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Impacts of organic and inorganic nitrogen fertilisers on sweetpotato (*Ipomoea batatas*) sprout production

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Abstract

Australian production of sweetpotato (*Ipomoea batatas)* achieve the highest yields in the world and has seen a 1700% increase in growth over the last 20 years. These advancements have been a result of the implementation by industry to use pathogen tested planting material (sprout cuttings) that is grown on farm in specialised nurseries called seedbeds. The effects of different organic and inorganic nitrogen sources; ammonia and nitrate on sprout weight, sprout count and seedbed breakdown were investigated in this study. Two different inorganic fertilisers and an organic based fertiliser at 3 different rates were used on two different cultivars of sweetpotato Beauregard and Bellevue.

The study was conducted at the Gatton Research Facility and ran from November 2016 to May 2017 for 202 days. Data was collected over the growing season at seven cutting intervals. Fertiliser treatments were applied at each cut and at planting. This study was undertaken to explore gross effects of N nutrition on sweetpotato sprout productivity, and scope what effects are possible, which would then be followed by more detailed and expensive investigations if warranted.

Findings indicated that Beauregard cultivar responded positively to nitrogen application regardless of the composition. The Bellevue cultivar showed no significant difference to number of sprouts produced or total biomass compared to that control which had no N addition. Bellevue did show signs of premature degradation which could be a factor of seedbed aeration, temperature or carbon dioxide/oxygen respiration of storage roots. Further research into Bellevue seedbed degradation is required to see how maintain seedbed health.

Declaration

This thesis describes the original work of the author, except otherwise stated. It has not been submitted previously at this or any institution

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1.0 Introduction

Sweetpotatoes are vegetatively propagated through sprout cuttings. These sprouts are grown in 'seedbeds' that require specialised agronomy separate to commercial production of the marketable product. The potential yield of marketable sweetpotato can be highly dependent on the quality of sprout cuttings, making this an important area of research (Henderson et al. 2014).

Research and development focused on seedbed production, is relatively new to Australian growers, only being explored in the last 5 years. There is still no single ideal method that has been defined. This is due to variations in the seedbed agronomy being influenced by farm size, time of planting and length of growing season, regional climate and machinery and labour availability (Henderson et al. 2014).

Generally, for the process of seedbed production storage roots which are grown from pathogen tested propagation material are planted above ground level in a raised bed and covered with a thin layer of soil (DAF 2014). Sprouts take 3-5 weeks of warm weather to grow to an optimal length for transplanting. Fertiliser is applied to the seedbed after every cut to increase and reinvigorate the production of sprouts (Maltby et al. 2006).

There is a recommendation from the Queensland Department of Agriculture and Fisheries DAF (2014) that an application of 100 g/m^2 of a complete fertiliser be applied after each seedbed cut, without specifications on which fertiliser is considered optimal. The form of fertilisers varies across the sweetpotato industry, particularly in the form in which nitrogen (N) is supplied to the crop. A range of complete, Nitrogen-Phosphorous-Potassium (NPK) fertilisers are currently on the market and are available for use in the industry. However, a consensus in the industry is that organic fertilisers help to create a better quality shoots over synthetic NPK fertilisers (Henderson & Dennien 2016).

Despite this, there has been little research on how seedbed production is affected by N in the different forms. N uptake in plants is predominantly in the form of nitrate (NO_3^-) with some uptake of in the form of ammonia ($^{NH4+}$) (Villagarcia et al. 1998). Inorganic fertilisers such as potassium nitrate and Nitrophoska Special®, which are nitrate based are considered to be readily available for plant uptake. Organic manure based fertilisers like Organic Extra® are ammonia based and require microbial activity to break down NH4+ into NO3- as plant uptake in ammonia is low (USDA nd).

Currently, there is a need for the Australian sweetpotato industry to establish key principles for quality seedbed production, with practical methods for producers to address variations is sprout quality. In particular, the effect of the rate and form of N fertiliser needs to be investigated to increase the understanding of bed growth, with potential to assist growers in consistently producing quality sprouts.

This study investigated the sequential production of acceptable sprouts (Short and Optimal lengths) and sprout biomass. Seedbeds were side dressed with a selection of fertilisers that are being used in industry. The study examined the effects of N on

seedbed longevity and overall sprout productivity across the whole growing season from November through to May.

2.0 Literature review

2.1 Overview of sweetpotato production

Over 110 million tonnes of sweetpotatoes are produced annually throughout the world. China by far is the largest producer of sweetpotatoes with a production of 100 million tonnes per year which is 90% of world production (*FAO Stat* 2014). Sweetpotato is a common staple in many developing and low income countries which accounts for 95% of total production, Tanzania, Nigeria, Ethiopia and Indonesia are in the top 5 world producers of sweetpotatoes. Sweetpotatoes ranked 7th in the list of important food crops because it can be grown in a variety of arid to tropical conditions (*Sweet Potato - Crop Trust* 2015). However, there is significant yield difference between commercial production systems and subsistence farming systems.

Australia's sweetpotato production ranks low on global production as it only produces 100 000 tonnes, a gross value of $90 million (Henderson et al. 2014). Sweetpotato is predominantly grown in the eastern states of Australia, in tropical and subtropical climates (*Australian Sweetpotato Industry-Key Facts* 2014). All Australian production is grown commercially and for domestic consumption. Importations of sweetpotato is small and in processed forms. The main regions of production are the Atherton Tablelands, Rockhampton, Bundaberg Queensland and Cudgen in northern New South Wales (DAF 2014)

Sweetpotatoes are vegetatively propagated in the form of vine cuttings or sprouts (root slips) and are genetic clones of their parent material (Rao & Campilan 2002). The quality of propagation material is a significant contributor to potential yield of a crop as well as agronomic factors such as irrigation, pest and disease management, weed control, nutrients and natural phenomenon such as climate (Atu 2014). Yield reduction in sweetpotato is majorly affected by the passing of pests, diseases and viruses from one generation to the next as it is propagated. There are over 20 known viruses that are found in sweetpotatoes worldwide (Loebenstein et al. 2009). Sweetpotato viruses can cause yield losses from 20-80% (Hahn 1979; Ngeve & Bouwkamp 1991; Kano & Nagata 1999).

There are five that can significantly reduce yield when one or more is present in a plant (Loebenstein 2012). Sweetpotato feathery mottle virus (SPFMV) does not reduce yield however when a plant is also infected with sweetpotato sunken vein virus (SPSVV) it causes an infection called sweetpotato disease virus (SPDV), and yields decline by 50%. An experiment in Brazil by Pozzer et al. (1995) found that sweetpotato meristems free of (SPFMV) and SPSVV yield up to 118% higher in field trials. Sweet potato mild mottle virus (SPMMV) can cause stunting, leaf mottling and loss of yield. Rate of yield reduction can be highly variable between cultivars. Sweetpotato latent virus (SPLV) has symptoms of mild chlorosis and is widespread throughout China. Symptoms are only visible during the early stages of infection. Furthermore, virus infected sweetpotato plants were found to be much more susceptible, than the healthy plants, to fungi *Monilochaetes infuscans* and

Ceratocystis fimbriata, and nematodes *Pratylenchus coffeae* (Yang et al., 1998; Wang et al., 2000)

2.2 Pathogen testing in Australia

Australian sweetpotato production relies heavily on irrigation, nutrient application and the associated infrastructures, making Australian production the most intensively farmed in the world. However, over the last 20 years Australian production has grown substantially by 1700% and currently achieve the world's highest yield at 70.5 t/ha. (Henderson et al. 2014) The increase in yield and subsequent growth is also due to the pathogen testing (PT) programs introduced in Australia in the late 1990s. In the PT scheme, over 95% of sprout production comes from 1st generation PT planting material. However, it should be noted that other countries that use similar PT schemes such as the United States (average yield is 24.5 t/ha) produce over 8 times more tonnage than Australia and demand for clean PT material well and truly outstrips supply (FAOstat 2014.)

