Irish Journal of Agricultural and Food Research **53:** 91–99, 2014

A note on the effectiveness of selenium supplementation of Irish-grown *Allium* crops

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Onions and other *Allium* crops contain high levels of dietary phenolics and, unlike many other crops, accumulate the beneficial mineral selenium. Selenium-enhanced *Allium* crops are of interest both from a public good perspective and as a market positioning strategy for growers. Field trials were carried out to i) identify onion and scallion varieties that contain high levels of health-promoting phenolic and flavonoid compounds as potential targets for selenium supplementation and ii) investigate selenium supplementation in the widely-grown commercial onion variety 'Hyskin' at different application rates of nitrogen fertilizer. Levels of selenium in onion bulbs were significantly increased from 0.5–5.9 µg/g dry weight (DW) to 40.6–70.0 µg/g DW.

Keywords: Allium; bioactive compounds; flavonoids; phenolics; selenium

Introduction

Bulb onions and scallions rank among the top ten Irish grown field vegetables in area and market value (Bord Bia 2009). Production area has increased in recent years although it is difficult for growers to compete with imports on production price. The genus *Allium* includes bulb onions (*Allium cepa*); shallots (*Allium cepa* var. *aggregatum*); Welsh onion (*Allium fistulosum*); garlic (*Allium sativum*); leek (*Allium porrum*); and chives (*Allium schoenoprasum*). According to the most recent census 359 ha were planted to onions, leeks, shallots and scallions in Ireland in 2008 with a farm gate value of over €7.7 million (Bord Bia 2009). Two classes of phytochemical found in onion and other *Alliums* show health-promoting activity. These are phenolic compounds (in particular

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the flavonols); and sulphur containing alk(en)yl sulfoxides. The main flavonols found in onion are quercetin, kaempferol and their glucosides (Desjardins 2008). Quercetin and its derivatives make up over 90% of the flavonol content in onion. Anthocyanins also occur in red onions (Slimestad, Fossen and Vagen 2007; Desjardins 2008). Numerous studies show significant health benefits associated with *Allium* consumption. These include reduced risks of cancer and cardiovascular disease, reduced inflammatory response, anti-asthmatic, anti-diabetic, antioxidant, antibiotic and antifungal properties (Patil, Pike and Hamilton 1995a; Lanzotti 2006; Desjardins 2008). The precise mechanisms are unclear and the antioxidant hypothesis in chemoprevention has recently been challenged (Watson 2013) but there is evidence that quercetin can function as an antioxidant, radical scavenger and chelating agent. Antibiotic and antifungal activities of onion are thought to be primarily attributable to the alk(en)yl sulfoxide isoalliin, whilst anti-asthmatic properties have been attributed to thiosulphonate compounds (Arai *et al*. 2000; Desjardins 2008).

Selenium is an essential mineral for humans and animals with deficiency symptoms if intake is insufficient. In animals, deficiency diseases include white muscle disease and ill-thrift in calves and lambs and hepatosis dietetica in pigs (Oldfield 2002). In humans, selenium is required for immune functioning and deficiency diseases include Keshan disease and Kashin-Beck disease (Broadley *et al*. 2006). Health-promoting effects have been reported for intake levels above nutritional requirement levels (Finley, Davis and Feng 2000; Finley *et al.* 2001; Broadley *et al.* 2006; Finley 2007; Lavu *et al*. 2012). The UK recommended daily intake of selenium for adults is 50–70 µg. Selenium is toxic at high levels and the recommended tolerable upper limit is set at 400 µg per day. Selenium levels are low in many soils worldwide, including China, Russia and parts of Europe. Human dietary intake is often low and reported adult intake in the UK is estimated at 35 µg per day. Incorporation of selenium into multinutrient fertilizers for agriculture has been required in Finland since 1984 to address low dietary intake (reviewed in Broadley *et al.* 2006). Globally, soil selenium concentration ranges from extremely low up to 100 ppm (Oldfield 2002). In alkaline well-drained soils it often occurs as selenates (Se^{6+}) which are highly available to plants (Broadley *et al.* 2006) and in some regions can accumulate to toxic levels. In acid and poorly-drained soils, however, selenium often occurs in a ferric (iron) selenate form which is less biologically available (Oldfield 2002). In most plant species, high levels of selenium are toxic to the plant; however, *Brassica* and *Allium* species are able to utilise selenium and are referred to as seleniferous plants or 'selenium accumulators' (Irion 1999). Selenium is taken up via the sulphur assimilation pathway and in non-tolerant plants is metabolised to seleno-methionine (Se-Met) and seleno-cysteine (Se-Cys) (analogues of the sulphur-containing amino acids). Non-specific incorporation of these analogues into plant proteins causes aberrant protein secondary structure and consequent toxicity to the plant (Broadley 2006). In contrast, in selenium accumulator plants, seleno-cysteine is methylated to methylseleno-cysteine (MeSeCys) which prevents aberrant protein formation (Terry *et al.* 2000; Broadley *et al*. 2006). In human health, methylseleno-cysteine (MeSeCys) (the dominant form of selenium in *Brassica* and *Allium* crops) shows better health-promoting biological effects (bioactivity) than seleno-methionine (Se-Met) which is the predominant form in non-accumulating crops (Finley *et al.* 2001; Broadley *et al.* 2006) due to its incorporation into essential selenoproteins.

