Proceedings Crop Production in Northern Britain 2020 INCREASES IN SHELFORD POTATO TUBER YIELD RESULTING FROM STABILISING UREA NITROGEN CAN BE MANIPULATED VIA TIMING OF APPLICATION...

Conference Paper · February 2020

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INCREASES IN SHELFORD POTATO TUBER YIELD RESULTING FROM STABILISING UREA NITROGEN CAN BE MANIPULATED VIA TIMING OF APPLICATION TO INFLUENCE SIZE DISTRIBUTION

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Summary: Technologies for stabilising urea N in fertiliser, preventing its breakdown to pollutants, and prolonging its availability for plants, have been developed. We have already shown that chemically stabilising ureic amine N in foliar fertilisers applied to potato crops in the field increases yield. Greenhouse trials demonstrated that this was due to increases in root growth and leaf chlorophyll content. Here we report results from UK field trials on *Solanum tuberosum* L. *cv*. Shelford showing that stabilised amine-induced increases in yield can be manipulated to shift tuber size distribution. Overall yield is greatest when four rather than three applications are made over the growing season. However, when applications are limited to three, a greater percentage of the harvested tubers falls within the 60-80 mm size category when a final application, at tuber bulking, remains within the program. When the final bulking application is left out, and a tuber initiation application remains within the program, a greater percentage of the harvest is comprised of tubers within the 40-60 mm size category. The ability to target a particular size category can enable farmers to match fertiliser regime to market; for example large tubers for chipping, or small tubers for seed and salad.

INTRODUCTION

Whilst crop growth is broadly aligned to the amount of nitrogen fertiliser applied to it, evidence is accumulating that the form of the nitrogen (N) in which it is supplied – nitrate, ammonium, urea, amine – can significantly influence growth and yield even when the total amount of N supplied is the same. This is because plants fertilised with a particular form of N are characterised by a specific architectural appearance, or phenotype, generated by a specific set of N-dependent plant regulatory functions. For example nitrate stimulates leafy growth and apical dominance rather than lateral root production (Wilkinson *et al*. 2019a, b). In experimental conditions ammonium fertilisation gives rise to plants with a similar amount of total tissue biomass as those treated with nitrate, but more of this is found underground as root tissue, with less partitioned above ground (Andrews *et al.* 2013). Shorter plants with more roots are more resistant to stresses such as drought or lodging (Wilkinson *et al.* 2019a). Plants with more roots are also able to scavenge a greater volume of soil for the nutrients and water required for above ground growth at later developmental stages. However in the field it is hard to distinguish between these phenotypes, because several N forms are present at any one time, and because conventional fertilisers (ammonium nitrate or urea) are not environmentally stable, such that most of the N applied is degraded to nitrate, regardless of its original form.

We have demonstrated, in glasshouse experiments, that urea amine N gives rise to a third phenotype, or rather a range of phenotypes which alter with developmental stage (Wilkinson *et al.* 2019a, 2019b). This phenotype group (see below) has been difficult to isolate in past experimental analyses, because bacteria that break urea down are ubiquitous in soil and on leaf surfaces, even in compost used in pot-based indoor studies. Urea is converted to CO_2, ammonia and ammonium (and eventually nitrate) within hours to days of its application, whether it is added via soil or in foliar sprays (e.g. Hoult & McGarity, 1986). However, the fertiliser industry has developed techniques to stabilise urea and prevent some of its degradation, such that it is available in this form for longer, and lower levels of polluting breakdown products are emitted (see Wilkinson *et al.* 2019a). We have used a chemical method to stabilize ureic amine such that it persists in this form when applied to plants via soil or leaves, and we found that this third phenotype is readily generated in a range of species including potato. Yields of field grown Sassy (Marks *et al.* 2018), Shelford and Rooster (Wilkinson *et al.* 2019b) were increased in UK and Irish field trials by this technology (termed 'Limin', developed by Levity Crop Science, UK). We demonstrated experimentally that the yield increase was a result of the generation of the specific ureic amine phenotype in Casablanca (Wilkinson *et al.* 2019b), which is characterised as follows, in comparison to un-stabilised urea and/or conventional N fertiliser: increased root production, reduced stem elongation and increased chlorophyll content prior to / during tuberisation; and increased above-ground biomass and chlorophyll content at bulking. Higher yields correlated with root proliferation, slow shoot extension, and increased chlorophyll content prior to tuberisation; and with increased shoot biomass and increased chlorophyll content at bulking. We describe here how this technology can be used in the field to manipulate tuber size distribution within a Shelford crop, by changing the number and scheduling of its applications.

