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Agronomic biofortification of maize with selenium (Se) in Malawi

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ABSTRACT

Suboptimal dietary Se intake is widespread in Malawi due to low levels of plant-available Se in most soils and narrow food choices. The aim of this study was to determine the potential for biofortifying maize using Se-enriched fertilisers in Malawi. The response of maize to three forms of selenate-Se fertiliser was determined. Crops were treated with a liquid drench of $Na_2SeO_{4(aq)}$ (0–100 g Se ha^{-1}), a compound NPK + Se fertiliser (0–6 g Se ha^{-1}), or Se-enriched calcium ammonium nitrate (CAN + Se; 0–20 g Se ha^{-1}). Experiments with $Na_2SeO_{4(aq)}$ and NPK + Se were conducted at six field sites, and at a subset of three sites with CAN + Se, in 2008/09 and 2009/10 (i.e. 30 experimental units). The increase in grain Se concentration was approximately linear for all Se forms and application rates ($R^2 > 0.90$ for 27 of the 30 experimental units). On average, whole-grain Se increased by 20, 21 and 15 μg Se kg^{-1} for each gram of Se applied as $Na_2SeO_{4(aq)}$, NPK + Se and CAN + Se, respectively. Grain and stover yields were unaffected by Se applications. An application of 5 g Se ha^{-1} to maize crops in Malawi would increase dietary Se intake by 26–37 μg Se $person^{-1}$ d^{-1} based on national maize consumption patterns. Agronomic biofortification with Se in Malawi is feasible in theory through the existing national Farm Input Subsidy Programme (FISP) if deemed to be economically and politically acceptable.

1. Introduction

Selenium (Se) is an essential element for humans and is derived primarily from dietary sources (Fairweather-Tait et al., 2011). Habitual suboptimal dietary Se intake leads to reduced Se status, which is associated with a range of adverse health outcomes including cardiovascular disorders, impaired immune function, and some forms of cancer. In Malawi, where subsistence agriculture is widespread and food choices are relatively narrow, there is evidence of widespread suboptimal dietary Se intakes (Donovan et al., 1992; Eick et al., 2009; Chilimba et al., 2011) and status (van Lettow et al., 2004). In Malawi, over 50% of dietary calorie intake (2,172 kcal $person^{-1}$ d^{-1}; 2007 data; FAO, 2011) is derived from maize grain, equating to 0.354 kg person d^{-1} based on trade and production statistics (FAO, 2011). Consumption of animal products with higher Se concentrations (fish, meat, offal, fats, milk and eggs) accounts for just 64 kcal $person^{-1}$ d^{-1} (FAO, 2011). From nationwide surveys of farmers' fields, the median maize grain Se concentration of 0.019 mg Se kg^{-1} (range 0.005–0.533 mg Se kg^{-1}) represents an intake of only 6.7 μg Se $person^{-1}$ d^{-1} from maize based on national consumption patterns (Chilimba et al., 2011). Low Se concentrations in edible crop material produced in Malawi are due to the widespread occurrence of highly weathered acid soils with low total and plant-available Se concentrations. In these soils, most Se is likely to be present in organic and mineral-occluded forms which are not directly available to plants, with most of the remainder present as strongly adsorbed $Se^{(IV)}$ species, which are poorly available compared to $Se^{(VI)}$ (Chilimba et al., 2011).

Suboptimal Se intake can be addressed through dietary diversification, food imports, supplements, food fortification and biofortification (Rayman, 2004, 2008; Broadley et al., 2006, 2010; Fairweather-Tait et al., 2011). Dietary diversification is an attractive option in terms of general protein, mineral and vitamin intake. In Burundi, greater consumption of fish, meat and offal among more affluent groups has been linked to higher Se intakes (Benemariya et al., 1993). However, access to diverse diets is not possible in many socio-economic contexts. Similarly, despite clear links between the Se composition and the geographic origin of staple foods such as wheat and rice (Thomson, 2004; Williams et al., 2009; Johnson et al., 2010; Fairweather-Tait et al., 2011), altering trade patterns is undesirable in many contexts. Supplementation of diets or foodstuffs with inorganic or organic forms of Se is again feasible (Rayman, 2004), although the production and equitable distribution of Se supplements is logistically challenging and expensive, and robust

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Table 1
Site location, soil characteristics and type of Se fertiliser applied.