Broccoli contains the important bioactive compound sulforaphane, and attempts have been made to increase levels of both sulforaphane and selenium in broccoli. These indicate however that there is an inverse relationship between selenium and sulforaphane accumulation (Finley *et al.* 2005; Robbins *et al.* 2005). An antagonistic interaction between sulphur (S) and selenium uptake has been reported in *Brassicas* (White and Broadley 2009); although in onions a synergistic effect is noted with sulphur uptake enhanced by selenium availability (Kopsell and Randle 1997). In a study on hydroponically-grown onions, increased nitrogen (N) fertilization had a significant effect on flavour compounds, resulting in lower onion bulb boron, calcium, and magnesium content and a quadratic increase in bulb sulphur and potassium (K) content. Additionally onion flavour and pungency compounds (pyruvic acid and cysteine sulfoxides) generally increased with increasing N, although a decline was seen at very high nitrogen levels (Randle 2000). A number of recent studies examined the effect of selenium supplementation on crops including *Alliums* and on sprouted seeds and mushrooms (Pyrzynska 2009; Arscott and Goldman 2012; Lavu *et al.* 2012). To our knowledge, however, only three selenium-enhanced crops (potatoes, tomatoes and Brussels sprouts) have been developed commercially in the UK and Ireland (M&S 2011).

The objective of the present study was to determine the effectiveness of selenium supplementation of *Allium* crops grown in Ireland. Several studies on onion and other cultivated *Allium* crops have shown that levels of phenolic and flavonoid compounds can vary between different varieties (Patil, Pike and Yoo 1995b; Marotti and Piccaglia 2002; Yang *et al.* 2004). In this study, levels of total phenolics and flavonoids in 10 scallion and 24 onion varieties (22 bulb and two shallot types) were evaluated in 1-year trials in order to identify any differences in dietary polyphenol content between varieties. Subsequently a selenium supplementation trial was carried out using the onion variety 'Hyskin' which is widely grown as a commercial crop in Ireland. The latter experiment was repeated over 2 years to account for climatic variation and selenium application was used at two N fertilizer rates to investigate any interaction between N and selenium.

Materials and Methods

Field trial experiments

Field trials were carried out at Teagasc, Kinsealy (53°25'N, 6°10'W) in north county Dublin, Ireland. Following a soil test, N, phosphorus (P) and potassium (K) were applied according to Teagasc recommendations (Lalor and Coulter 2008). Fertilizer was applied as calcium ammonium nitrate (CAN), single super-phosphate and sulphate of potash. Onions and scallions were sown as multi-seed modular transplants and transplanted at 3 rows per 60 inch (1.52 m) bed with 25 cm in-row spacing. Weed and pest control treatments were in accordance with commercial growing practices (Alexander 2011). Scallion and onion variety trials were carried out for one season only and were a randomised complete block design with six and three replicates, respectively. For the selenium supplementation experiment, a factorial design with two levels of N fertilizer application (70 and 140 kg/ha) with or without selenium treatment was used (n=3). The trial was repeated over 2 years to account for climatic variation. Nitrogen fertilizer treatments were applied as a base dressing of 70 kg/ha with two 35 kg/ha top dress applications in June and July of both years. For the selenium treatment 100 mL of a treatment solution (3.8 L distilled water containing 1.5 g each of sodium selenate and sodium selenite) was applied around the base of treatment plants with an equivalent amount of distilled water applied to control plants. Treatments were applied twice at 4 and 2 weeks before expected harvest date. Climatic conditions during the growing season for trial years are shown in Table 1. Samples for analysis were composite samples comprising 3 bulbs or 3 trimmed scallions from each plot.