MATERIALS AND METHODS

Solanum tuberosum L. *cv*. Shelford (FL1625 x Hermes) was used in field trials designed to test the efficacy of four N fertilisation programs, on a commercial farm in 2016 in Hampshire, England, UK. The previous crop was wheat; soil type was sandy (no ridging required); beds were ploughed and de-stoned and fertiliser was broadcast at a rate of 180 kg N h^{-1} as urea. The crop was irrigated; and dates of crop treatments, from sowing to harvest; are described in Table 1. Five replicate plots (4.0 m^2) for each of four experimental treatment programs were laid out in a randomised block design: 1) commercial urea controls, 2) foliar stabilised amine nitrogen (SAN) applied four times (SAN x 4), 3) SAN applied three times, including at tuber initiation but excluding at late bulking (SAN x 3 + early), 4) SAN applied three times, excluding at tuber initiation but including at late bulking stage (SAN x 3 + late). Foliar SAN applications were carried out at a rate of 5.0 L ha^{-1} in 200 L water (0.1 mmol m^{-3}).

Maincrop marketable yield data (40-80 mm tubers) is presented as tuber number per hectare, and tuber yield weight per hectare (metric tonnes: t ha^{-1}). Tubers were graded into categories by size: 40-60 mm and 60-80 mm. Means and standard errors of yield data for the four treatment programs (control, SAN x 4, SAN x 3 + early, SAN x 3 + late) are displayed as bar charts. The significance of any difference from the control treatment was calculated using a one-tailed *t*-test for two independent means, and where treatments are significantly different from controls, (denoted by differing letters ‘a’, ‘b’, or ‘c’), the *p* value is displayed above the appropriate column on the graphic representations of the data.

Table 1. Chronology of field activities from sowing to harvest of crop (*Solanum tuberosum L. cv*. Shelford).

Date	Activity
03.05.2016	Fertiliser broadcast, crop sown
24.06.2016	SAN treatment 1 (omitted for SAN x 3 + late)
08.07.2016	SAN treatment 2
22.07.2016	SAN treatment 3
05.08.2016	SAN treatment 4 (omitted for SAN x 3 + early)
26.08.2016	Harvest

RESULTS

Fig 1 A demonstrates that all SAN treatment programs significantly increase mean marketable tuber number per hectare in comparison to conventionally fertilized controls, and that this is greatest when all 4 applications are included across the season (16.1 %). When only 3 applications are given, excluding the last, numbers are increased by 8.2 % (SAN x 3 + early); and when 3 applications are given, excluding the first, numbers are increased by 10.5 % (SAN x 3 + late). Fig 1 B shows that all 3 SAN treatment programs increase mean marketable yield weight (t ha^{-1}), but that this is only statistically significant, at 5.9%, when all 4 applications are included. When only 3 applications excluding the last are given, the increase is 2.6 %, and with 3 applications excluding the first, the increase is 4.8%.

Figure 2A shows that, when only the smaller size category of 40-60 mm tubers is included in the analysis, tuber numbers are increased significantly in all SAN treatment programs, this being greatest when all 4 applications are included (32.1 %). When only 3 applications are included, excluding the last, numbers are increased by 16.7 %, and when the first application is excluded, numbers are increased by 9.8%. Figure 2B shows that yield weight is increased the most in the SAN x 4 treatment program, by 18.3%, and this is a significant result. When the final application is excluded from the treatment regime (SAN x 3 + early), yield weight is also significantly increased, by 10.4 %. However when the first application is excluded from the program (SAN x 3 + late), total yield weight of smaller tubers is not increased at all, even though there are greater numbers of these than in controls (Fig 2A).