The designing of the PT scheme in Australia began in the Redlands and Gatton Horticultural Research Stations by the Department of Agriculture and Fisheries (DAF). Plants that are being tested for virus are first grafted onto *Ipomoea setosa* (when the first two true leaves are produced). For indexing. *Ipomoea setosa* is from the family Convulvacea from which sweetpotato is a member. I. setosa, commonly known as Brazilian morning glory is a native of Brazil, that had been used as a virus indicator for sweetpotato virus. The reason for using *I. setosa* is the ability to express virus symptoms and its lack of the inhibitor in sweetpotato that is responsible for inhibiting virus expression on sweetpotato (Moyer & Salazar 1989). *I. setosa* grafted plants are observed from 2-3 weeks after the graft. Leaves with symptoms are sampled for serology using ELISA and PCR testing for SPFMV, Virus G, Phytoplasma and Begomovirus Sweetpotato feathery mottle virus (SPFMV), Sweetpotato mild mottle virus (SPMMV), Sweetpotato mild speckling virus (SPMSV), Sweetpotato latent virus (SPLV), Sweetpotato caulimo-like virus (SPCaLV), Sweetpotato chlorotic stunt virus (SPCSV), Sweetpotato chlorotic fleck virus (SPCFV), Sweetpotato virus G (SPVG) and Cucumber mosaic virus (CMV).

Confirmation of presence of sweetpotato virus then lead to heat treatment at average temperatures of 39°C. If any of the samples came back positive then the associated germplasm undergoes 4 weeks of heat treatment with average temperatures at 39^{0}C to remove any virus. After heat treatment the tip cuttings are removed and the meristems are placed in tissue culture. Meristems are used because they have no vascular tissue and are therefore unable to harbour any virus. The heat accelerates the growth of the plant at the same time reducing the replication of virus (Dennien et al. 2013). When the number of tissue cultured plantlets have been bulked up enough, they are de-flasked and transplanted in insect screened igloos where they grow to a vine length of 30-45cm and are cut every 2-3weeks depending on growth rate and planted out to produce Pathogen tested virus free storage roots to be used in seedbeds.

Finding or breeding varieties of sweetpotato that are resistant to viruses has had little success, making PT material the highly recommended practice. Production of PT seed for commercial growers is carried out by Australian Sweetpotato Seed based in

Rockhampton. Virus free mother plants are multiplied in controlled conditions then grown out in the field for root production. The roots are harvested and graded so that small, oversize and misshapen roots are not used for seedbeds. The seed roots are then cured to allow for any cuts/abrasions acquired to heal over and therefore reduce possible entry points for disease (Clark et al. 2009b). The roots are occasionally coated with a fungicide (fludioxonil,) from the benzimidazole group before being distributed to growers.

2.3 On farm seedbed production

On farm seedbed production to create sprouts for propagation, is a high input process that requires good soil preparation, chemical treatment, storage root placement and planting, nutrition, pest management, and sprout harvesting. Sprout production require roots to be planted on raised beds approximately 1 m wide, with roots placed on the bed at a rate of 20-30 kg/m^2. Roots should have a small gap and not be touching one another in an attempt to prevent disease dispersion throughout the seedbed. The bed is then covered with a thin layer of soil 2-3cm thick. Too much soil over the top can hinder sprout emergence and root health (Henderson 2015).

Research by Henderson and Dennien (2016) showed that there was no difference in sprout yield per square meter from seedbeds that used irregular roots in comparison to uniform roots. However, oversize roots could be susceptible to early breakdown of root in unfavourable conditions such as waterlogging and high temperatures as well as depending on the cultivar. Smaller roots might not produce vigorous sprouts and can be impractical when covering the bed with 1.5-2cm of soil causing uneven sprout emergence (Clark et al. 2009a).

Beds are lightly irrigated to encourage sprout emergence. Water monitoring is an important aspect of seedbed agronomy as poor watering has a high yield penalty and excessively wet soil is linked to bacterial root rot of sweetpotato (Khan & Doty 2009)

seedbeds are first planted from about July onwards. Early season beds may be warmed using plastic covers, which require aeration to prevent over-heating, CO2 toxicity, or excessive humidity (Henderson 2015; *Growing Your Seedstock* n.d). Plastic is removed once sprouts are growing well as daytime temperature can cause excessive burning of sprout tips. Once sprouts begin to appear, growers may trim the initial flush to promote uniform regrowth and increase overall sprout density. Sprouts are generally harvested when the bulk of the bed comprises sprouts with tips between 35 and 45 cm in length, cut 2-3cm above ground. Australian seedbed cuts can achieve a minimum of 4 cuts and up to 8 cuts of commercial material in a season (Clark et al. 2009b).

Young leaves found at the terminal ends of vine i.e. tips are known to have higher levels of the auxin indole acetic acid (IAA). IAA has a known role in activating adventitious root growth on many plant cuttings and it is found in higher levels in young and newly developed leaves (Salsbury and Ross 1992). Back cuttings (taken approximately 50 cm from the tip) therefore lack this auxin and this has clearly

affected the back cuttings performance in commercial production. The demonstration of these effects was a turning point in the reduction of back cutting use in Australia and showed the importance of producing cuttings that have growing points adding to the importance of having a productive seedbed (Coleman 2006). A threshold for the age of a seedbed in terms of production penalty has not yet been established by the industry. Seedbeds are harvested until they decline in vigour, often due to fungal and viral diseases or when conditions become too cold (Clark et al. 2009a).

Australia is a unique sweetpotato producer, in that it is using an annualised storage root / plant bed system to produce sweetpotatoes for an extended period, in contrast to other temperate areas, where there is a clearly defined planting period of around 2-3 months. Australia's sweetpotato production season start in September through to May (Henderson & Dennien 2016).

2.4 Plant and root breakdown in seedbeds

Sclerotium rolfsii (Sclerotium blight) can cause circles of infection on roots and display white mycelia on the top of the plant bed and on the base of the sprouts. Sclerotium tends to be more prevalent in wet humid conditions when there is decaying leaves from a previous cut left on top of the seedbed (Henderson 2015).

Monilochaetes infuscans – (Scurf) can cause shallow purple-brown blotches, blotches, known as starts, on the periderm of storage roots. Scurf stars on the storage root but spreads upward onto the lower section of sprouts within the first centimetre above ground and if the sprout is cut to low during the seedbed harvest, the fungus is transferred into the propagated field. Infection and spread can be heightened by animal manure incorporation in the soil. (Henderson 2015).

Fusarium oxysporum f. sp. Batatas - (Fusarium wilt), infects the vascular tissue of the sprouts, causing wilting and eventual death. In storage roots, this disease usually associated with vascular discolouration near the proximal end of the roots (Clark et al. 2009a).

Fusarium solani – (stem canker and Fusarium wilt) causes a 'dry' rot in storage roots. This disease can be easily transferred among bedded roots tightly packed that are carrying the infection. This disease can infect emerging sprouts, and cause cankers in the lower portions of the stem (Clark et al. 2009a). It can thus readily spread into commercial fields. Storage roots that are harvested particularly in cold and wet conditions can be infected if they become damaged or scratched as the disease can survive for long periods of time in the soil. Co-infection with *Dickeya dadantii* (Erwinia chrysanthemi) is particularly aggressive, and can decimate a plant bed or commercial crop (Henderson 2015).

Rhizopus stolonifer– (Rhizopus soft rot) is a rapidly progressing soft rot of wounded storage roots. Generally occurs during harvest and storage, prior to bedding, thus diseased roots are generally removed from the PT chain. However, as these are ubiquitous fungi in air and soils, freshly wounded bedding roots in the plant bed may be vulnerable. Crush wounds are more vulnerable than clean cuts (Khan & Doty

2009). As with other diseases, many cultivars grown in Australia, are variably resistant to this organism.

Soil borne pathogens are the predominant cause of storage root breakdown in seedbeds (Clark et al. 2009a). Some pathogens can be the result of a carryover of infection from when the roots were harvested as disease can be exacerbated from physical damage during handling. This poses a major barrier to sweetpotato seedbed yield. Soil-borne disease management is one of the most important agronomic roles in seedbed production.

2.4.1 Seedbed pests

Cylas formicarius (Sweetpotato weevil), Elateridae spp. and Tenebrionidae spp. (wireworms) larvae infest and damage sweetpotato stems, crowns and storage roots (Akers, McCrystal et al. 2014). In plant bed systems, infected roots should never be planted. The current Australian PT system means G1 storage roots should not be infested with any insects (Reddy et al. 2014). Thus management is around preventing infestation in the plant bed itself. The other critical element is management of virus vectors, particularly aphids and whiteflies, which are the key vectors of the sweetpotato viruses currently known in Australia (Dennien, Homare et al. 2013; Loebenstein, Thottappilly et al. 2009)

2.4.4Gas exchange and degradation

The raised levels of carbon dioxide (CO_2+ and lower oxygen (O_2) can cause the growth of lignified roots during development (Eguchi & Yoshida 2007). In early spring when seedbeds are first planted temperatures might not be warm enough to reach optimal growth rates. Therefore, it's common practice for growers to cover seedbed with plastic to increase soil temperatures and speed up growth(Edmunds et al. 2008). However care needs to be taken to allow the seedbed to 'breathe' by uncovering the seedbed from the plastic for a few hours a day to release any build up of CO_2, otherwise it can cause premature breakdown in storage roots (Dennien et al. 2013).

