Journal of Horticulture and Postharvest Research

University of Birjand

Stabilising urea nitrogen enhances flowering, nitrogen use efficiency, and growth habit for stress tolerance in ornamental plants

Sally Wilkinson[1*], Anna Kate Weston[2] and David James Marks[3]

1, 2, 3 Levity Crop Science Ltd., The Rural Business Centre, Myerscough College, Bilsborrow, PR3 0RY, UK

A R T I C L E   I N F O

*Corresponding author:
Levity Crop Science Ltd., The Rural Business Centre, Myerscough College, Bilsborrow, PR3 0RY, UK.
 E-mail: sally@levitycropscience.com

A B S T R A C T

Purpose: Supplying plants with nitrogen in ammonium nitrate- or urea-based fertiliser is wasteful: much is degraded before acquisition, releasing environmental pollutants. Preventing urea degradation can reduce pollution and improve crop nitrogen use efficiency. We investigate benefits to ureic stabilisation, on flowering and stress tolerance, as organic nitrogen sources favourably alter biomass partitioning in this regard. **Research Method**: We test effects of adding chemically stabilised urea to soil, on the physical form and flowering of containerised, greenhouse-grown pelargonium, petunia, pansy and marigold, when transplanting seedlings to larger pots. Efficacies of stabilised urea, non-stabilised urea and industry standard fertiliser are compared under identical total nitrogen supply. The significance of treatment differences is calculated using a one-tailed *t*-test. **Findings**: Development is favourably altered by ureic stabilisation. Earliest changes measured are increased root lengths, leaf growth rates and chlorophyll concentrations. Plants then develop more shoots and 25-130% more flowers. Improvements arise partially through increased nitrogen longevity in soil, and partially through positive effects of urea itself on biomass partitioning between organs, and on plant physiology; giving rise to improved commercial attributes (more branches and flowers) and tolerance to stress (more root, less apical dominance, more chlorophyll). **Research Limitations**: Further research could measure leachate nitrogen content, and compare different methods of ureic stabilisation in more crops. **Originality/Value**: Urea stabilisation can increase fruit and flower yields, whilst reducing vulnerability to erratic climates, and fertiliser-derived pollution. We propose that urea's effectiveness arises because plants have evolved strategies to proliferate whilst competing with micro-organisms for organic nitrogen.

INTRODUCTION

In most natural environments and crop and nursery production systems, nitrogen (N) is the primary limiting factor for plant growth and development (Kraiser et al., 2011). Plants constantly monitor nitrogen availability as it fluctuates in soil, and modulate their development accordingly. N that is available in all soils for plants to use falls into two categories: mineral (inorganic) N, and organic N. Mineral N consists of ammonium, nitrite and nitrate; which are inert end-products of the biochemical activity of an array of soil micro-organisms that induce either nitrification of soil organic matter or fixation of gaseous nitrogen from the atmosphere. Organic or biological N consists of urea, and other amino acid-based molecules such as peptides and proteins. In natural systems urea enters the soil from urine excreted by animals (Barthelemy et al., 2018); and bulky organic matter is broken down via decay, to smaller organic molecules that plants can access, again by arrays of soil organisms and micro-organisms, such as fungi and bacteria (Neff et al., 2003; Schimel & Bennett, 2004; Walch-Liu et al., 2006).

From the mid-20th century and into the first decade of the 21st century, soil fertilisation by humans for intensive agriculture and horticulture was based mainly on supplying mineral N in the form of manufactured ammonium nitrate, nitrogen solutions, and anhydrous ammonia. Despite being a natural organic N source, urea can also be manufactured, and since it is relatively cheap and contains 46% N it is now the major fertiliser for crop production at a global level. However urea is rapidly converted to ammonium and nitrate by enzymes released by soil microbes, and nitrate from this and all other man-made or natural sources is soluble and labile, such that a significant proportion leaches into the ground water. Nitrification of ammonium also releases greenhouse gases such as carbon dioxide, nitrous oxide and methane to the atmosphere, and ammonium is prone to immobilisation in some soil types. Thus these methods of supplying N to plants are very inefficient as well as environmentally costly (Colangelo & Brand 2001; Liu et al. 2013): nitrate in water systems can be toxic and/or lead to algal blooms and eutrophication, and the gases released to the atmosphere contribute significantly to global warming.

Over recent decades a huge research effort has been directed towards increasing crop yields. A major part of this has focussed on crop nitrogen nutrition, and has already led to the development of novel fertilisers and the implementation of more sophisticated ways to apply these. Some important in-roads have been made towards the goals of reducing N waste, of preventing damage to the environment; and of increasing yield both per unit N, and *per se*. As part of this effort, studies of organic N uptake, utilisation and effects in plants have attracted increased attention (e.g. Witte, 2011; Nardi et al., 2016), because it has been repeatedly demonstrated that plants have direct access to these forms of N in the natural environment, and that they have evolved sophisticated methods for competing for, acquiring and utilising them (Mérigout et al., 2008; Paungfoo-Lonhienne et. al., 2012; Ma et al., 2018). It is now becoming clear that plants are not totally reliant on inorganic N, or on microbial activity to release inorganic N from organic matter (Neff et al., 2003). This has energized scientists, entrepreneurs and agronomists to discover ways of exploiting this knowledge to improve cropping systems. Methods of stabilising highly soluble urea in the soil, and slowing its breakdown to ammonium and nitrate by the ubiquitous bacteria-sourced soil enzyme urease, have been sought (Bhogal et al., 2003). Urease inhibitors can be added to soil along with the urea, and this can promote nitrogen use efficiency (Arkoun et al., 2012). Coatings on urea granules such as sulphur and polyurethane can reduce some of the degradation to other N forms by forming a physical barrier to slow urea solubilisation. Yields can either be

maintained at lower N input, or, importantly, increased (Tiana et al., 2018; Wang et al., 2015). We describe here effects of an alternative, chemical method of stabilising urea-sourced amine N in soil, developed by Levity Crop Science Ltd. (Preston, UK), on the efficiency with which ornamental plants can access and use nitrogen for improved hardiness and flower production. This technology (termed 'LimiN') has recently been used to induce greater tuber yields in potato crops in the field (Marks et al., 2018).

It is now additionally being recognized that different N-forms have different effects on plant phenotype, some of which are more beneficial than others (in our case for flower production and stress tolerance). Under organic N nutrition plants appear to develop an enhanced root to shoot ratio in comparison to nitrate N, amplifying the plant's capacity to scavenge soil for the water and nutrients required for continued above-ground growth (Zerihun et al., 1998; Cambui et al., 2011; Nardi et al., 2016; Franklin et al., 2017). Reduced apical dominance and increased lateral shoot branching are also induced. On this basis we surmised that supplying stabilised urea to plants would eventually lead to increases in their reproductive capacity, and produce phenotypes with more resistance to stress: more root mass per unit of shoot mass, and more branching as opposed to a single tall stem. This structure will enhance resistance to lodging, flooding, drought and transplant stress, for example.

Underlying the effect of N form on plant phenotype is the fact that the short-term allocation of absorbed N differs among nitrate, ammonium and organic N (Andrews et al., 2001; Andrews et al., 2013). A larger fraction of absorbed nitrate N is, in many cases, allocated to above-ground structures; whereas N derived from urea, amino acids and ammonium is more likely to be allocated to roots, leading to a larger root mass fraction (Ingestad & Ågren, 1991; Franklin et al., 2017). This is because different N forms are broken down and assimilated in different organs by different biochemical pathways (Zerihun et al., 1998), to provide the more basic N molecules that are needed for the synthesis of important proteins such as chlorophyll for photosynthesis, hormones that alter development, and the basic building blocks for tissue growth. Absorbed urea is rapidly hydrolysed in the root cytosol by urease, releasing ammonium and carbon dioxide. This ammonium is rapidly assimilated by a second cytosolic pathway involving a glutamate dehydrogenase, a glutamine synthase and an asparagine synthetase (Mérigout et al., 2008; Witte, 2011; Pinton et al., 2016). On the other hand the utilisation of inorganic nitrate requires the operation of the nitrate reductase pathway, which consumes more of the plant's energy, and carbon (C) from photosynthesis, than any other N assimilation mechanism (Sunil et al. 2013; Franklin et al., 2017). Thus absorption and metabolism of organic N as urea and amino acids is much more energy efficient. More energy, metabolite, and C are available for the many processes leading ultimately to reproduction, which, we propose, is the reason for the more 'luxuriant' flower-bearing phenotype that we predict will occur under stabilised ureic N provision.