Fig 3 shows the effects of the SAN treatment programs on number and weight of tubers in the larger 60-80 mm range category. Only the treatment program supplying 3 SAN applications that excludes the earliest (SAN x 3 + late) gives rise to an increase in tuber numbers (3A) in comparison to control treatments, of 11.6%, although this is not significant.

The SAN x 4 and the SAN x 3 + early treatment programs slightly decrease numbers of tubers in this larger category. The SAN x 3 + late treatment regime significantly increases 60-80 mm tuber numbers compared to the treatment program that includes all 4 SAN applications across the season, by 19.1 %. It significantly increases tuber number in comparison to the program that excludes the late application, by 16.7 %. Figure 3B demonstrates the effects of treatment programs on yield weight of the larger tuber category. Whilst there are no significant differences between all 4 programs, there is an increase in the total weight of large tubers compared to all other treatments, when the first SAN application is excluded. This increase is 11.9% compared to controls, 17.4 % compared to SAN x 4, and 16.5 % compared to the program which excludes the latest application (SAN x 3 + early).

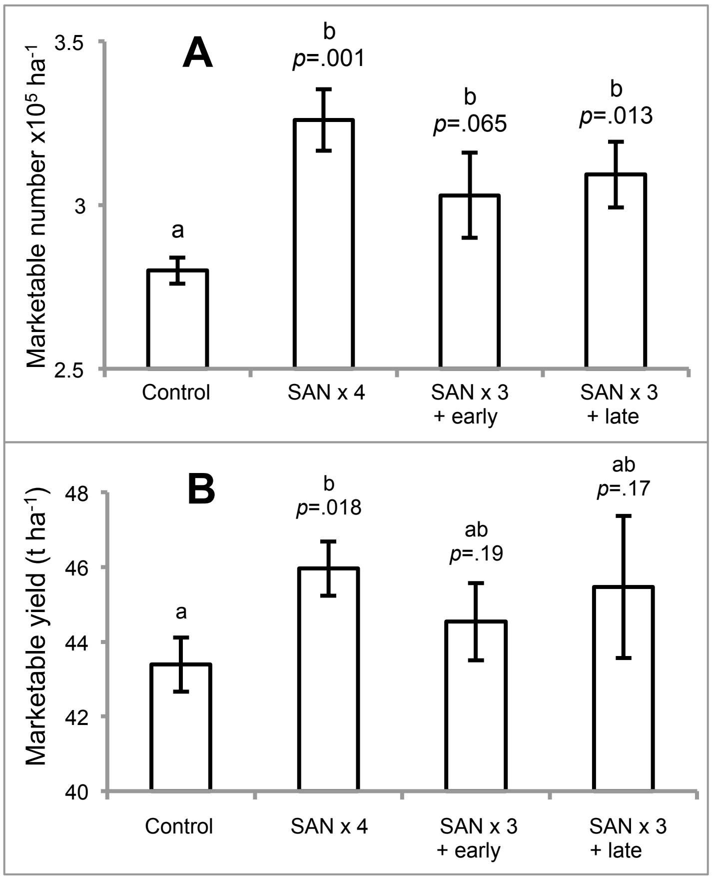

Figure 1. Effect of foliar SAN application program on marketable tuber number (A) and yield (B) compared to conventionally fertilized controls. SAN was applied either 3 (SAN x 3) or 4 times (SAN x 4). Means ± standard errors are depicted; significance (*p*) of differences from the control are shown above columns.

Figure 4 details the composition of the SAN-induced changes in yielding (positive or negative) from the control. The positive change induced by the inclusion of the early SAN application consists entirely of a greater number and total weight of small (40-60 mm) tubers, both in the SAN x 4 and the SAN x 3 + early regimes. However this is slightly off-set by a reduction in the number and total weight of large (60-80 mm) tubers, even when the bulking stage final application is included (SAN x 4). On the other hand the positive change in tuber number resulting from the exclusion of the initial SAN application, and the inclusion of the late application, consists of both large and small tubers. However in terms of yield as weight, 100% of the positive change in the SAN x 3 + late treatment consists of 60-80 mm tubers.