2.4.2 Sweetpotato varieties

Beauregard is a high-quality orange fleshed sweetpotato cultivar and has a smooth pink skin that is easy to peel. It can be consistently high yield over a variety of soils and up until 2016, was the most commonly grown cultivar throughout Australia. Beauregard is a medium maturing plant usually harvested after 20 weeks (Arnold 2016). Beauregard was first developed in the 1980s in Louisiana in the U.S and was first grown in Australia in the late 1990s. The roots of Beauregard can have some irregularity when filling out, in heavy soils roots may struggle to become completely round and lighter soils tent to cause roots to become long and irregularly shaped when planted in dry conditions. Beauregard has strong resistance to Streptomyces soil rot and is highly susceptible to root hnot nematode. According to the cultivar release notes, it is resistant to Fusarium wilt, and Rhizopus soft rot; moderately

resistant to Soil rot and Sclerotial blight. It is however very susceptible to Root knot nematode and Bacterial root rot. (DAF 2014; Rodney Wolfenden 2014)

Bellevue sweetpotato cultivar is an orange fleshed, smooth copper skinned cultivar that has superior shape and disease resistance than that of Beauregard. Bellevue storage roots are elliptical and uniform in shape which has low rates of lobbing (HARLER 2016). The yields of Belleview has yields that are equal to or greater than Beauregard and has a superior resistance of root knot nematode, which Beauregard is prone to. Belleview sprout production from seedbeds can be weak unless they are pre-sprouted and can be 20-30% lower in sprout yield than that of Beauregard. The lower performance of seedbed production can be due to rate of bacterial rot infection (La Bonte et al. 2015). “Yet other varieties that perform well, like Bellevue, are not accepted or understood by consumers, which occasionally makes them difficult to sell. Consumers just don’t really know what to do with them,” (Pritchard 2016).

2.5 Sweetpotato responses to nitrogen

Sweetpotatoes can be grown on marginal land with little N inputs (Nshimiyimana et al. 2013) However, a baseline amount of N is required for photosynthetic activity, shoot development and storage root growth. This amount can vary from different soil types and years as soil N can vary (Harper et al. 2005).

When growing a commercial crop of sweetpotatoes a high amount of available N does not necessarily reflect in a yield response in root growth. The Department of Agriculture and Fisheries recommend that optimal yields are achieved with 100kg/ha of N and that rates higher than this can cause reduced root weight (DAF 2014). Excess N application can trigger copious production of vine and leaves at the expense of starch accumulation in storage roots (Osaki et al. 1995). Sweetpotato response to N is also a varietal factor. Jones and John (1992) found that cultivars that had been developed in soils of low fertility where soil amendments are not normally used, responded negatively to an N application of 60kg/ha however cultivars that had been developed in the United States showed an increase in yield when more N fertiliser was applied.

Research by Villordon et al. (2009) found that N based fertilisers are best applied in split doses during critical points of the growth stages. The trial had 40% of the plants total N applied as a basal unit followed by 2 side dressing applications of the remaining 60% at 5 and 8 weeks after transplanting. The number of roots produced/ha is unaffected with N application, however there is a higher yield of marketable roots/ha.

Storage root initiation is when there is when secondary meristematic activity begins in adventitious roots. Which heralds the initial development of storage root formation, which is a defining moment for potential yield. High levels of N are not required in storage root initiation, which can occur as early as 13 days after transplant (DAT) (Villordon et al. 2012).

Appropriate fertilisation of a seedbed will increase the vigour and size of sprouts produced as well as the number of sprouts produced per root. (Clark et al. 2009b; Dennien et al. 2013; Henderson 2015; *Growing Your Seedstock* n.d). Seedbed nutrition is a pioneering development requiring a complete fertiliser for optimal growth.

2.5.1 Nutrient sampling.

Normally leaf samples are collected for analysis, however as leaves they accumulate nutrients. Therefore, it is important to sample at the same physiological stage of growth to ensure analysis is consistent between samples. Many crops use the first fully unfolded leaf to be sampled, however once leaves are unfolded in sweetpotato they continue to expand. The physiological maturity cannot be justified on a fully unfolded leaf. There is no specific method for leaf sampling in sweetpotatoes so there is variation in different sources of literature (O'Sullivan et al. 1997). There are numerous methods that take different parts or samples of the leaf for analysis making it hard to compare.

The concentration of nitrate-N in the petiole is often used to assess the N status of crops. Lorenz (1965) quoted nitrate-N concentrations in the petiole of the sixth leaf of sweet potato in mid-season, as 1500, 2500 and 3500 mg/kg for deficient, intermediate and sufficient N status, respectively. However, Walker and Woodson (1987) found that, while petiole nitrate-N concentration was sensitive to N supply, it was highly variable among cultivars and with age of sampling, and was a poor predictor of root yield.

A large body of data indicates that the N distribution between the leaves of a canopy is not uniform (Grindlay, 1997). Individual leaves in a canopy experience different light environments due to shading by upper leaves; they also differ in age. In addition, different leaves in the canopy may develop under different conditions of N supply because of fluctuations in soil N supply during crop growth whilst leaf production remains continuous. All these aspects will potentially lead to the observed non-uniform N distribution (Gastal & Lemaire 2002)

2.6 Plant uptake nitrate vs ammonium

N uptake in plants is predominantly in the form of nitrate (NO_{3-}) with some uptake of in the form of ammonia ($NH4_{+}$). Ammonium is the principle form of N found in manure or compost based fertilisers. When applied to the soil microbial communities break down the ammonium as it undergoes mineralisation to become nitrate (Kaupa & Rao 2014).

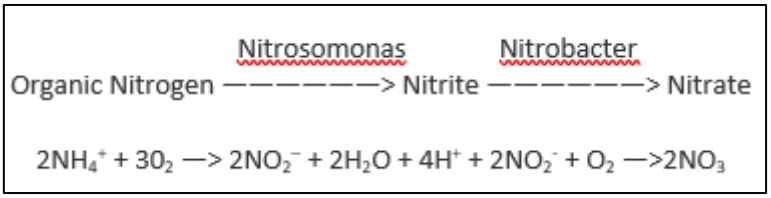

Figure 1 The nitrification process, adapted from (Summit Fertilisers nitogen n.d)

The uptake of nitrate is high across a range of soils but is favoured in soil with a low pH. According to Tisdale et al. (1993, Soil Fertility and Fertilizers), the rate of NO3- uptake is usually high and is favoured by low-pH conditions. NH4+ uptake proceeds best at neutral pH values and is depressed by increasing acidity.

Marchner (1995, Mineral Nutrition of Higher Plants), states that higher growth rates are achieved with mixed supply of both form of N. When both forms of N are

supplied, it is easier for the plant to regulate intracellular pH and to store some of the N at low energy costs.

The assimilation of NH4+ is more energetically efficient when compared with NO3-, because NH4+ can be directly incorporated into glutamate via an NH4+ assimilation pathway. Nitrate, on the other hand, must first be modified via a reduction pathway before assimilation (Fig 1). However, NO3- is usually more available for uptake in many ecosystems, owing to its higher mobility. Nitrate can be incorporated into organic compounds in both root and leaf tissues whereas NH4+ is only synthesized into amino acids in the root tissues near the site of uptake to avoid toxic accumulation. Either NH4+ or NO3- can dominate the inorganic N pool of an ecosystem (USDA nd). For example, in most mature undisturbed forests, the soil inorganic N pools are dominated by NH4+. In well aerated agricultural soils or other frequently disturbed sites, NO3- is the principal inorganic N source (*Summit Fertilisers nitogen* n.d).

The use of high input of N can decrease the amount of root growth but increase vegetate growth. In seedbed production this can be advantageous for sprout development as sprouts can reach their desired length quicker. The less time that it takes for each consecutive cut can help increase the longevity of a seedbed allowing growers to get more cuts per season. However there is little information as to whether an increased amount of N on a seedbed could potentially increase the breakdown of roots from soil borne pathogens bacteria, fungi, worms, sweetpotato weevil, wireworm and viruses Harper et al. (2005).