Another layer of complexity arises when two or more forms of N are simultaneously present, which realistically is most of the time in natural systems and also in agriculture and horticulture. Pinton et al. (2016) show that entirely different sets of genes are induced in *Arabidopsis* and maize when both N sources are simultaneously available, compared to those that are up-regulated when each is supplied alone. When urea and nitrate are both available in the external solution, the induction of the uptake systems of each N source is actually reduced, despite which, plant growth and N utilization is promoted. The authors hypothesize that this increase in primary assimilation reflects cooperation amongst acquisition processes, through activation of different N assimilatory pathways, for tight control of overall nitrogen uptake and use.

We test here whether a novel chemical method of stabilising urea in the soil ('LimiN'; Levity Crop Science Ltd.), can increase the efficiency with which four popular ornamental species use nitrogen, and convert this to increased floral bud and flower production. We investigate whether a second, important consequence of stabilising ureic amine in soils - alterations in plant structure - can increase resilience to some types of stress. Re-potting module-grown seedlings or plants raised from cuttings to larger pots under nursey conditions can be detrimental to subsequent establishment and performance of the maturing plant, particularly when these were initially supplied with large amounts of fertiliser in the warm, humid conditions found under glass or polythene. High N, particularly as nitrate, can give rise to tall, 'leggy' plants with small root systems (see above). When transplanted these may not grow as well as plants with more compact and bushy forms above-ground, and/or those with larger root systems, as the accompanying tissue water loss and mechanical damage are more likely to have a negative impact (Kaczperski et al., 1996). Furthermore newly transplanted seedlings - especially of tender annual and semi-annual varieties - are particularly vulnerable to adverse environmental conditions such as low temperature, dry soil, and heavy rain- and wind-induced lodging. Zandstra and Liptay (1999) and Franco et al. (2005) have reviewed literature demonstrating the benefit of high root-shoot ratios for reducing transplant stress itself, and for improving post-transplant resilience. Therefore we propose that, in addition to increasing flower numbers, using stabilised amine can alter plant physiology in such a way as to allow tender plants to tolerate a variety of stressful conditions, by virtue of their altered form.

MATERIALS AND METHODS

Pelargonium (*Pelargonium zonale*), petunia (*Petunia* x *hybrida* Grandiflora), pansy (*Viola tricolor* Hortensis) and french marigold (*Tagetes petula* Red Boy) were grown in modules (1.5 cm diameter x 2.5 cm deep, or 6.0 cm x 5.0 cm) and treated via the surrounding soil with nitrogen (N) fertiliser as either industry standard N-P-K controls (IS), stabilised amine nitrogen (SAN) in a formulation called 'Lono' (supplied by Levity Crop Science Ltd., Preston, UK), or standard urea. SAN was supplied at 0.05 or 0.1 mmol m^{-3}, equivalent to a rate of 2.5 or 5.0 L ha^{-1} in 200 L water. IS was supplied at 0.7 or 1.4 mmol m^{-3}. Urea was supplied at 0.03 mmol m^{-3}. Treatments were applied either before and/or after transplantation of the module-grown plants to larger 0.5 or 1.0 L pots containing J. Arthur Bowers John Innes No. 2 compost (Westland Horticulture Ltd., Co. Tyrone, UK). The pH of this compost is 5.5-6.0, and it provides appropriate macro- and micro-nutrients to plants in all treatments, including boron and calcium, which are also present in SAN. Solutions were applied evenly to the soil using a pipette. SAN contains 15% N (by weight), and the IS and urea treatments were designed to provide the same amount of N to the plants (given that IS contains 24% N and urea contains 46% N by weight). IS contains a mixture of ureic and ammonium nitrate N. All three treatments were supplemented with industry standard N-P-K (IS) at one quarter recommended strength every 8-10 days, approximately mid-way between main treatment dates, ensuring access to sufficient micronutrients (and P-K in the case of urea-treated plants). Where SAN was supplied as a concentration range, the N content of IS was matched to the highest SAN concentration, thus in some cases IS-treated plants received more total N than SAN-treated plants. Plants in plugs and pots were treated with N fertiliser at the concentrations and times detailed in the Results section and in Table 1, at a rate of 20 cm^3 per m^2 planting area.

Table 1. Time courses showing treatments and measurement activity during the experiments, for pelargonium (a), petunia (b), pansy (c), and marigold (d).

a) Pelargonium

Days from start	Activity	Figure no.
0	N treatment	
1	Transplant	
3	Chlorophyll analysis	**1a**
5	Leaf 6 area, Leaf 6 growth rate	**2a, 2b**
8	Chlorophyll analysis	**1b**
15	Treatment 2	
22	Number of leaves over 20cm^2	**2c**
40	Treatment 3	
45	Branch count	**3a**
56	Flower count	**5a**
57	Photography	**8a, 8b**
58	Weigh harvested tissue	**6a,7a**

b) Petunia

Days from start	Activity	Figure no.
0	Transplant + N treatment	
4	Chlorophyll analysis	**1c**
13	Treatment 2	
14	Chlorophyll analysis	**1d**
18	Shoot count	**3b**
19	Flower count	**5b**
21	Weigh harvested tissue	**6b, 6c, 7b**

c) Pansy

Days from start	Activity	Figure no.
0	Transplant + N treatment	
3	Chlorophyll analysis	**1e**
15	Treatment 2	
25	Chlorophyll analysis	**1f**
28	Leaf width	**2d**
33	Treatment 3	
40	Flower count	**5c**
50	Treatment 4	
60	Weigh harvested tissue	**6d, 6e, 7c**

d) Marigold

Days from start	Activity	Figure no.
0	Transplant + N treatment	
14	Treatment 2	
26	Flower count	**5d**
27	Chlorophyll analysis	**1g**
28	Root measurement	**4**

Experiments were carried out in northern England in spring, summer and autumn, in a heated and ventilated glasshouse under natural light (PPFD 200-800 µmol m^2 s^{-1}). Night-time temperature was 12-20 °C, and day-time temperature was 15-32°C. Plants were watered by hand to soil capacity as required. Over the course of the experiments (ranging from four weeks to two months) plants were treated with the appropriate N compound every 2-3 weeks (as specified in Table 1), and a range of measurements were made on each plant over this time: leaf relative chlorophyll content; leaf length, extension rate, width and an estimation of area (of actively expanding leaves); lateral branch or shoot count; floral bud and flower numbers; and root length (of roots protruding from the basal openings of the pots). At the end of the experiment shoots were harvested and tissues were separated (vegetative tissue, reproductive tissue) and weighed immediately.

Relative chlorophyll content was measured in leaves as an index, with a FieldScout CM 1000 Chlorophyll Meter (Spectrum Technologies Inc., Illinois, USA). "Point-and-shoot" technology instantly measures the reflectance of ambient and reflected 700 nm and 840 nm light in a conical viewing area on the adaxial leaf surface 30-180 cm from the light receptor.

Laser guides outline the edges of the sampling area, allowing replication of the position of this between plants (we chose a 0.5 cm diameter area mid-way between the midrib and the leaf edge). The light receptor comprises four photodiodes; two for ambient light and two for reflected light from the leaf. Measurement units are calculated as an index of relative chlorophyll content, 0-999 ± 5%. Leaf lengths were hand-measured with a ruler from tip to base, and leaf widths were hand-measured with a ruler at the widest point. Leaf area was estimated by multiplying leaf length by leaf width. Root lengths were hand-measured with a ruler.

Each treatment comprised 6-20 replicates, and means and standard errors of each measurement type per treatment are displayed as bar charts. The significance of the differences between treatments was calculated using a one-tailed *t*-test for two independent means, and where treatments are significantly different from each other (at a probability level of less than 0.1%), this is denoted by 'a', 'b' and/or 'c' above the appropriate column on the graphic representations of the data.

RESULTS

Leaf relative chlorophyll content
Fig. 1 demonstrates that in pelargonium, petunia, pansy and marigold, N fertiliser supplied as stabilised amine nitrogen ('SAN') increased relative chlorophyll content of leaves in comparison to industry standard treatment (IS controls); and in marigold SAN increased chlorophyll content in comparison to plants supplied with the same amount of N in the form of standard urea (in this case matched to 0.05 mmol m^{-3} SAN).