Quantification of total phenolics, total flavonoids and selenium

For the determination of total phenolics a modification of the Folin-Ciocalteau method (Lowry *et al.* 1951) as described in Reilly *et al.* (2014) was used. The absorbance of methanolic extracts at 725 nm (A_{725}) was determined relative to a blank containing 80% (v/v) methanol instead of extract, and the concentration was determined from a calibration curve using gallic acid as a standard. Results are expressed as gallic acid equivalents [GAE mg/100 g fresh weight (FW)]. Determination of flavonoids was according to Marinova, Ribarova and Atanassova (2005). The absorbance at 510 nm (A_{510}) was determined from methanolic sample extracts, and flavonoid concentration was determined from a standard curve using catechin as a standard. Results are expressed as catechin equivalents (CE mg/100 g FW). For selenium analysis, onion samples were freeze-dried in an analytical freeze-drier (Labconco Freezone 2.5, Kansas, USA) and transferred to Eurofins Food Testing UK Ltd (Wolverhampton) for accredited selenium analysis.

Data analysis

Statistical analyses of data were carried out with SAS 9.3 (Cary, NC). Variety and selenium trials were analysed using an ANOVA linear or mixed model, respectively, with Tukey adjustment. For the linear model ANOVA, variety (var) and block were the main effects. For the mixed model ANOVA, N, selenium and their interaction were the terms in the model.

Results and Discussion

Onion and scallion variety trials

Levels of total phenolics ranged from 98.1 GAE (mg/100 g FW) in the heritage

Table 1. Climatic conditions during the 2010 and 2011 growing seasons

2010	T	TM	Tm	H	PP	V	RA	2011	T	TM	Tm	H	PP	V	RA
May	10.2	14.7	4.8	78.3	29.2	15.3	20	May	11.3	15.1	7.8	75.3	37.1	27.4	28
June	14.5	19.2	8.9	78.8	57.4	14.5	17	June	12.3	16.9	7.1	79	63.5	17.6	24
July	15.7	19.6	12.1	80.4	**77**	19.5	28	July	14.2	18.4	9.2	79.1	41.2	16	23
August	14	18.3	9.6	80.8	47.5	17	22	August	13.6	17.9	9.2	81	35	16.5	25
September	13.2	17.1	9.1	84.7	**106**	17.7	22	September	14	17.5	10.6	80.5	53.1	23.8	24

T = Mean temperature (°C), TM = Average maximum temperature (°C), Tm = Average minimum temperature (°C), H = Mean humidity (%), PP = Precipitation amount (monthly total) (mm), V = mean wind speed (km/h), RA = Indicator for occurrence of rain or drizzle (days).
Excessive monthly rainfall values in July and September 2010 are shown in bold.

bulb onion variety 'De La Reine' to 565.5 GAE (mg 100 g^{-1} FW) in 'Wellington' (Table 2). Total flavonoids ranged from 45.3 to 152.5 CE (mg/100 g FW) depending on cultivar. These data are in agreement with previously reported contents of phenolic compounds in onion (Patil *et al.* 1995b; Slimestad *et al*. 2007). As noted elsewhere (Marinova *et al.* 2005; Desjardins 2008), levels are high compared to reported values for other vegetable crops, and onions provide an excellent source of dietary polyphenols. Variety had a significant effect on phenolic but not flavonoid content and individual phenolic content was significantly different only for 'Wellington' compared to 'De La Reine'.