3.0 Materials and methods

3.1 Planting site and preparation

The experiment was conducted at the Department of Agriculture and Fisheries (DAF) Gatton Research Facility (map coordinates -27.543259, 152.327093). The soil type at the field sight classified as a Hooper black earth, a weakly self-mulching, cracking dark clay (Powell 1982). Initial soil tests of the planting area had nitrate levels at 20 ppm and electrical conductivity of 0.17dS/m. Gypsum was applied at 1kg/m^2 as standard practice. The gypsum was incorporated using a rotary hoe whilst forming the beds (Fig 2).

Figure 2 Preparation of trial site

3.2 Planting material

Storage roots from two gold cultivars (Beauregard and Belleview *Ipomoea batatas*) were sourced from the seed supplier 'Australian Sweetpotato Seed' as per grower practice. The storage roots were delivered in bulk bins, had been treated with fungicide and cured. (Fig.3).

Figure 3 A sample of storage roots used in seedbeds

3.3 Planting

30kg of roots were selected at random from each bin, making sure no damaged roots are included. Roots placed in the 1m^2 datum section. Buffer zones were then filled with any leftover roots. The beds were then covered with 2-3cm of topsoil and plot areas were remarked. The nitrogen fertiliser treatments (Table 7) was then applied to their designated plot (Fig 4) via hand broadcasting which was then followed by an initial overhead irrigation of 10mm to help soil root contact.

Figure 4 Seedbed ready for soil covering

Table 1 Summary of the 3 different fertilisers used in the trial (Multi-K potassium nitrate fertilizer 2014; Nitrophoska Special n.d; Organic Xtra n.d)

Type of fertiliser	Multi K® Potassium nitrate	Nitrophoska Special ®	Organic Xtra®®
Overview	Readily available for plant uptake Prilled form	Ammonium nitrate based source of N Prilled from 5% nitrate 7% ammonium Nitrate form immediately available for plant uptake Ammonium fraction is taken up by roots or gradually converted to nitrate by soil microorganisms.	Poultry manure based fertiliser Powdered form Almost exclusively ammonia based and require microbial activity to break down into nitrate
NPK Ratio	13%N 0%P 46%K	12%N 5.2%P 14%K	4%N 1.5%P 3%K
Nitrogen source	Nitrate (NO_3^-)	Ammonium nitrate (NH_4NO_3)	Ammonium (NH_4^+).

- Both the low organic and potassium nitrate have the same 6 units of N,
- The same principle goes for treatments 3 and 5 –Nitrophoska special and medium organic.
- We did not use a higher rate of inorganic fertiliser to match the highest organic treatment as it was thought that there would be a fertiliser burn damage

3.4 Sprout establishment

Careful management of irrigation to avoid excessive soil moisture where possible was vital in the first 4 weeks of sprout establishment. A thin layer of soil crusting can hinder the initial emergence (Fig. 5), so the crust that was formed after heavy rainfall was broken using a rake, taking care to not damage any pre-sprouted roots.

Figure 5 crusting effecting initial emergence

3.5 In-crop agronomic practices

Boron levels were low for sweetpotato requirements, so a boron based fertiliser Solubor® (25% boron) was applied in-crop.

Apart from the experimental treatments, the plant bed area was kept free of insect pests by regular additions of insecticides, as per common grower practice. It was particularly important to keep out sweetpotato virus vectors, including aphids, whiteflies and jassids.

3.6 Sprout cutting

The first cut of the seedbed occurred 5 weeks after planting, the cutting of the seedbed was undertaken in early morning when conditions were cool and moist to avoid the sprouts wilting in hot conditions. A sharp knife or snips were used to cut the sprouts at 4cm above ground level. Consecutive cuts were made just above the initial slit with every cut in the following months. Harvested sprouts were placed in crates (two crates per plot) and transported to the cold room where they were kept at 14^{o}C before grading and data collection. Straight after cutting fertiliser treatments were applied accordingly via hand broadcasting. Time between sprout cuts depended on temperature as growth only occurs above 15.6^{o}C soil temperature (Henderson & Dennien 2016). Ideal cutting spacing was every 4 weeks for sprouts to be of a length that would be cut in industry. Sometimes (as in commercial practice) cuts were brought forward or delayed by weather events or labour availability. The 7th cut was delayed by an additional 4 weeks as temperatures below 15 ^{o}C reduced in April and May slowed down regrowth (Fig 7.).

3.7 Experimental measurements

A day before the seedbed was cut, 40 leaf samples (first fully mature leaf) were taken at random from each plot. The samples are dried at 60-70 C° for a minimum of 12 hours or until dry. Dried leaf samples were then sent to Nutrient Advantage (Victoria) for the analysis of total nutrients, nitrate, ammonia and total N for each treatment.

Sprouts will be assessed based on their length, diameter and the number of nodes in the first 20cm from the cut end of the sprout

The harvested sprouts from each plot is weighted and processed according to the following categories

- Optimal (28cm to 40cm)
- Short acceptable (20cm to 28cm)
- Undersized sprouts (<20cm)
- Damaged or diseased
- Back cuttings Any sprouts that are <40cm are cut back to 40cm with the cut portion deemed a back cutting

A count was taken of the number of sprouts in the optimal and short acceptable categories, whilst only a weight was recorded for the other categories. The number of nodes in the first 20cm (from the cut end of the sprout) will be graded from 2<, 3, 4 to >5 nodes in 20cm (Fig 6.). Because of time constraints, only the results for the numbers and biomass of acceptable sprouts, total biomass production and N contents of sprouts are discussed in this thesis.

Figure 6 Optimal sprout cutting 20-28cm in length

Figure 7 Seedbed cut

3.8 Experimental design

Each seedbed was divided into 12 plots, each plot is 2m x 1m, with 0.5m of buffer from each end and 1m^2 datum.

The experiment was a randomized split block design with 24 plots at 2m spacing allowing for 1m^2 of buffer area and 1m^2 of datum. The 2 cultivars Beauregard and Bellevue were separately planted on 2 blocks 1.2x24m, each block had 6 treatments and 2 replicates. Each replication plot was randomised. The 6 treatments were a series of organic and inorganic nitrogen fertilisers that are currently being used in the sweetpotato industry (table 2.1). There was a control (no fertiliser), Nitrophoska special and 3 different rates of an organic chicken manure based fertiliser ‘Organic Extra’.

3.9 Statistical analysis.

Statistical analysis was completed using Minitab and SAS statistical packages. A one-way ANOVA was used separately on each cultivar. With 6 treatments and only 2 replications, there were just 6 degrees of freedom for error. This meant with conventional analyses, the power of the experiment was low, with little capacity to detect small treatment differences. Other more complex regression and time series analyses may be able to provide additional clarity; however, these are beyond the scope and timeframe of this thesis. This initial experiment was undertaken to explore gross effects of N nutrition on sweetpotato sprout productivity, and scope what effects are possible, which would then be followed by more detailed and expensive investigations if warranted.

Dunnets multiple comparisons with a control was used to compare the treatments with the control.

4.0 Results

4.1 Sprout production

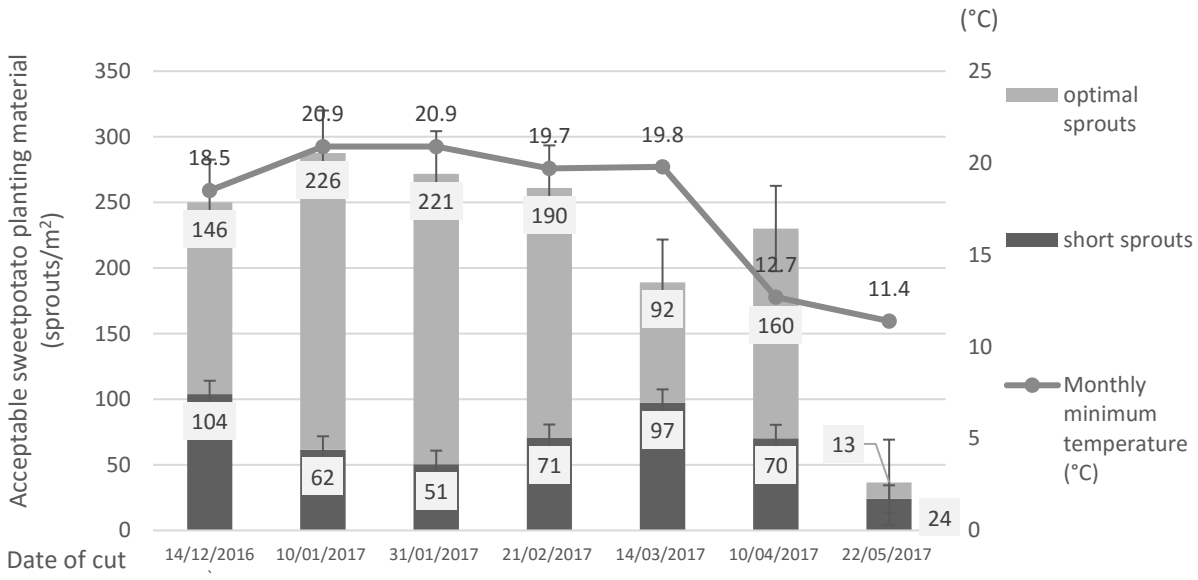

Figure 8 Average production of Beauregard sprouts per cut.