The relative chlorophyll content of 5th-7th leaves of pelargonium was significantly higher than IS at 0.1 mmol m^{-3} SAN. Fig. 1c-d demonstrates that the relative chlorophyll content of petunia leaves was significantly higher at both SAN concentrations (c) four days post-transplant, and (d) two weeks post-transplant. In pansy the initial SAN-induced increase in relative chlorophyll content (e) was only significant at the higher concentration of SAN, however after 25 days 0.05 mmol m^{-3} SAN also increased chlorophyll index significantly (f). Fig. 1g shows that leaves of marigold plants had a significantly higher chlorophyll index when treated with 0.05 mmol m^{-3} SAN, than when treated with IS or urea (0.7 mmol m^{-3} and 0.03 mmol m^{-3} respectively). Urea also increased the chlorophyll index compared to controls, but not to the extent of SAN.

Leaf size
N addition increased leaf size of pelargonium and pansy (Fig. 2) to a greater extent when applied as SAN in comparison to IS, although both treatments contained the same amount of N by weight. Where effects of the lower SAN concentration (0.05 mmol m^{-3}) were tested, IS controls were actually supplied with more N. Despite this, in some cases, SAN still significantly increased leaf size.

When 6th leaf length was measured 24 h post-transplant of pelargonium plug plants to larger pots, there was no effect of SAN above that of controls (not shown). However five days post-transplant leaf 6 was longer on SAN-treated plants, and this was significant at 0.1 mmol m^{-3} (Fig. 2a). The growth rate of the 6th leaf between one and five days post-transplant was more rapid in the presence of SAN, and this was significant at 0.1 mmol m^{-3} (Fig. 2b). The number of leaves per plant with an estimated area over 20 cm^2 was significantly greater in the presence of SAN 22 days post-transplant, exhibiting a concentration-dependent effect (Fig. 2c). Fig. 2d shows that pansy plants, which had been treated with SAN twice, had larger leaves than those of controls: plants had more leaves wider than 2.0 cm, and this was

significant at 0.05 mmol m^{-3}. Fig. 2c-d demonstrates that SAN increased leaf size even when soil was supplied with less N than controls.

Branching
Fig. 3 demonstrates that SAN increased branching in comparison to IS in pelargonium (a) and increased shoot number in petunia (b). The increase in branching in pelargonium was significant in the presence of 0.05 mmol m^{-3} SAN, and in petunia was significant at 0.1 mmol m^{-3}.

Rooting
N added to marigold plants via the soil as SAN (0.05 mmol m^{-3}) increased root length compared to IS or urea (Fig. 4), when supplied twice over a four-week period. The increase in root length induced by SAN was significant compared to that in urea-treated plants.

Bud and flower numbers
Fig. 5 shows that SAN fertilisation increased flower and/or floral bud numbers per plant by up to 130% compared to IS controls, in (a) pelargonium, (b) petunia, (c) pansy, and by 40% compared to urea (d) in marigold. The SAN-induced increase in flower number in pelargonium showed the same significance at both 0.05 and 0.1 mmol m^{-3}, and in petunia was significant at 0.05 mmol m^{-3}. Fig. 5c shows a significant increase in numbers of both senesced and current pansy flowers per plant in the presence of 0.05 mmol m^{-3} SAN. Marigold produced greater numbers of floral buds (5d) when fertilised with 0.05 mmol m^{-3} SAN, and this was significant compared to urea-fertilised plants.

Reproductive and vegetative shoot tissue weights at harvest, and ratios between them
When N-form was tested on final shoot fresh tissue weights at harvest (Fig. 6), plants fertilised with SAN (0.05-0.1 mmol m^{-3}) had more reproductive tissue by weight than IS-treated plants. Pelargonium exhibited a significant concentration-dependent increase (a), with up to twice as much reproductive tissue as controls; petunia had approximately 25% more reproductive tissue, and this was significant at 0.1 mmol m^{-3} SAN (b); and 0.05 mmol m^{-3} SAN significantly increased flower weight by over 100% in pansy (d).

The effect of N-form on vegetative tissue weight was not as straightforward: in pelargonium (6a) this was significantly increased in the presence of SAN compared to IS; in petunia (6c) it was reduced by SAN, although not significantly; and in pansy it was increased by 0.05 mmol m^{-3} SAN (6e).

When this data was expressed as a ratio of reproductive to vegetative tissue weight, the ratio was higher (indicating less vegetative tissue per unit weight of reproductive tissue) when the plants were fertilised with SAN (Fig. 7) than with IS. The increase in the ratio was significant at both 0.05 and 0.1 mmol m^{-3} SAN in pelargonium (7a), at 0.1 mmol m^{-3} SAN in petunia (7b), and at 0.05 mmol m^{-3} SAN in pansy (7c).