It has been suggested that older and landrace crop varieties could contain higher levels of bioactive compounds (for review see Leonti 2012 and references therein; Reilly 2013 and references therein). In this study, however, modern commercially-grown varieties generally contained higher levels of phenolics and flavonoids, although as noted above the differences were not statistically significant in most instances. This is likely due to the high variability between replicates for many of the varieties. A larger trial with additional replicates would give more sensitive discrimination between varieties and would be of interest. However it is clear that currently grown commercial varieties would

Table 2. Total phenolic and total flavonoid content in bulbs of 24 cultivated onion and shallot varieties

Variety	Total phenolics (GAE mg/100 g FW)	Total flavonoids (CE mg/100 g FW)
Wellington	565.5±174.9[a]	139.7±35.1[a]
Hyfort	488.7±156.7[ab]	104.5±36.4[a]
Hypark	485.7±55.8[ab]	106.1±15.8[a]
Balstora	477.3±72.9[ab]	117.3±13.7[a]
Hyskin	462.6±68.2[ab]	101.3±1.6[a]
Sprinter	437.1±88.8[ab]	96.5±23.9[a]
Hybing	427.4±65.4[ab]	77.3±4.2[a]
Hybelle	415.4±103.3[ab]	98.1±38.4[a]
Redspark	405.6±19.9[ab]	146.1±20.8[a]
Hytech	405.3±73.5[ab]	104.5±28.9[a]
Lagergold	385.5±27.8[ab]	117.3±30.5[a]
Napoleon	378.5±70.6[ab]	85.3±22.2[a]
Iceni	373.1±51.1[ab]	152.5±69.8[a]
Ambition	354.4±81.3[ab]	117.3±23.9[a]
Buan	343.2±81.9[ab]	99.7±19.2[a]
Reliance	339.6±65.9[ab]	136.5±23.1[a]
De Mazeres	321.9±55.4[ab]	99.7±20.9[a]
Exhibition	275.7±72.3[ab]	88.5±21.0[a]
Allina	272.0±36.4[ab]	96.5±23.1[a]
Zittauer Gelbe	257.2±83.2[ab]	86.9±35.1[a]
Picador	245.5±28.6[ab]	75.7±8.3[a]
HRI 5991	186.9±35.3[ab]	58.1±16.0[a]
Ailsa Craig	118.5±4.1[ab]	62.9±21.5[a]
De La Reine	98.1±2.4[b]	45.3±15.3[a]

Data shown are mean±s.e. Means with the same letter within columns were not significantly different ($P<0.05$). Heritage and landrace varieties are shown in bold. Shallot types (*Allium cepa* var. *aggregatum*) are in italics.

be suitable targets for supplementation with selenium, to produce a "functional" food crop that is high in both selenium and antioxidant polyphenols.

Bunching onions or scallions are harvested as immature onions prior to bulb filling and may be *A. cepa, A. fistulosum* or crosses *A. cepa* × *fistulosum* depending on the variety. Levels of phenolics and flavonoids in 10 scallion varieties are shown in Table 3. In this trial, variety had a significant effect on both phenolic and flavonoid content (P<0.0001). Levels of total phenolics were in the range 72.7 to 343.8

Table 3. Total phenolic and total flavonoid content in leaf tissue of 10 scallion varieties

Variety	Total phenolics (GAE mg/100 g FW)	Total flavonoids (CE mg/100 g FW)	Species
Napoleon	343.8±35.0[a]	178.0±10.5[a]	*A. cepa*
Wellington	228.6±45.3[b]	161.9±10.0[a]	*A. cepa*
Sprinter	171.4±29.6[bcd]	135.8±14.0[ab]	*A. cepa*
White Lisbon	186.0±24.9[bc]	132.0±11.7[ab]	*A. cepa*
Ramrod	72.7±13.5[d]	122.9±19.0[ab]	*A. cepa*
Apache	157.7±15.3[bcd]	122.5±17.1[ab]	*A. cepa*
Eiffel	101.2±18.0[cd]	95.3±12.6[bc]	*A. cepa*
Guardsman	107.4±22.4[cd]	91.6±15.3[bc]	*A. cepa* × *fistulosum*
Summer Isle	114.0±19.7[cd]	46.0±6.4[c]	*A. fistulosum*
Sentry	112.3±19.9[cd]	31.8±6.0[c]	*A. fistulosum*

Data shown are mean±s.e. Means with the same letter within columns were not significantly different (P<0.05).