In Fig.8, production of acceptable Beauregard sprouts peaked at Cut 2, and then slowly declined in successive cuts. There was a major, significant reduction in acceptable sprouts at Cut 5, and particularly by Cut 7. Once the weather started to cool, the time between cuts was also extended. The most productive cuts also had the highest proportions of Optimal sprouts.

There was significant difference in sprout production in all cuts for Short and Optimal sprouts ($P<0.0001$)

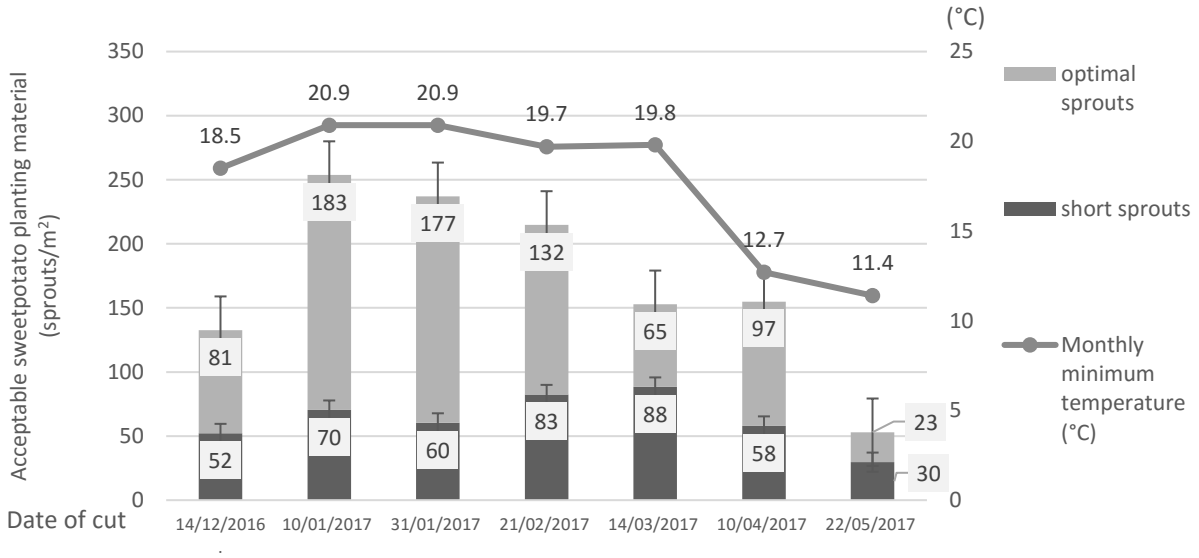

Figure 9 Average production of Bellevue sprouts per cut

Production of acceptable Bellevue sprouts peaked at Cut 2 before gradually declining after each successive cut. As to with Beauregard, Bellevue's most productive cut had the highest proportion of sprouts. Bellevue cut 5 had a significant reduction of optimal sprouts at cut 5 before increasing in cut 6. There was significant difference in sprout production in all cuts for Short and optimal sprouts ($P<0.0001$)

Although not statistically significant (because of the layout) Beauregard always produced more optimal and acceptable (Short + Optimal) sprouts at every cut except cut 7. Overall, Bellevue produced 28% fewer optimal sprouts and 8% fewer short sprouts than Beauregard.

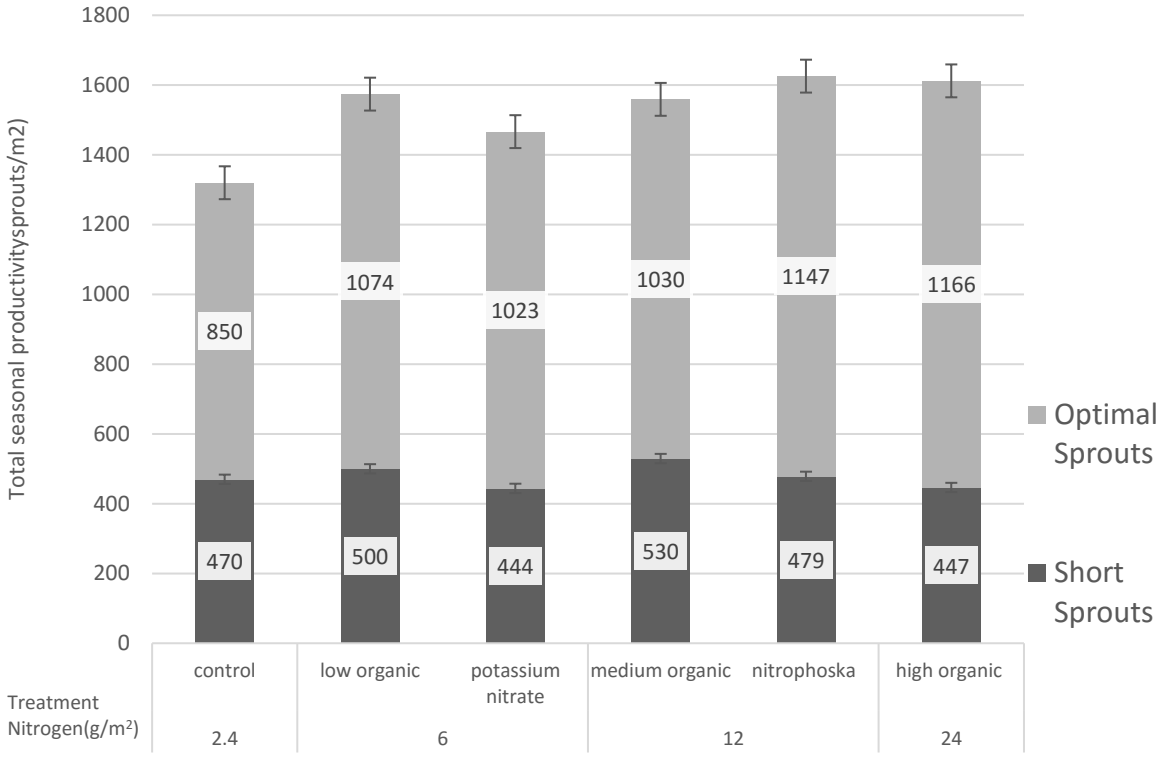

Figure 10 Total seasonal production of Beauregard sprouts

The six nitrogen treatments have been arranged showing increasing additional N applications at each cut from left to right. Note that the Control treatment, with no additional N application, started with an estimated 2.4 g/m^2 of total N in the upper 10 cm of the seedbed profile, based on initial soil analysis.

There were no significant differences between treatments for numbers of Short sprouts ($p<0.631$) or numbers of Optimal sprouts ($p<0.113$). However, the organic fertiliser treatments (4, 5 and 6) and the Nitrophoska Special® produced significantly more total acceptable sprouts (Optimal + Short) than the Control ($P<0.016$). Interestingly, Treatment 2, where only potassium nitrate was applied, was not significantly better than the unfertilised treatment.