The weight of smaller tubers is slightly reduced. Retention of the final SAN application within the SAN x 4 program, could not override an effect of the inclusion of the initial application to set final tuber size at 40-60 mm, in 100% of the SAN-induced yield increase.

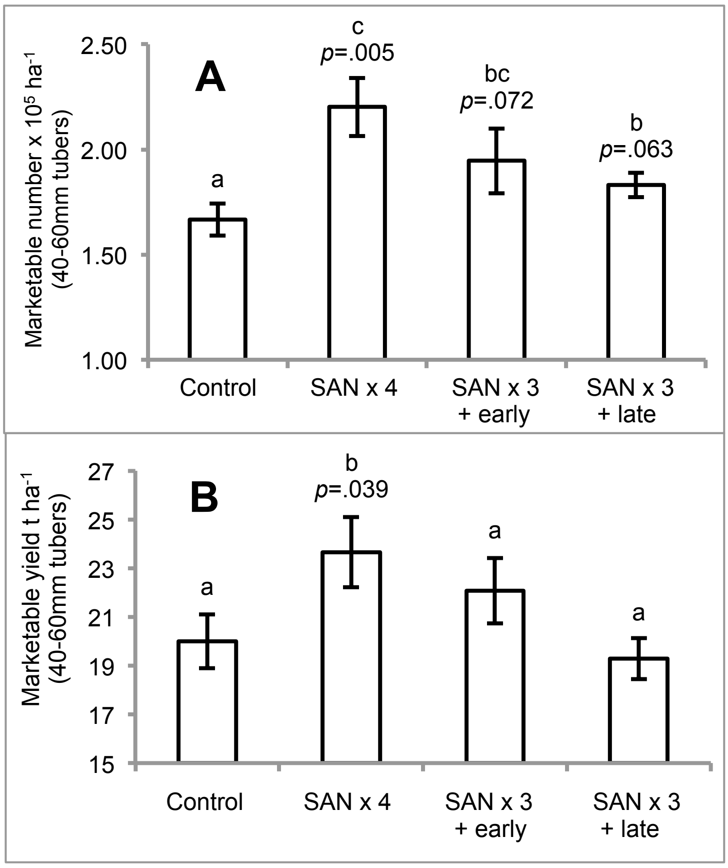

Figure 2. Effect of SAN application program on marketable tuber number (A) and yield (B) within the 40-60 mm tuber size category, compared to conventionally fertilized controls.

DISCUSSION

Manipulation of tuber size distribution within a crop is an important agronomic aim when targeting a specific market (crisping, frying, salad). Both seed potato characteristics and field management practices can affect tuber size and/or size distribution at yield. Seed potato influences include weight, physiological age, and apical dominance (reflected by shoot number). Seed planting density and depth, and timing of haulm destruction can be controlled in the field (e.g. Knowles & Knowles, 2015; Struik *et al*. 1990). Here we describe a novel technique for manipulating tuber size distribution within an increased overall yield, which consists of the use of specified application schedules of stabilized amine nitrogen (SAN) over the course of the growing season. Namely, inclusion of an early, pre-tuberisation application of SAN within the fertilization program gives rise to yields consisting of a high percentage of smaller tubers (40-60 mm); and exclusion of an early application is necessary for the bulking stage application to increase the percentage of large (60-80 mm) tubers. The shift in yield content to large tubers (SAN x 3 + late) does not come at the price of a smaller yield number or weight *per se*; rather, yield is still increased over and above that attained using standard agricultural practice.