The effect of SAN to promote flowering, in comparison to IS controls supplied with the same amount of N, can clearly be seen in Fig. 8 in photographic form. Fig. 8a depicts three individual pelargonium plants representative of each of three treatments: from left to right these are IS, 0.05 mmol m^{-3} SAN, and 0.1 mmol m^{-3} SAN. Fig. 8b shows a group of six IS-treated pelargoniums on the right, and a second group on the left which had been treated with 0.1 mmol m^{-3} SAN.
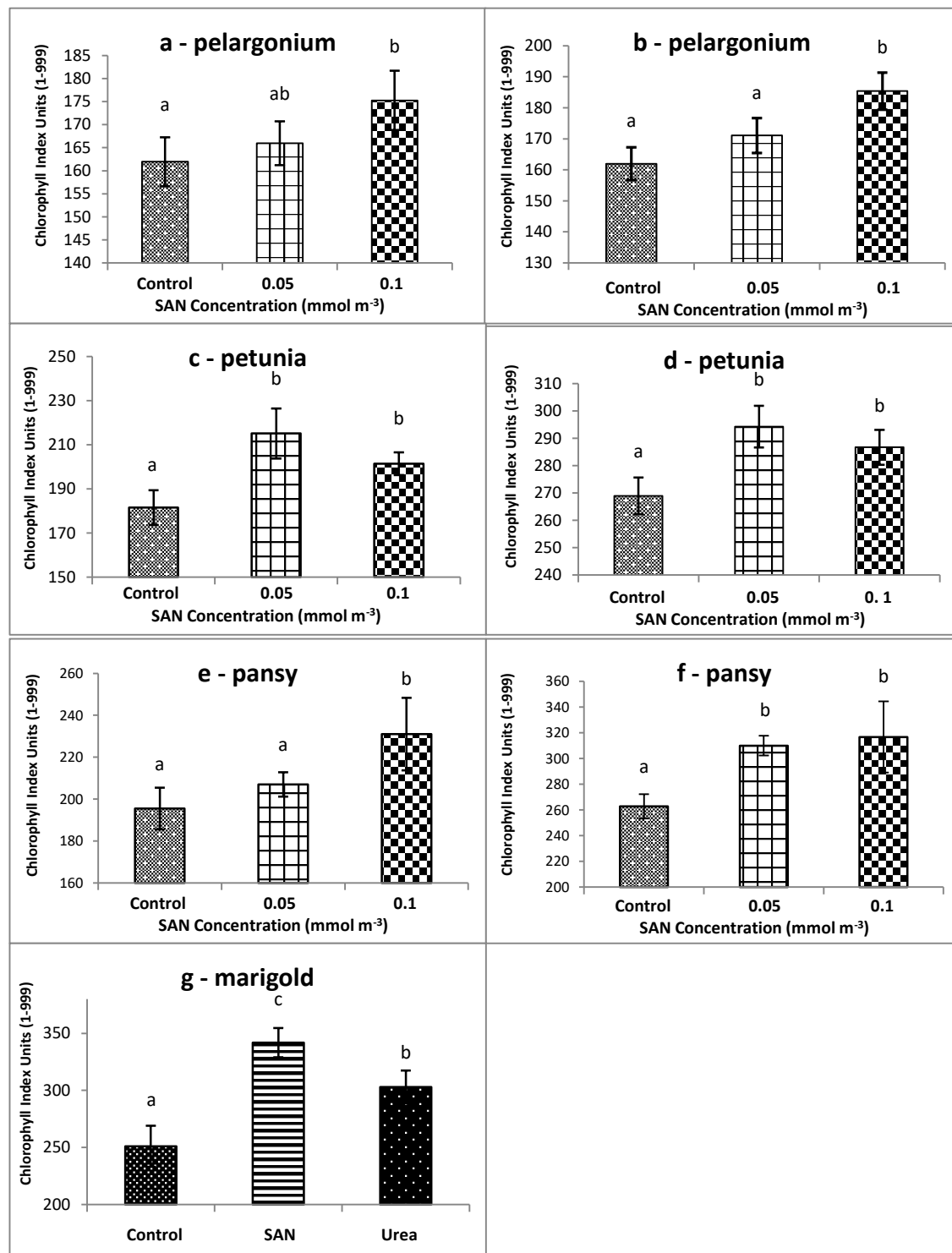
Fig. 1. Effect of fertiliser nitrogen (N) form on leaf chlorophyll content of pelargonium, petunia, pansy and marigold. Stabilised amine nitrogen (SAN) at two concentrations (0.05 and 0.1 mmol m^{-3}), is compared to industry standard NPK (IS) or non-stabilised urea, on leaf relative chlorophyll content following transplant of plugs into larger pots: pelargonium (a, b); petunia (c, d); pansy (e, f); and marigold (g). Means +/- standard errors are shown (n=6-20), and significant differences between treatments are denoted by different letters above each column (p<0.1 maximum).
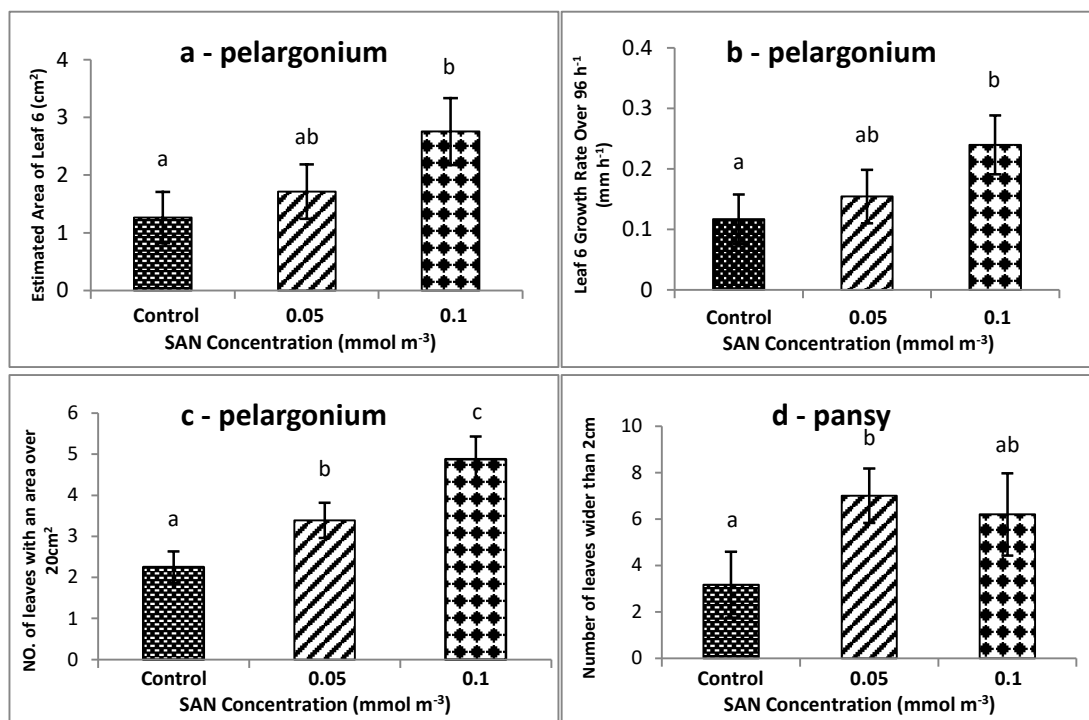
Fig. 2. Effect of fertiliser N-form on leaf growth and dimensions in pelargonium and pansy. The effect of SAN (0.05-0.1 mmol m^{-3}) compared to IS (1.4 mmol m^{-3}) is shown on (a) leaf 6 estimated area (cm^2), (b) leaf 6 growth rate over 96 hours (mm h^{-1}), and (c) the number of leaves per plant over 20 cm^2 in area, after transplant of pelargonium from plugs to larger pots; and on (d) the number of leaves over 2 cm wide in pansy after transplant to larger pots. Means +/- standard errors are shown (n=6-20), and significant differences between treatments are denoted by different letters above each column (p<0.1 maximum).
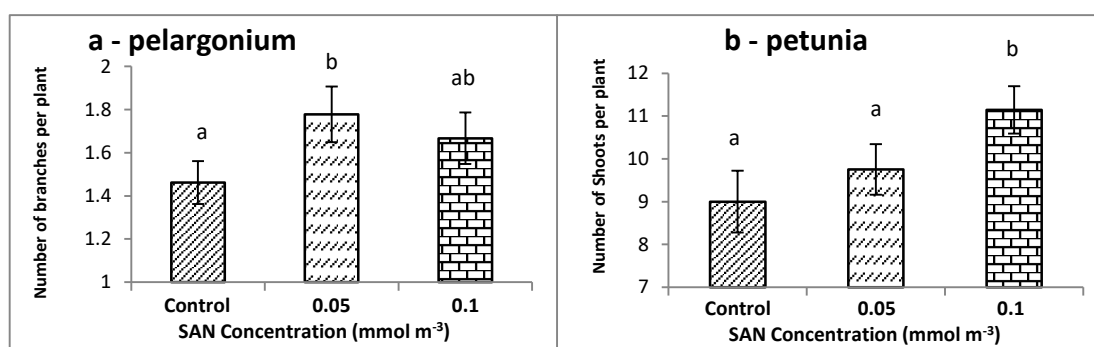

Fig. 3. Effect of fertiliser N-form on branching in pelargonium (a) and shoot number in petunia (b). Fertilisers as IS (1.4 mmol m^{-3}) with N content matched to the higher SAN concentration, and SAN (0.05 and 0.1 mmol m^{-3}), were tested on growth habit after transplanting seedlings to larger pots. Means +/- SE (a - n=20; b - n=7) are shown, with significant differences between treatments denoted as differing letters above each column.
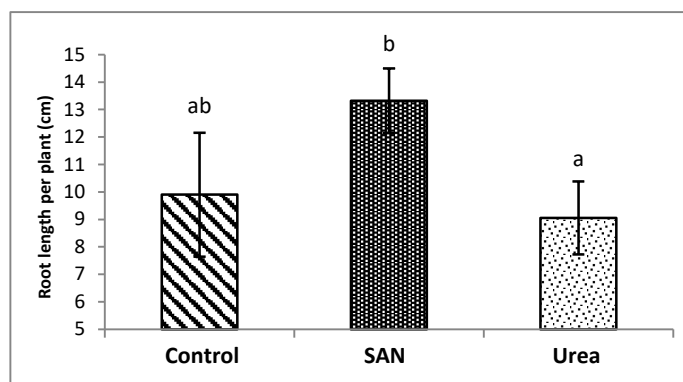
Fig. 4. Effect of fertiliser N-form on root length of marigold plants. SAN (0.05 mmol m^{-3}), urea (0.03 mmol m^{-3}) and IS (0.7 mmol m^{-3}) were supplied twice after transplant, and root lengths were measured four weeks after the initial treatment. Means +/- SEs (n=6-12) are shown, with significant differences between N forms designated by different letters above each column.

DISCUSSION

Fertilisation of ornamental plants with stabilised amine nitrogen (SAN), as compared with industry standard soluble N-P-K (IS controls), improves a) their aesthetics for retail – more branches and flowers; b) their ability to tolerate transplantation stress – more roots, branches and chlorophyll; and c) equips developing plants with more of the attributes known to aid avoidance of and/or tolerance to stresses such as low or high temperature, wet or dry soil, and high wind- and heavy rain-induced damage or lodging – longer roots, and more branches and chlorophyll. It is clear from Figs. 5-8 that plants develop 25-130 % more flowers in the presence of SAN, such that a greater proportion of shoot fresh matter is made up of reproductive tissue (Fig. 7). When transplanted to larger pots, SAN-fertilised plants grew more rapidly and exhibited higher chlorophyll contents, larger leaves and more shoots (Figs. 1-3).

These results demonstrate that the form of the nitrogen, rather than the amount, is the basis for the positive effects described above, as SAN-treated plants were supplied with the same amount of total N by weight as IS-treated plants (or indeed slightly less). As described in the Introduction, stabilising ureic amine prevents N loss from soil (Wang et al., 2015; Tiana et al., 2018), thus even though all plants had access to the same amount of total N each time they were fertilised, those given standard soluble N-P-K or non-stabilised urea would actually have had access to less total N between treatment dates. This is due to leaching and volatilisation, as occurs frequently in agriculture and horticulture, including under containerised nursery plant production (Huett & Morris, 1999; Colangelo & Brand, 2001; Bhogal et al., 2003).