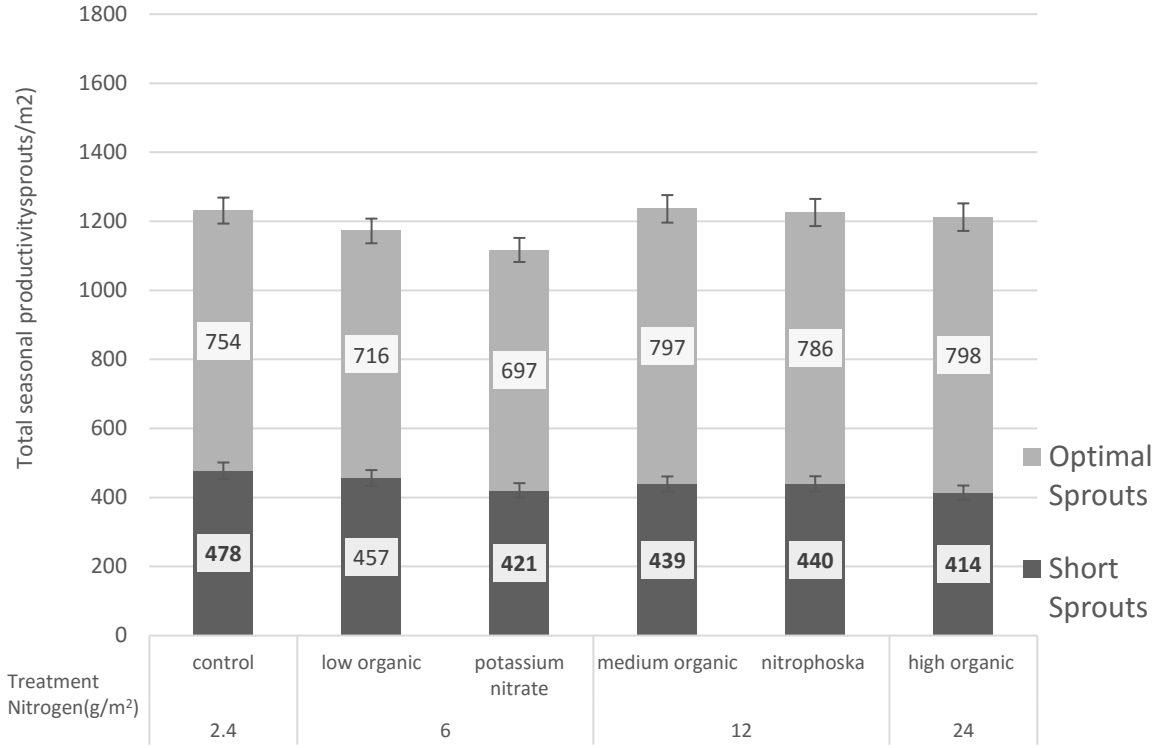

Figure 11 Total seasonal production of Bellevue sprouts

There were no significant differences between treatments for numbers of Short sprouts ($p<0.839$) or numbers of Optimal sprouts ($p<0.113$). The organic fertiliser treatments (4, 5 and 6), the potassium nitrate and Nitrophoska Special® did not produce significantly different total acceptable sprouts (Optimal + Short) than the Control ($P<0.821$).

5.2 Biomass production

Table 2. Average sprout weights of Beauregard and Bellevue (g)

Treatment	Beauregard		Bellevue	
	Short	Optimal	Short	Optimal
control	10	15.9	10.2	15.6
low organic	9.2	15.1	9.9	16.3
potassium nitrate	9.7	15.8	9.7	15.1
medium organic	10.1	15.3	9.9	15.2
Nitrophoska Special	8.9	16.1	10	16.1
high organic	9.7	15.4	10.2	16.3

In Table 2. There was no significance between treatments of cultivars, for both short and optimal sprouts

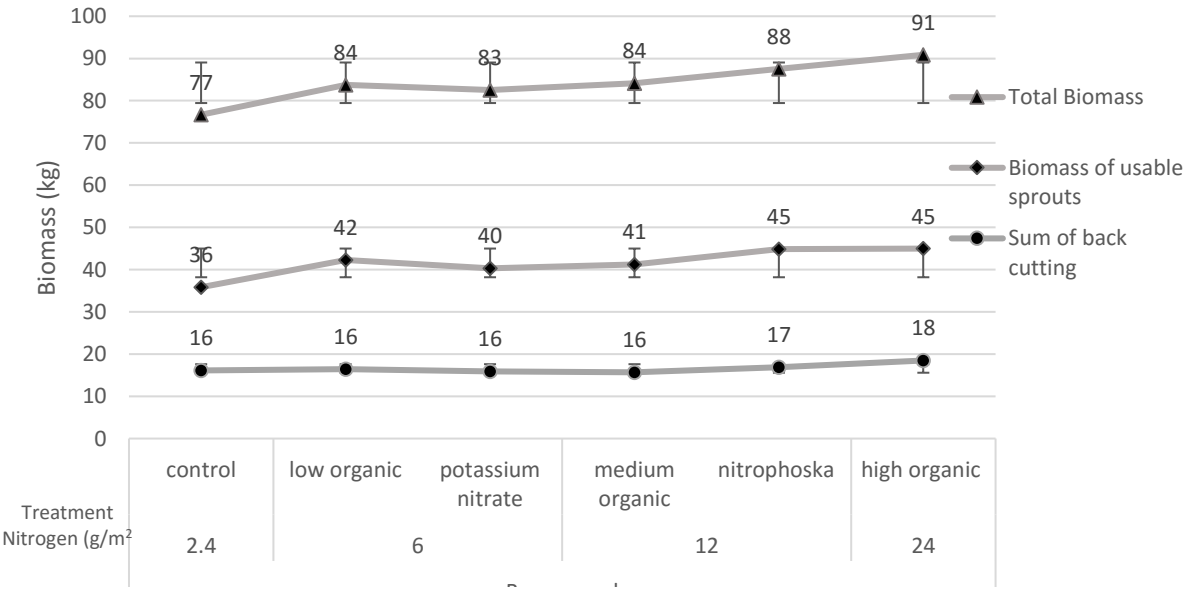

Figure 12 Beauregard biomass per treatment

The total seasonal production of Beauregard biomass for each treatment displayed no significance (P=0.096) between treatments, as did the sum of back cuttings (P=0.834). However, the biomass of usable sprouts showed significance (P=0.031) in the highest organic N treatment and the Nitrophoska Special® treatment.

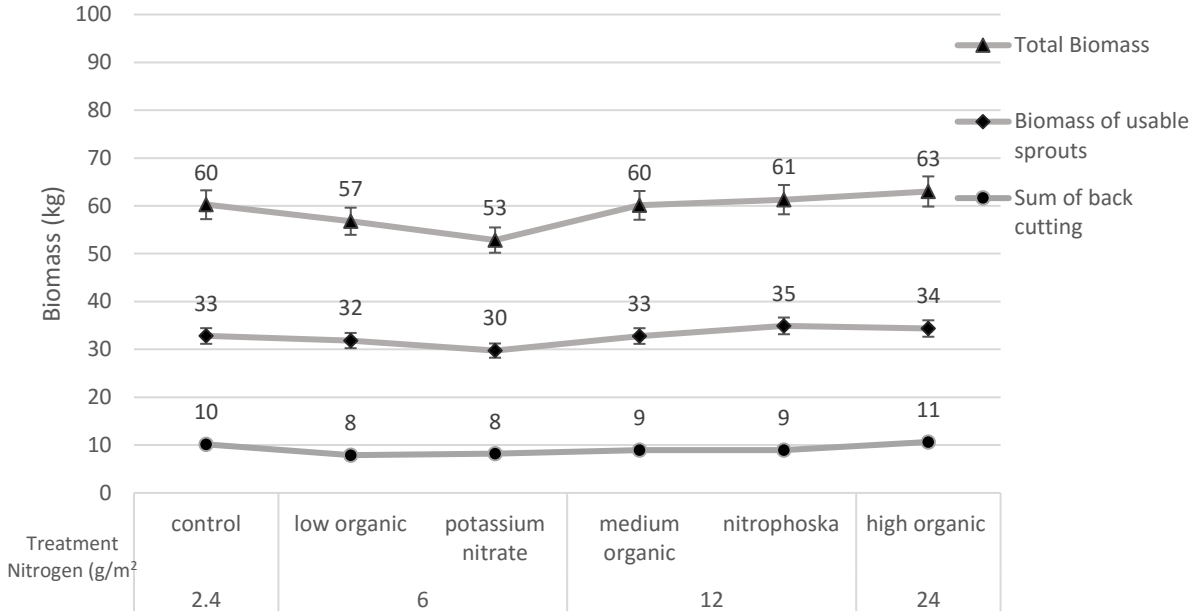

Figure 13 Bellevue biomass per treatment

As with the Beauregard, Bellevue's most productive treatments were the Nitrophoska Special® and high organic. However, no significance was found in any of the variables total biomass, usable sprout biomass and back cuttings (P=0.393, P=0.542 and P=0.266).