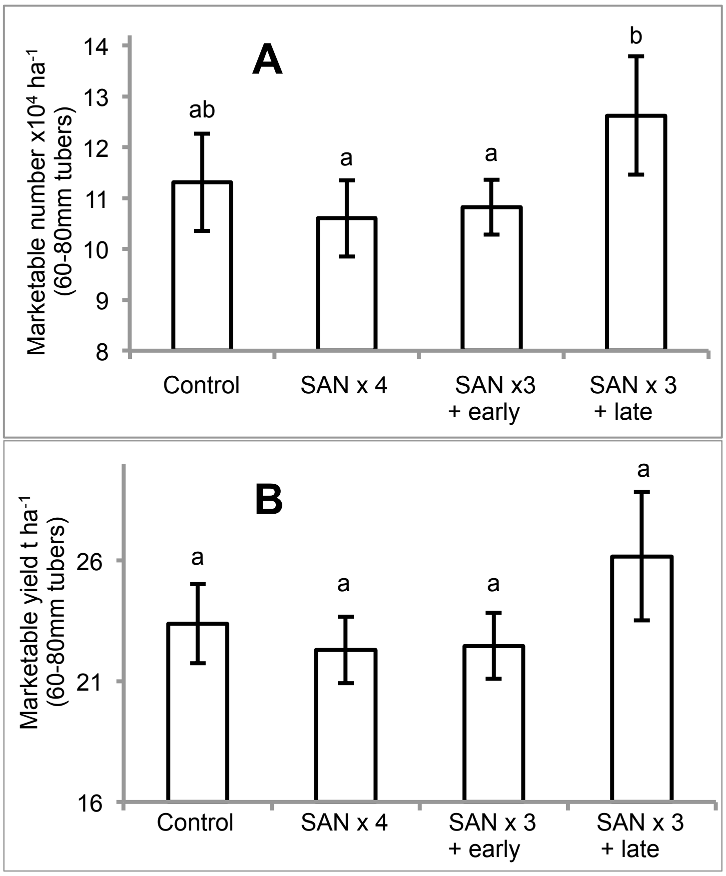

Figure 3. Effect of SAN application program on marketable tuber number (A) and yield (B) within the 60-80 mm tuber size category, compared to conventionally fertilized controls.

Effects of nitrogen fertilization programs designed to test whether tuber size distribution within a potato crop could be influenced have previously been reported. For example, Qsaki *et al.* (1995, field) and Gao *et al.* (2014, greenhouse) demonstrated that final yield in terms of weight was unaffected by nutritional N form when comparing nitrate and ammonium N. Differences in tuber size distribution were nevertheless apparent: nitrate nutrition led to the development of many small tubers; and ammonium nutrition induced the formation of large tubers, although these were less numerous. More stolons (specialized underground shoots) were generated pre-tuberisation under nitrate nutrition, increasing the number of tubers initially set, because this N form is partitioned to, and assimilated within shoot tissue (Qiqige *et al*. 2017). N sourced from ammonium is preferentially used for root growth, such that less N is available for stolon growth, and fewer tubers are formed. At bulking, later in the season, above-ground vegetative growth continued to be stimulated by nitrate in comparison to ammonium nutrition, and shoots attained a greater size. The authors hypothesized that little resource remained to allow all the tubers set earlier in the season to bulk to marketable size. On the other hand, ammonium nutrition increased tuber bulking compared to nitrate treatment, albeit in the lower number of tubers set under this N form, as above-ground growth was comparatively limited later in the season, freeing resource for tuber growth.