Additionally, because a greater proportion of the N available to SAN-treated plants is effectively organic - being ureic amine - we can conclude that less energy and carbon are needed to assimilate this N to usable forms in comparison to that in IS-treated plant (Cambui et al., 2011; Franklin et al., 2017). In IS-treated plants nitrate is likely to be the predominant N form, particularly when the ureic element of IS becomes degraded between treatment dates. Thus SAN effectively provides more resource for photosynthesis, leaf growth, branching and root growth; and ultimately for reproductive development. This is at least a part of the reason for the improved traits described (in addition simply to the stabilisation-induced retention of

more of the supplied N in the soil), because SAN also alters plant phenotype: the ratio of reproductive to vegetative above-ground tissue by weight is greater in SAN-treated plants (Fig. 7), there are more lateral shoots and longer roots (Figs. 3-4); and even though plants are not necessarily shorter, these traits are characteristic of less apical dominance (Ingestad & Ågren, 1991; Andrews et al., 2013).
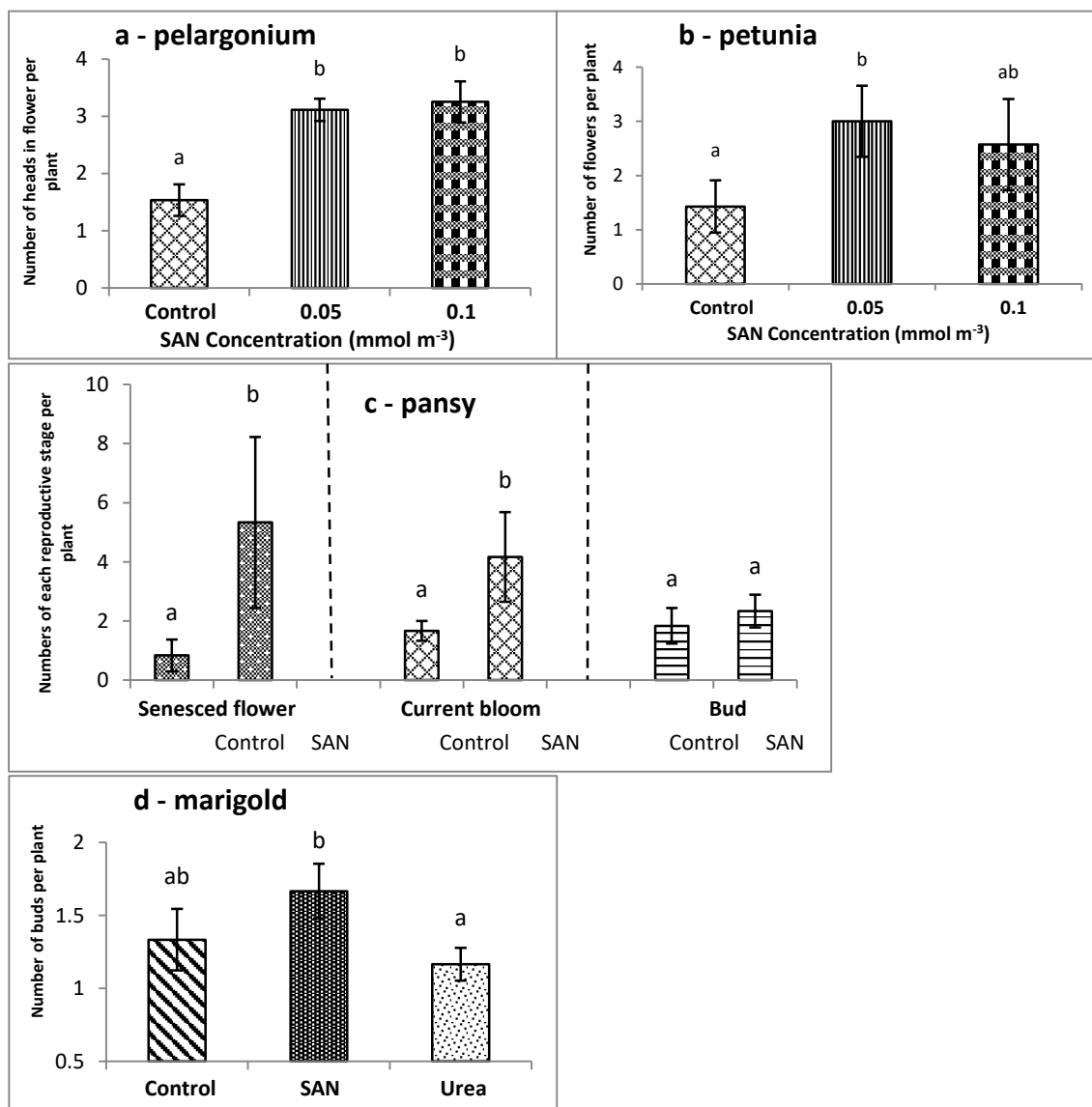