5.3 Leaf samples

Leaf samples from each treatment have had their replicates combined, this was due to larger size of sample required for processing the treatment, and because of the cost of the treatment.

Table 3 Nitrate samples in Beauregard and Bellevue

Nitrate concentration (mg/kg)		
Treatment	**Beauregard**	**Bellevue**
control	*756*	*924*
low organic	*597*	*1037*
potassium nitrate	*634*	*1643*
medium organic	*421*	*1299*
Nitrophoska Special	*1137*	*1834*
high organic	*724*	*1864*

Table 3 shows that nitrate samples in Bellevue were much higher compared to Beauregard

Table 4 Total nitrogen content in leaf samples

Total nitrogen in leaf samples (%)		
Treatment	**Beauregard**	**Bellevue**
control	*4.91*	*4.86*
low organic	*5.09*	*5.27*
potassium nitrate	*4.74*	*5.03*
medium organic	*4.86*	*4.81*
Nitrophoska Special	*4.90*	*4.90*
high organic	*4.97*	*5.09*

Table 4 is a summary of the total nitrogen content from leaf samples in each treatment. There was no significance.

5.4 Sclerotium rolfsii

In cuts 4 and 5 sclerotium rolfsii was present on in both cultivars. The 2 replicates of the high organic in the Beauregard cultivar had blight present during both cuts. Bellevue had sclerotium rolfsii recorded in a potassium nitrate treatment at cut 4 and a high organic treatment plot during cut 5.

Figure 14 displayed the white mycelia symptoms of Sclerotium rolfsii

Table 5Summary of plots with symptoms of S. rolfsii

Cultivar	Plot and treatment	Date and Cut	
Beauregard		Cut 4. 21/02/2017	Cut 5. 14/03/2017
	1 control	no	no
	2 normal organic	no	no
	3 potassium nitrate	no	no
	4 control	no	no
	5 Nitrophoska ®	no	no
	6 low organic	no	no
	7 normal organic	no	no
	8 Nitrophoska ®	no	no
	9 high organic	yes	Yes
	10 low organic	No	no
	11 high organic	yes	yes
	12 potassium nitrate	no	no
Bellevue	13 low organic	no	no
	14 control	no	no
	15 potassium nitrate	no	no
	16 normal organic	no	yes
	17 high organic	no	no
	18 normal organic	no	no
	19 Nitrophoska ®	no	no
	20 potassium nitrate	yes	no
	22 low organic	no	no
	23 high organic	no	yes
	24 control	no	no

Table 5 shows plots displaying white mycelium visual symptoms of sclerotium rolfsii. Only cut 4 and 5 are included as they were the only cuts where symptoms had been seen.

5.0 Discussion

5.1 Temperature and cutting influences on acceptable sprout production

It took 42 days for the initial cut to be of a cutting length (Table 8, Fig 8). This on average was an additional 20 days of growth compared to the intervals for cuts 2-6. While initial temperatures after the November planting were lower than peak production, they were still in growing temperature range (Loretan et al. 1994). Cut 1

required the additional days of growth due to the preliminary period of sprout imitation and emergence.

The standard time for seedbed planting can be as early as July, generally to provide sprouts as early as possible in spring. However there may be an additional benefit from avoiding high daily maximum temperatures, which can adversely affect initial sprout emergence (Loader et al. 1999). This trial was planted later than what other growers would be doing and so faced some harsher conditions at first growth, with temperatures in November slightly above average, as well as erratic rain events that caused soil crusting. This was only a problem for cut 1 as all other cuts 2-7 were re-sprouting only a few mm above the previous cut section of the sprout.

The study showed that temperature was a key regulator of sprout yield and seedbed productivity. particularly the reduced temperatures for Cuts 6 and &. This reduced growth is probably associated with reduced rates of CO_2 assimilation. Cen and Sage (2005) found that sweetpotato CO_2 assimilation was dramatically reduced between temperature ranges 10°C and 15°C, and 35°C and 40°C. Cut 6 and Cut 7 had minimum temperatures that were down to 12.7 ^oC and 10.7 ^oC.

Cut 2 and Cut 3 had the highest minimum and maximum temperatures and highest sprout number and biomass. Not only is CO_2 assimilation affected by temperature but so too is the source and sync ratios of the plant. A study of dry matter accumulation at different temperature by Eguchi et al. (1994) concluded that root dry matter accumulation was highest at 24 ^oC, however the dry matter accumulation in leaf and petiole biomass was increased at higher temperatures of 30 and 32^oC, with storage root accumulation reduced. It is possible that the high temperatures for Cuts 2 and 3 drove more photosynthate into sprout production, while later cuts partitioned some photosynthate into new roots below the sprout and even back into the originating bedding roots. Cut 5 had more short acceptable sprouts than optimal sprouts in both cultivars (Fig 8, Fig 9). This indicates that the cutting time should have been delayed for an additional week to so that most sprouts were of optimal size. However, there were constraints in finding available labor for the following week.

Cut 7 had a total 43 days of growth at the lower temperature and recorded the lowest number of sprouts for both cultivars. Sweetpotato growers in subtropical regions would normally cease seedbed production in April as sprouts planted would face increased risks from frost (Loader et al. 1999). This study aimed to continue cuts past this time so that any symptoms of breakdown could be observed as the seedbeds continued to age. More cuts could have been viable if the seedbed was planted in mid September 2016, however logistically the resources were not available in that time frame.

5.2 Cultivar is a major influence on sprout production

Both cultivars followed the same pattern of peak production in Cuts 2-3, and falling away after that. Sprout emergence for Bellevue was slow compared to Beauregard

and continued to lag at every cut (Fig 8, Fig 9). The maximum number of acceptable sprouts produced by the Bellevue storage roots was lower than that of Beauregard, a feature others have also observed as a physiological characteristic of Bellevue (Thompson et al. 2010).

5.3 High initial soil fertility reduces likelihood of major N responses

The relatively high fertility of the experimental site could have reduced the response to N fertiliser. The soil was a Hooper black earth, a weakly self-mulching, cracking dark clay, with available nitrate in the top 10cm at 33ppm. In commercial growing trials (not seedbeds) most cultivars can grow well on soils with only 20 ppm nitrate levels for commercial root production (Rodney Wolfenden 2014). It should be noted that the 20 ppm nitrate level is used to maximise growth of storage roots; the appropriate levels for seedbed production to maximise sprout numbers is still uncertain. Also Rodney Wolfenden (2014) conducted the bulk of these growing trials in traditional sweetpotato growing area of Bundaberg. These soils have a much lower cation exchange capacity and nutrient holding ability. This further complicates comparisons with the black earth soils of Gatton.

5.4 Additional fertiliser response in Beauregard

The application of Nitrophoska Special® and the three Organic Xtra® fertilisers in Beauregard increased the number of sprouts produced (Fig 10). The potassium nitrate treatment did not show a significant increase in sprouts produced compared to the control. Current literature suggests that a complete fertiliser should be applied to seedbeds for optimal growth (Loader et al. 1999; Smith et al. 2009; Lebot 2010; Henderson 2015). Potentially one of the other nutrients was also marginally limiting production, apart from N and potassium.

5.5 Additional fertiliser response in Bellevue

The application of N fertiliser to the Bellevue seedbed had no significant effect on either the numbers or acceptable sprouts harvested, or total biomass production (Fig 13, Fig 11). Perhaps Bellevue was unable to utilise any extra nitrogen applied, as there was already adequate levels in the soil to achieve maximum production. In addition, because Bellevue’s peak production of sprouts was lower than beauregard, so too perhaps was the nitrogen requirement to achieve peak physiological production (La Bonte et al. 2015). Conducting a nitrogen rate trial in a soil with only trace amounts of N may better help determine deficiency point, compared to the fertile soil used in this experiment.

5.6 Leaf samples

Although there was a trend in for slightly higher nitrate levels in the treatments with larger N application, the nitrate levels were highly variable. There were consistently higher nitrate levels in Bellevue (compared to Beauregard) (Table 3.) however these differences could not be statistically evaluated. There could be a better mechanisms for nitrate uptake in Bellevue than Beauregard, and potentially this could explain the lack of N response in Bellevue sprout production, as discussed previously.