Table 4. Mean bulb weight, total phenolic, total flavonoid and selenium content in bulbs of onion 'Hyskin' in 2010 and 2011

Year	N (kg/ha)	Se	Mean bulb weight (g)	Total phenolics (GAE mg/100 g FW)	Total flavonoids (CE mg/100 g FW)	Selenium (µg/g DW)
2010	70	–	213.3±13.1[a]	583.1±157.5[a]	47.8±11.4[a]	0.6±0.2[b]
	70	+	230.0±21.1[a]	663.2±133.4[a]	61.3±14.1[a]	45.3±7.9[a]
	140	–	204.6±9.2[a]	630.6±31.2[a]	63.2±11.5[a]	0.5±0.2[b]
	140	+	198.4±14.4[a]	532.7±72.5[a]	51.0±7.2[a]	40.6±10.8[a]
ANOVA P values						
	N		0.2047	0.7528	0.8486	0.695
	Se		0.7225	0.9460	0.9608	**0.0003**
	N *Se		0.4524	0.5063	0.3523	0.7070
2011	70	–	120.0±8.8[b]	654.3±183.1[a]	81.3±13.0[a]	5.9±4.8[b]
	70	+	123.3±3.8[b]	841.1±26.4[a]	82.5±0.9[a]	55.4±6.1[a]
	140	–	223.3±23.1[a]	571.3±172.8[a]	58.7±8.8[b]	1.0±0.1[b]
	140	+	246.7±22.2[a]	565.3±153.3[a]	56.1±9.8[b]	70.0±8.2[a]
ANOVA P values						
	N		**0.0001**	0.3228	**0.0471**	0.1817
	Se		0.3563	0.6067	0.9436	**<0.0001**
	N *Se		0.4820	0.5839	0.8544	0.1522

Data shown are mean±s.e. Means with the same letter within columns were not significantly different (P<0.05). ANOVA P values <0.05 are shown in bold.

GAE (mg 100 g^{-1} FW) and flavonoids ranged from 26.0 to 178.0 CE (mg 100 g^{-1} FW) depending on cultivar. Flavonoid content was higher in *A. cepa* types. Levels of flavonoids in the scallions were generally higher than levels found in onion bulbs. These data indicate particular scallion varieties could be selected to develop bioactive-enriched crops and would be an excellent source of flavonoids.

Fertilizer trial

Levels of total flavonoids and total phenolics in bulbs of the onion variety 'Hyskin' ranged from 51.0–82.5 CE (mg/100 g FW) and 532.7–841.1 GAE (mg/100 g FW), respectively (Table 4). Detected levels of total phenolics and flavonoids were not affected by selenium application in either year. Levels of flavonoids were reduced by high N application rates in 2011 ($P<0.05$) but not in 2010. Similarly mean bulb weight was increased by N application rate in 2011 but not in 2010. It is likely that the extremely heavy rainfall during the trial in 2010 (Table 1) would have resulted in leaching and movement of N in the soil which may account for these differences between years.

Base levels of selenium in onions which did not receive supplementation treatments were in the range 0.5–5.9 µg/g dry weight (DW). Levels were boosted significantly to 40.6–70.0 µg/g DW by two selenium supplementation treatments during the growing season. Similar levels of selenium in enhanced *Brassica* sprouts were previously shown to have anti-carcinogenic effects in animal intervention studies (Finley *et al.* 2001). Levels of selenium in onion bulbs were not affected by N fertiliser in our study and differences were consistent between years. These data indicate that relatively simple interventions could be used to increase the bioactive potential in Irish grown onions. As noted previously, selenium accumulating plants such as onions would be expected to contain selenium in the optimal methylseleno-cysteine (MeSeCys) form (Broadley *et al*. 2006) which would be desirable for potential health promoting effects. Our data indicate that widely grown commercial bulb onion varieties such as 'Hyskin' already contain high levels of dietary phenolics and could be easily supplemented to produce "functional" onions high in both selenium and antioxidant polyphenols. Data from the scallion variety trials indicate that scallion varieties with high flavonoid content could be identified as optimum targets for selenium supplementation and this warrants further investigation.

Acknowledgements

The authors thank Christy Roberts and Philip Dunne for assistance in implementing and maintaining the trials, and Warwick HRI and Irish Seedsavers for seed of traditional onion varieties. This study is funded by the Department of Agriculture and Food through the Network and Team Building Initiative of the Food Institutional Research Measure (FIRM Reference Number 06/NITARFC6).

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Received 11 January 2013