Fig. 5. Effect of fertiliser N-form on flower numbers in (a) pelargonium, (b) petunia, (c) pansy, and (d) marigold. Plants were fertilised with IS (1.4 mmol m^{-3} [a,b] or 0.7 mmol m^{-3} [c,d]), SAN (0.05-0.1 mmol m^{-3} – a,b; 0.05 mmol m^{-3} – c,d), or urea (0.03 mmol m^{-3}, d). Flower numbers were counted 3-8 weeks after transplant to larger pots, with final N treatments occurring 5-10 days before counting reproductive structures on each plant. Data are presented as means +/- SE (n=6-20), and significant differences between treatments are denoted by differing letters above columns.
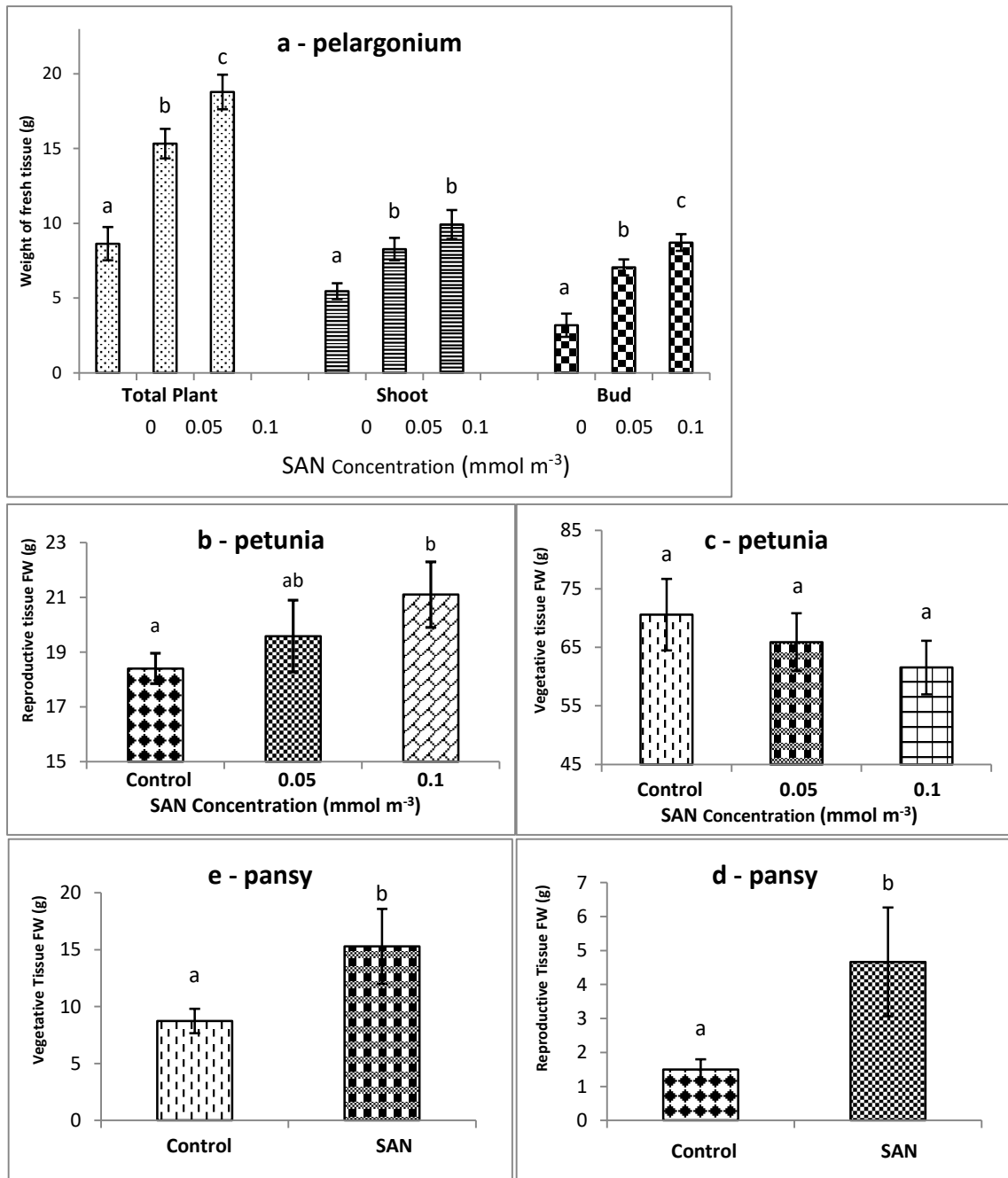
Fig. 6. Effect of fertiliser N-form on shoot tissue weights of pelargonium (a), petunia (b - reproductive, c - vegetative), and pansy (d – reproductive, e – vegetative). Plants were transplanted to larger pots and fertilised on 2-4 occasions with IS (1.4 mmol m^{-3}), or SAN (0.05-0.1 mmol m^{-3}). Shoots were harvested and weighed 3-10 weeks after first N treatment. Data are presented as means +/- SE (n=6-20), and significant differences between treatments are denoted by differing letters above columns.
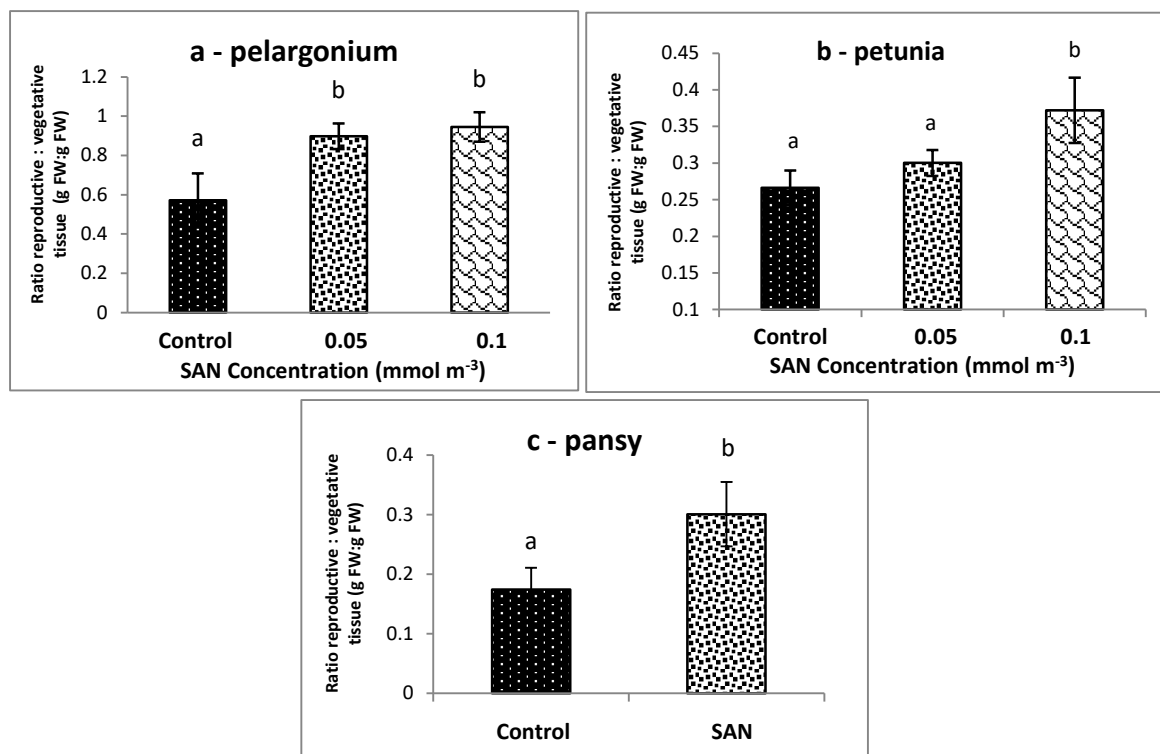
Fig. 7. Effect of fertiliser N-form on the ratio between reproductive and vegetative shoot tissue weights in pelargonium (a), petunia (b), and pansy (c). Plants were fertilised with IS (1.4 mmol m^{-3}), or SAN (0.05-0.1 mmol m^{-3}), and harvested and weighed, as described in Fig. 6. Data are presented as means +/- SE (n=6-20), and significant differences between treatments are denoted by differing letters above columns.

In the field it has been shown that the coating method of stabilising urea can increase yields of grain crops, garlic and cotton (Wang et al., 2015; Tiana et al., 2018). We have previously shown that the chemical method of inducing ureic stability used here as SAN (also known as 'LimiN') increases potato yield in UK field trials (Marks et al., 2018). When equivalent amounts of ureic N were supplied to potato plants as foliar sprays, resources were partitioned to tubers more effectively when the urea was stabilised. Gou et al. (2017) demonstrated that foliar application of urea along with a urease inhibitor could significantly enhance drought-tolerance in maize. This occurred through up-regulation of the antioxidant defence system. Although SAN plants were not subjected to drought, it was certainly the case that chlorophyll concentrations were higher (Figs. 1-2). It has not previously been demonstrated, as shown here, that stabilised urea can increase flowering (Figs. 5-7), and resistance to transplantation stress.

Effects of N-form on resource partitioning
Franklin et al. (2017) synthesized results from multiple experiments, on N-form effects on plant phenotype, producing a robust plant growth model that could explain all aspects of the data. This confirmed that the carbon cost of assimilating organic N into proteins is lower than that for inorganic N. This carbon bonus even occurred when organic N availability to the roots was very low – up to 70% lower for *Arabidopsis* seedlings. At equal growth rate, root-shoot ratio was up to three times higher and nitrogen productivity up to 20% higher for

organic than inorganic N. In this regard it is pertinent that nitrate at sufficiency is actively involved in the inhibition of early lateral root development (Forde, 2002; Walch-Liu et al.). Despite the fact that Franklin et al. (2017) demonstrated that a larger proportion of biomass production was initially allocated to roots (in our case SAN increased root length – although total biomass was not measured – Fig. 4), according to the model organic N still eventually gave rise to a higher relative growth rate above ground. SAN-treated plants had faster growing, larger leaves (Fig. 2); and greater shoot biomass (Fig. 6); which agrees with this prediction.

Nitrogen deficiency has very different effects on plants, regardless of form; including induction of lateral root growth through nitrate signalling (Andrews et al., 2001; Forde, 2002; Gojon et al., 2011). None of the plants in our experiments experienced N deficiency. There is much evidence for lateral root proliferation within concentrated but isolated patches of both nitrate nitrogen (Remans et al., 2006), and amine nitrogen (such as glutamate – Walch-Liu et al., 2006), when the nitrogen status of the whole plant is low, and when this fact is signalled to, or sensed within the root. Local proliferation of roots is a key factor in interspecific competition for non-uniformly distributed supplies of organic N in natural habitats. Hodge et al. (2002) found that when two grasses (*Lolium perenne* L. and *Poa pratensis* L.) were grown in competition, root proliferation and root length density in an organic patch was more important for N capture than alterations in N uptake capacity per unit of root mass. Although both species proliferated roots in the patches, *L. perenne* produced greater root length densities than *P. pratensis*, and also captured more N. However, Fig. 4 shows that organic nitrogen (SAN) also increases root growth in N-sufficient plants compared to standard fertilisation, and we can assume that this was available homogeneously rather than in 'patchy' form.

Until recently, it was generally accepted that the terrestrial nitrogen cycle was based primarily on net mineralization, such that plants were thought mainly to use inorganic N and to compete poorly against soil microbes for this commodity. However, soil N cycling has recently been shown to be driven heavily by the de-polymerization of N-containing polymers by microbial enzymes (such as urease) in the soil (Schimel & Bennett, 2004), without a necessity for mineralization. This releases organic N-containing monomers, and it is now known that plants can compete with microbes very successfully for this N. In this context the most efficient cost versus benefit phenotype is a high root-shoot ratio for competitive uptake of organic N, that then gives rise to greater above and below ground plant biomass – mirroring effects of SAN. Ma et al. (2018) found that root diameter exerts the strongest influence on root trait variation. They proposed that plants have evolved thinner roots and thus more total root surface area since they first emerged on land, to improve their efficiency of soil exploration and ability to compete with microbes per unit of carbon invested.