Moreover, there were no significant differences in total N concentration in the indicator leaves in sprouts, suggesting neither major deficiency or luxury consumption of N (Table 4.). Nitrate levels are generally a short-term measure of the availability of N, whilst total N is an indication of medium term, cumulative growing conditions (Walker & Woodson 1987; O'Sullivan et al. 1997).

5.7 Bedding root breakdown

In industry there had been some concerns about using high rates of fertiliser also increasing the breakdown of roots in the seedbed. While no formal statistical measurements had been analysed in the trial for breakdown (Table 5), weekly photographic observations were taken of each plot. One replicate in the potassium nitrate treatment in Bellevue showed signs of breakdown just 2 weeks after planting and was consistently the lowest optimal sprout yielding plot (Fig. 16 Fig. 18). However, there was no evidence throughout the experiment of any consistent treatment effects. Bellevue consistently deteriorated more after each cut than Beauregard. By cut 7 it appeared that Bellevue was at an advanced stage of breakdown in the seedbed, however there were no obvious symptoms delineating casual bacterial or fungal infections such as those described by (Edmunds et al. 2008; Clark et al. 2009a).

Further research into the contributions of aeration of seedbeds and respiration/gas exchange of Bellevue storage roots could be useful. Less soil coverage could be required to allow for more aeration, but could also cause more sun or heat damage on roots that aren't covered deep enough. A theory that can be put into though here is that Bellevue skin is much harder than Beauregard. When sweetpotatoes are being harvested from a commercial crop the top biomass must be cut off from the roots at ground level, so the storage roots lose enough moisture for the cortex to dry and harden therefore reducing the amount of damage that could be sustained during harvest, washing and packing. Beauregard requires 10-20 days for the skin to harden whereas Bellevue is able to be harvested and handled with little to no damage 2-3days of top chopping, suggesting an inherently harder (and potentially less permeable skin). Coleman (pers. Comm.) has also indicated much higher CO_2 generation in storage rooms packed with Bellevue storage roots, compared to Beauregard storage roots, indicative of greater respiration rates, which may also contribute to premature breakdown.

6.0 Conclusion

Conducting a trial with more replicates to achieve results that will be statistically relevant will help to confirm that type of N does not affect sprout production.

For experimental purposes, reducing the amount of available soil nitrate by planting crops to remove nutrients from the soil could assist with determining adequacy levels for sprout production in seedbeds. Because seedbeds are such a small area of the farm, the actual cost of fertilises to generate planting material is relatively trivial. The experiment confirmed there is no apparent production penalty form high rates of fertiliser addition. The number of sprouts produced is the data that is relevant to the growers. It does not appear likely that seedbed productivity can be adversely affected by even high rates of organic fertiliser in terms of creating conditions of excessive fungal diseases. The current DAF recommendations of 100g/m^2 of a complete fertiliser (5:6:5) seem adequate for Beauregard seedbeds. There is potential in conducting research on how Bellevue seedbeds can maintain productivity for longer without breaking down. The impacts of soil aeration, irrigation and soil temperature effects (e.g. using plastic row covers) on storage roots O_2 and CO_2 gas exchange could be useful.

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8.0 Appendix

Table 6 Summary of treatments

Treatment number	Treatment	Type of fertiliser	Fertiliser rate (g/m^2)	Nitrogen ($g/^{m2}$)
1	Control (no fertiliser)	Nil	Nil	20 ppm nitrate N= 2.4g in soil
2	Best practice	Potassium nitrate	15	6.0
3	High inorganic	Nitrophosca	100	12.0
4	Low organic	Organic Xtra	150	6.0
5	Medium organic	Organic Xtra	300	12.0
6	High organic	Organic Xtra	600	24.0

Table 7 Summary of the Cut dates and monthly average temperature

Cut and Date	Days between cuts	Monthly minimum and maximum temperature for each cut
Planting 2/11/16		15.9-33.1ºC
Cut 1 14/12/16	43 days	18.5-33.8 ºC
Cut 2 10/01/17	27 days	20.9 -37.7ºC
Cut 3 31-01-17	21 days	20.9-37.7ºC
Cut 4 21/02/17	21 days	19.7-35.1ºC
Cut 5 14/03/17	21 days	19.8-30.7ºC
Cut 6 10/04/17	27 days	12.7-25.8ºC
Cut 7 22/05/17	42 days	11.4-24.3ºC

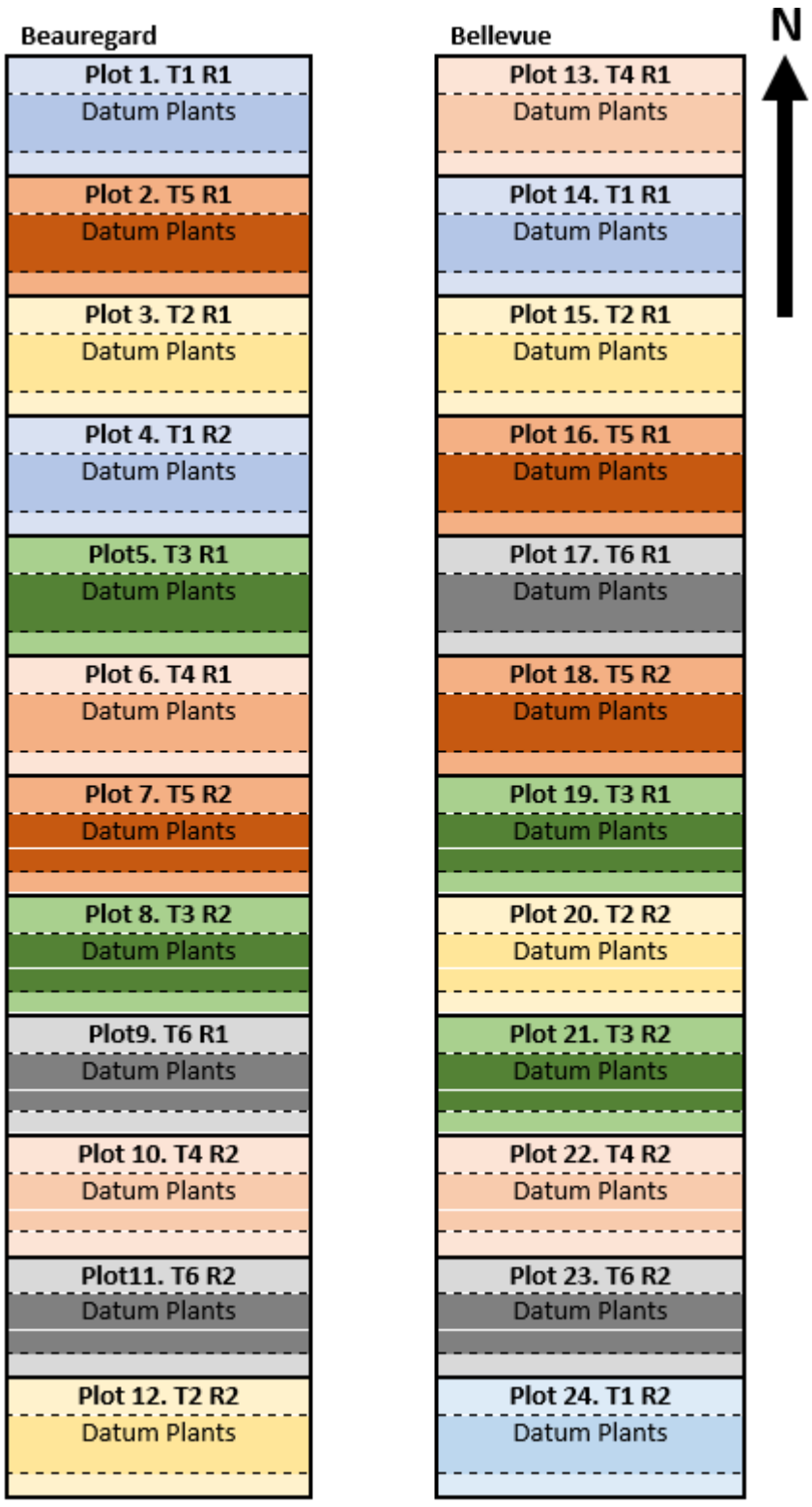

Figure 15 Map of trial site

Figure 16. Potassium nitrate treatment in Bellevue with premature degradation

Figure 17. Close up of breakdown