	YaraMila™		Red	78.9 (70.8,87.0)	108.3 (97.3,119.4)
13	Urea CO(NH ₂) ₂		Green	79.9 (71.9,88.0)	104.3 (93.2,115.3)

Table A7 Mean Mildew Score (95% confidence limits).

	Treat	CAN	Colour	Score
1	Control	0	White	0.5 (0.1,0.8)
2	CAN	2 x 20g	Yellow	1.3 (1.0,1.7)
3		2 x 40g	Orange/Black	1.5 (1.1,1.9)
4		4 x 20g	Pink/Black	1.1 (0.8,1.5)
5		6 x 20g	Neon Orange	1.1 (0.7,1.5)
6		8 x 20g	Blue/White	1.0 (0.6,1.4)
7		10 x 20g	Blue	1.3 (1.0,1.7)
8	KNO ₃		Black Dot	1.1 (0.8,1.5)
9	Ca(NO ₃) ₂		Yellow/Black	0.7 (0.3,1.1)
10	NH ₄ NO ₃		Blue/Black	1.5 (1.1,1.9)
11	(NH ₄) ₂ SO ₄		Orange/White	1.3 (0.9,1.6)
12	YaraMila™		Red	1.1 (0.7,1.4)
13	Urea CO(NH ₂) ₂		Green	1.4 (1.1,1.8)

Table A8 Percentage expression for each treatment for Leaf and Bud scars (95% confidence limits).

		CAN rate	Colour	Leaf	Bud
1	Control	0	White	15.0 (2.6,53.8)	36.8 (25.2,50.0)
2	CAN	2 x 20g	Yellow	73.9 (61.2,83.6)	85.1 (73.1,92.3)
3		2 x 40g	Orange/Black	71.4 (57.1,82.4)	85.0 (68.5,93.7)
4		4 x 20g	Pink/Black	78.9 (65.3,88.2)	85.7 (71.3,93.5)
5		6 x 20g	Neon Orange	81.1 (68.2,89.6)	86.3 (72.3,93.8)
6		8 x 20g	Blue/White	77.4 (65.4,86.1)	87.0 (75.5,93.5)
7		10 x 20g	Blue	82.4 (70.7,90.1)	96.8 (86.5,99.3)
8	KNO ₃		Black Dot	72.6 (57.0,84.1)	75.0 (59.3,86.0)
9	Ca(NO ₃) ₂		Yellow/Black	71.4 (57.1,82.4)	86.8 (73.2,94.0)
10	NH ₄ NO ₃		Blue/Black	82.8 (70.1,90.8)	93.0 (81.2,97.6)
11	(NH ₄) ₂ SO ₄		Orange/White	83.8 (66.8,93.0)	82.9 (64.7,92.7)
12	YaraMila™		Red	84.5 (71.7,92.1)	88.3 (76.0,94.8)
13	Urea CO(NH ₂) ₂		Green	75.5 (60.9,85.9)	89.6 (75.6,96.0)

Note that the lower number of inoculated leaf scars for the control is the reason why the confidence limits for the control are proportionately larger than those for the other treatments.

Table A9 AUDPC for each treatment for Leaf and Bud scars (95% confidence limits).

		CAN rate	Colour	Leaf	Bud
1	Control	0	White	480 (161,800)	474 (234,713)
2	CAN	2 x 20g	Yellow	1006 (757,1254)	1192 (948,1436)
3		2 x 40g	Orange/Black	1031 (772,1290)	1151 (870,1432)
4		4 x 20g	Pink/Black	1101 (844,1358)	1241 (975,1507)
5		6 x 20g	Neon Orange	1185 (930,1439)	1266 (1009,1523)
6		8 x 20g	Blue/White	1106 (859,1352)	1277 (1033,1522)
7		10 x 20g	Blue	1238 (992,1484)	1290 (1041,1538)
8	KNO ₃		Black Dot	925 (661,1188)	1047 (778,1316)
9	Ca(NO ₃) ₂		Yellow/Black	998 (739,1257)	1276 (1019,1533)
10	NH ₄ NO ₃		Blue/Black	1175 (921,1429)	1361 (1107,1616)
11	(NH ₄) ₂ SO ₄		Orange/White	1209 (926,1493)	1196 (901,1490)
12	YaraMila™		Red	1253 (996,1511)	1677 (1425,1929)
13	Urea CO(NH ₂) ₂		Green	1102 (841,1363)	1296 (1031,1561)