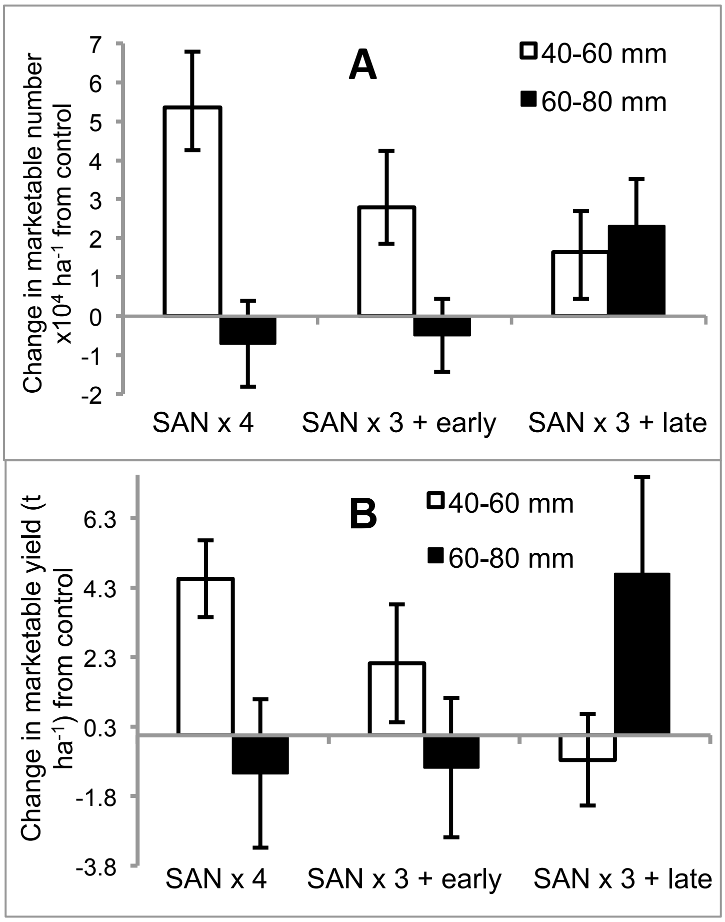

Figure 4. Effect of SAN application program on the change in marketable tuber number (A) and yield (B) from control values, within both the 40-60 mm and 60-80 mm tuber size categories.

We show here that pre-tuberisation stage SAN applications increase numbers of smaller tubers at yield in comparison to conventional fertilization (Figs 1-4), however we propose that this occurs via a different mechanism to the nitrate-induced effect described above. Wilkinson *et al*. (2019b) demonstrated that SAN nutrition increased root biomass and reduced shoot extension at this stage in Casablanca, compared to ammonium nitrate and un-stabilised urea, implying that a reduction in apical dominance was being induced (see also Wilkinson *et al.* 2019a). This reflects some interesting findings described by Dean *et al.* (2018), where seed treatments with plant hormones also altered apical dominance, which in turn affected tuber size. Application of Gibberellic Acid (GA) induced early shoot emergence in Alturas and Payette Russet, also decreasing apical dominance, which shifted tuber size distribution towards smaller classes. However inclusion of the auxin NAA in the seed treatment maintained apical dominance, and prevented the shift in tuber size to the smaller class. However, this begs the question: how do SAN treatments that include an early application provide enough resource to increase both root biomass (Wilkinson *et al*. 2019b) and tuber numbers; and therefore overall yield? The answer lies in the fact that the uptake and processing of urea and amide in root cells is much more resource efficient than assimilation and processing of either nitrate (in shoots), or ammonium (in roots) (see Wilkinson et al. 2019a, b). Thus, whole-plant pools of N-based protein, and chlorophyll content for photosynthetic provision of carbon resource, are increased. As the crop matures and tubers start to bulk, we surmise that the larger root system developed by SAN-treated plants, that persists from the early application (Wilkinson *et al.* 2019a, b), provides more soil-sourced resource in the form of a range of nutrients, as well as N, and water, to the shoots. As a result vegetative tissue also proliferates under SAN treatment, and provides enough resource to bulk all the tubers set earlier in the season, at least to the marketable 40-60 mm size category.

In the case of the SAN x 3 + late program, the improved yield in comparison to all other treatments comprised of a larger percentage of 60-80 mm tubers. We conclude that the absence of a SAN treatment prior to tuberisation means that the seed tubers that expressed an innate tendency for apical dominance (assuming that a portion of these were present even within a relatively uniform seed batch) generated fewer tubers per plant. These will inevitably grow large enough to be part of the 60-80 mm size category as a result of the final SAN application at bulking, as all SAN-treated plants (with or without seed-induced apical dominance) generate more total resource throughout the growing season than plants supplied with other N sources, as described above, as a result of the increased internal nitrogen utilization efficiency of urea amine (Wilkinson *et al.* 2019a, b).

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