Effects of mixed N supply

Pompeiano and Patton (2017) exposed greenhouse-grown zoysiagrass (*Zoysia* spp.) to five different ratios of nitrate and urea as N sources (% nitrate:% urea at 100:0, 75:25, 50:50, 25:75, 0:100). 10 weeks later both above- and below-ground dry weight production was greatest following treatments with 25:75 nitrate:urea, whereas 100% nitrate produced plants with the lowest dry weight. It will certainly be the case that, whilst urea is a major component of total N in the SAN-treated plants, N as nitrate will also have been present in the soil (from the soil itself, and from the background low rate N-P-K supplements); most likely giving rise to lower nitrate:urea ratios than plants treated as per industry standard. Different sets of genes are up-regulated when both N forms are present, increasing the efficiency of total N uptake, assimilation and use (Pinton et al. 2016).

Fig. 8. Effect of fertiliser N-form on appearance and flowering of pelargonium. Pelargonium were fertilised with IS (1.4 mmol m^{-3}), 0.05 mmol m^{-3} and 0.1 mmol m^{-3} SAN, and representative examples are shown as single plants from each treatment, from left to right (a); and as groups of six plants (b), fertilised with IS (left) or 0.1 mmol m^{-3} SAN (right).

CONCLUSION

Stabilising ureic nitrogen (N) in urea-based fertiliser induces beneficial attributes in containerised plants compared to industry standard and non-stabilised urea fertilisation, when all plants are supplied with identical amounts of N by weight: improved aesthetics, stress avoidance, stress tolerance, and N-use-efficiency. We argue that this arises not only from increased longevity of this N-form in soil, but also from impacts of ureic-N itself – effectively an organic N-source - on plant development. Our working hypothesis is that biomass growth occurs within an altered phenotype, initially favouring partitioning to roots, and subsequently increasing 'branchiness' above ground, because plants have evolved the ideal architectural form for proliferation within the constraints of competition with soil micro-organisms for organic nitrogen monomers (such as ureic amine). Since assimilation of organic N uses less energy and carbon (C) than that of nitrate N, it is more efficient for plants to acquire this N-form, such that increasing root surface area to compete with soil microbes is an initial priority. More resource is then available for chlorophyll production such that more C becomes available for further tissue growth. Since reduced apical dominance and increased 'branchiness' are more beneficial traits for stress tolerance, and – ultimately – for reproductive capacity, this developmental process and growth form is prioritised in unstressed plants. Stabilising urea could thus improve fruiting and flowering in many horticultural crops despite our increasingly stressful climate, whilst reducing the risk of N pollution.

CONFLICT OF INTEREST STATEMENT

In accordance with JHPR policy and my ethical obligation as a researcher, I am reporting that I am an employee of a company that may be affected by the research reported in the enclosed paper. I have in place an approved plan for managing any potential conflicts arising from that involvement.

ACKNOWLEDGEMENTS

REFERENCES

Andrews, M., Raven, J. & Lea, P. (2013). Do plants need nitrate? The mechanisms by which nitrogen form affects plants. *Annals of Applied Biology*, *163*, 174-199. https://doi.org/10.1111/aab.12045

Andrews, M., Raven, J.A. & Sprent, J.I. (2001). Environmental effects on dry matter partitioning between shoot and root of crop plants: relations with growth and shoot protein concentration. *Annals of Applied Biology*, *138*, 57-68. https://doi.org/10.1111/j.1744-7348.2001.tb00085.x

Arkoun, M., Sarda, X., Jannin, L., Laîné, P., Etienne, P., Garcia-Mina, J-M. … & Ourry, A. (2012). Hydroponics versus field lysimeter studies of urea, ammonium and nitrate uptake by oilseed rape (*Brassica napus* L.). *Journal of Experimental Botany*, *63*, 5245–5258 https://doi.org/10.1093/jxb/ers183.

Barthelemy, H., Stark, S., Michelsen, A., Olofsson, J. (2018). Urine is an important nitrogen source for plants irrespective of vegetation composition in an Arctic tundra: Insights from a ^{15}N-enriched urea tracer experiment. *Journal of Ecol*ogy, *106*, 367-378. https://doi.org/10.1111/1365-2745.12820

Bhogal, A., Dampney, P. & Goulding, K. (2003). Evaluation of urea-based nitrogen fertilisers. Report for Defra (UK) projects NT2601 and NT2602. http://www.envirobase.info/PDF/RES31087_final_report.pdf

Brackin, R., Näsholm, T., Robinson, N., Guillou, S., Vinall, K., Lakshmanan, P., …& Inselsbacher, E. (2015). Nitrogen fluxes at the root-soil interface show a mismatch of nitrogen fertilizer supply and sugarcane root uptake capacity. *Scientific Reports 5*, 15727. http://dx.doi.org/10.1038/srep15727

Cambui, C.A., Svennerstam, H., Gruffman, L., Nordin, A., Ganeteg, U. & Näsholm T. (2011). Patterns of plant biomass partitioning depend on nitrogen source. *PLoS ONE 6*. https://doi.org/10.1371/journal.pone.0019211

Colangelo, D.J. & Brand, M.H. (2001). Nitrate leaching beneath a containerized nursery crop receiving trickle or overhead irrigation. *Journal of Environmental Quality 30*, 1564-1574. doi:10.2134/jeq2001.3051564x

Forde, B.G. (2002). Local and long range signaling pathways regulating plant responses to nitrate. *Annual Review of Plant Biology*, *53,* 203-224. https://doi.org/10.1146/annurev.arplant.53.100301.135256

Franco, J., Martínez-Sánchez, J.J., Fernandez, J. & Bañón, S. (2006). Selection and nursery production of ornamental plants for landscaping and xerogardening in semi-arid environments. *Journal of Horticultural Science and Biotechnology, 81,* 3-17. 10.1080/14620316.2006.11512022

Franklin, O., Cambui, C. A., Gruffman, L., Palmroth, S., Oren, R. & Näsholm, T. (2017). The carbon bonus of organic nitrogen enhances nitrogen use efficiency of plants. *Plant, Cell & Environment*, *40*, 25–35. https://doi.org/10.1111/pce.12772

Gojon, A., Krouk, G., Perrine-Walker, F. & Laugier, E. (2011). Nitrate transceptor(s) in plants. *Journal of Experimental Botany*, *62*, 2299–2308. https://doi.org/10.1093/jxb/erq419

Gou, W., Zheng, P., Tian, L., Gao, M., Zhang, L., Akram, N.A. & Ashraf, M. (2017). Exogenous application of urea and a urease inhibitor improves drought stress tolerance in maize (*Zea mays L.*) *Journal of Plant Research*, *130*, 599–609. https://doi.org/10.1007/s10265-017-0933-5

Hodge, A., Robinson, D., Griffiths, B.S. & Fitter, A.H. (1999). Why plants bother: root proliferation results in increased nitrogen capture from an organic patch when two grasses compete. *Plant, Cell & Environment*, *22*, 811-820. https://doi.org/10.1046/j.1365-3040.1999.00454.x

Huett, D. O. & Morris, S. C. (1999) Fertiliser use efficiency by containerised nursery plants. 3. Effect of heavy leaching and damaged fertiliser prills on plant growth, nutrient uptake, and nutrient loss. *Australian Journal of Agricultural Research 50*, 217-222. https://doi.org/10.1071/A98084

Ingestad, T. & Ågren, G.I. (1991). The influence of plant nutrition on biomass allocation. *Ecological Applications*, *1*, 168-174. https://doi.org/10.2307/1941809

Kaczperski, M.P., Armitage, A.M. & Lewis, P.M. (1996). Performance of plug-grown geranium seedlings preconditioned with nitrogen fertilizer or low-temperature storage. *HortScience 31*, 361–363. http://hortsci.ashspublications.org/content/31/3/361.short

Kraiser, T., Gras, D.E., Gutiérrez, A.G., González, B., & Gutiérrez, R.A. (2011). A holistic view of nitrogen acquisition in plants. Journal of Experimental Botany, *62*, 1455–1466. http://dx.doi.org/10.1093/jxb/erq425

Liu, X., Zhang, Y., Han, W., Tang, A., Shen, J., Cui, Z. … & Zhang, F. (2013). Enhanced nitrogen deposition over China. *Nature*, *494*, 459–462. http://dx.doi.org/10.1038/nature11917

Ma, Z., Guo, D., Xu, X., Lu, M., Bardgett, R.D., Eissenstat, D.M. & Hedin, L.O. (2018). Evolutionary history resolves global organization of root functional traits. *Nature*, *555*, 94–97. http://dx.doi.org/10.1038/nature25783

Marks, D.J., Wilkinson, S. & Weston, A.K. (2018). Influence of foliar stabilised nitrogen on potato tuber yield. *Proceedings Crop Production in Northern Britain*, 225-230 (8, 20183245174); pub. by 'The Association for Crop Protection in Northern Britain, Dundee, UK'. The Dundee Conference. Crop Production in Northern Britain 2018, Dundee, UK,

Masclaux-Daubresse, C., Daniel-Vedele, F., Dechorgnat, J., Chardon, F., Gaufichon, L. & Suzuki, A. (2010). Nitrogen uptake, assimilation and remobilization in plants: challenges for sustainable and productive agriculture. *Annals of Botany*, *105*, 1141–1157. https://doi.org/10.1093/aob/mcq028

Mérigout, P., Lelandais, M., Bitton, F., Renou, J.P., Briand, X., Meyer, C. & Daniel-Vedele, F. (2008). Physiological and transcriptomic aspects of urea uptake and assimilation in *Arabidopsis* plants. *Plant Physiology*, *147*, 1225-38. 10.1104/pp.108.119339

Nardi, S., Pizzeghello, D., Schiavon, M. & Ertani, A. (2016). Plant biostimulants: physiological responses induced by protein hydrolyzed-based products and humic substances in plant metabolism. *Scientia Agricola*, *73*, 18-23. http://dx.doi.org/10.1590/0103-9016-2015-0006

Neff, J. C., Chapin, F. S. & Vitousek, P. M. (2003). Breaks in the cycle: dissolved organic nitrogen in terrestrial ecosystems. *Frontiers in Ecology and the Environment*, *1*, 205-211. https://doi.org/10.1890/1540-9295(2003)001[0205:BITCDO]2.0.CO;2

Paungfoo-Lonhienne C., Lonhienne T.G.A., Rentsch D., Robinson N., Christie M., Webb R.I., … & Schmidt S. (2008) Plants can use protein as a nitrogen source without assistance from other organisms. *Proceedings of the National Academy of Sciences, USA 105*, 4524–4529. https://doi.org/10.1073/pnas.0712078105

Pinton, R., Tomasi, N. & Zanin, L. (2016). Molecular and physiological interactions of urea and nitrate uptake in plants. *Plant Signaling and Behaviour*, *11*, 1. https://doi.org/10.1080/15592324.2015.1076603

Pompeiano, A. & Patton, A.J. (2017). Growth and root architecture responses of zoysiagrass to changes in fertilizer nitrate:urea ratio. *Journal of Plant Nutrition and Soil Science*, *180*, 528–534. https://doi.org/10.1002/jpln.201600401

Remans, T., Nacry, P., Pervent, M., Filleur, S., Diatloff, E., … Forde, B.G. & Gojon, A. (2006). The *Arabidopsis* NRT1.1 transporter participates in the signalling pathway triggering root colonization of nitrate rich patches. *Proceedings of the National Academy of Science USA*, *103*, 19206–19211. https://doi.org/10.1073/pnas.0605275103

Scheible, W.R., Lauerer, M., Schulze, E.D., Caboche, M. & Stitt, M. (1997). Accumulation of nitrate in the shoot acts as a signal to regulate shoot-root allocation in tobacco. *Plant Journal 11*, 671–691. https://doi.org/10.1046/j.1365-313X.1997.11040671.x

Schimel, J.P. & Bennett, J. (2004). Nitrogen mineralization: challenges of a changing paradigm. *Ecology*, *85*, 591-602. https://doi.org/10.1890/03-8002

Sunil, B., Talla, S.K., Aswani, V. & Raghavendra, A.S. (2013). Optimization of photosynthesis by multiple metabolic pathways involving inter-organelle interactions: resource sharing and ROS maintenance as the bases. *Photosynthesis Research*, *117*, 61-71. https://doi.org/10.1007/s11120-013-9889-z

Tiana, X., Lia, C., Zhang, M., Li, T., Lu, Y. & Liu L. (2018). Controlled release urea improved crop yields and mitigated nitrate leaching under cotton-garlic intercropping system in a 4-year field trial. *Soil and Tillage Research*, *175*, 158-167. https://doi.org/10.1016/j.still.2017.08.015

Walch-Liu, P., Liu, L-H., Remans, T., Tester, M. & Forde, B.G. (2006). Evidence that L-glutamate can act as an exogenous signal to modulate root growth and branching in *Arabidopsis thaliana*. *Plant and Cell Physiology*, *47*, 1045–1057. https://doi.org/10.1093/pcp/pcj075

Wang, S., Zhao, X., Xing, G., Yang, Y., Zhang, M. & Chen H. (2015). Improving grain yield and reducing N loss using polymer-coated urea in southeast China. *Agronomy for Sustainable Development, 35*, 1103–1115. https://doi.org/10.1007/s13593-015-0300-7

Witte, C-P., (2011). Urea metabolism in plants. *Plant Science 180*, 431-438. https://doi.org/10.1016/j.plantsci.2010.11.010

Zandstra, J.W. & Liptay, A. (1999). Nutritional effects on transplant root and shoot growth - A review. *Acta Horticulturae 504*, 23-32. http://dx.doi.org/10.17660/ActaHortic.1999.504.1

Zanin, L., Zamboni, A., Monte, R., Tomasi, N., Varanini, Z., Cesco, S. & Pinton R. (2015). Transcriptomic analysis highlights reciprocal interactions of urea and nitrate for nitrogen acquisition by maize roots. *Plant and Cell Physiology*, *56*, 532-48. https://doi.org/10.1093/pcp/pcu202

Zerihun A., McKenzie B.A. & Morton J.D. (1998) Photosynthate costs associated with the utilization of different nitrogen-forms: influence on the carbon balance of plants and shoot-root biomass partitioning. *New Phytologist 138*, 1–11.

افزایش گلدهی، کارایی مصرف نیتروژن و مقاومت به تنش در گیاهان زینتی از طریق پایدارسازی نیتروژن اوره

سالی ویلکینسون، آنا کیت ویستون و دیوید جیمز مارکس

چکیده:

تغذیه گیاهان با نیتروژن به فرم‌های کودی نیترات آمونیوم یا اوره کاری بی‌فایده است زیرا بخش زیادی از آن پیش از استفاده توسط گیاه تخریب شده و آلاینده‌های زیست محیطی را آزاد می‌کند. جلوگیری از تخریب اوره می‌تواند آلودگی را کاهش داده و میزان بهره وری مصرف نیتروژن را بهبود بخشد. با توجه به نقش منابع آلی نیتروژن در جزء بندی بیوماس، اثرات مفید پایدارسازی اوره بر گلدهی و تحمل به تنش بررسی گردید. در این آزمایش اثرات افزودن اوره پایدار شده به روش شیمیایی به خاک بر میزان گلدهی و شکل ظاهری گیاهان پرورش یافته در شرایط گلخانه‌ای شامل شمعدانی، اطلسی، بنفشه و جعفری در هنگام انتقال نشاءهای بذری به گلدان‌های بزرگتر مورد بررسی قرار گرفت. اثر بخشی اوره پایدار شده، اوره پایدار نشده و کود استاندارد صنعتی در تغذیه نیتروژن کل مقایسه شد. نتایج نشان داد که پایدارسازی اوره رشد و نمو گیاهان را تغییر داد. در تغییرات اولیه اندازه گیری شده صفات طول ریشه، میزان رشد برگ و غلظت کلروفیل افزایش داشتند. گیاهان سپس شاخه های بیشتری تولید کرده و ۲۵ تا ۱۳۰٪ گل بیشتری تولید کردند. این بهبودی تا حدی از طریق افزایش ماندگاری نیتروژن در خاک و همچنین بخشی به دلیل اثرات مثبت اوره به تنهایی در تشکیل بیوماس بین اندام ها و فیزیولوژی گیاه بوجود آمد که در نتیجه بهبود ویژگی‌های تجاری (گل و شاخه های بیشتر) و مقاومت به تنش (ریشه بیشتر، غالبیت انتهایی کمتر، کلروفیل بیشتر) ایجاد شد. پایدارسازی اوره می‌تواند علاوه بر افزایش عملکرد میوه و گل، آسیب پذیری در مقابل اقلیم‌های متغیر و آلودگی حاصل از کودها را کاهش دهد. افزایش کارایی اوره می‌تواند به دلیل توسعه و بهبود مکانیسم‌های گیاهان در رقابت با ریزجانداران برای نیتروژن باشد.

کلمات کلیدی: کود اوره، نیتروژن آلی، ریشه‌ها، شاخه‌های جانبی، کلروفیل