Site	Soil Se concentration (mg kg^{-1})[a]	Selenium form	Location (°) (Lat., Long.)	Soil type[b]	Texture class[c]	pH[d]	OM (%)[e]	Fertiliser applied (kg ha^{-1})[f]		
								N	P_2O_5	K_2O
Bvumbwe (Dwale)[g]	0.288	Na_2SeO_4	−15.92, 35.07	Chromic Luvisols	sl	5.2	1.08	92	20	10
		NPK + Se						100	20	20
		CAN + Se						130	45	23
Chitala (Chinguluwe)	0.362	Na_2SeO_4	−13.68, 34.28	Chromic Luvisols	scl	5.6	2.38	92	20	10
		NPK + Se						100	20	20
Chitedze (Chitsime)	0.300	Na_2SeO_4	−13.98, 33.63	Chromic Luvisols	scl	5.9	2.03	92	20	10
		NPK + Se						100	20	20
		CAN + Se						130	45	23
Makoka (Thondwe)	0.272	Na_2SeO_4	−15.52, 35.22	Chromic Luvisols	scl	5.4	1.87	92	20	10
		NPK + Se						100	20	20
Mbawa (Mbawa)	0.124	Na_2SeO_4	−12.12, 33.42	Haplic Luvisols	ls	5.7	1.86	92	20	10
		NPK + Se						100	20	20
Ngabu (Mikalango)[h]	0.217	Na_2SeO_4	−16.60, 34.35	Eutric Vertisols	c	7.9	2.64	92	20	10
		NPK + Se						100	20	20
		CAN + Se						130	45	23
Kasinthula (Mitole)[i]	0.197	Na_2SeO_4	−16.05, 34.81	Eutric Vertisols	sl	7.4	2.95	92	20	10
		NPK + Se						100	20	20
		CAN + Se						130	45	23

[a] Total soil Se.
[b] FAO classification (Green and Nanthambwe, 1992).
[c] sl = sandy loam, scl = sandy clay loam, ls = loamy sand, c = clay.
[d] Water.
[e] Organic matter.
[f] Na_2SeO_4, uniform NPK (base, 23:10:5 + 3S) and N (top, urea); NPK + Se (25:5:5 + 0.0012), varying Se splits; CAN + Se, uniform NPK (base 23:10:5 + 3S) and N (top, CAN/CAN + Se).
[g] Extension planning area (EPA) in parentheses.
[h] 2008/09 only.
[i] 2009/10 only (irrigated site).

controls are required to minimise risks of toxicity. The potential for genetic biofortification of crops through breeding is not yet clear. Lyons et al. (2005) screened cereal grain Se composition among modern wheat (*Triticum aestivum* L.), durum wheat (*Triticum dicoccum* (Schrank) Schubl.), wheat landraces, ancestral diploid relatives (*Aegilops tauschii* (Coss.) Schmal.), barley (*Hordeum vulgare* L.), triticale (x *Triticosecale* Wittmack ex A. Camus.) and rye (*Secale cereale* L.), all grown on soils with low bioavailable Se concentrations. A lack of breeding potential was noted, with cereal grain Se composition being associated primarily with non-genetic factors, as also seen in UK bread wheat ($n = 150$; Zhao et al., 2009). However, variation in grain Se composition among non-cultivated varieties and at higher bioavailable soil Se concentrations indicates that future breeding efforts may yet be possible (Lyons et al., 2005; Garvin et al., 2006; White and Broadley, 2009). In terms of agronomic biofortification, the Se concentrations of all fractions of cereal grains can be increased easily when Se is applied in its selenate form (Broadley et al., 2010; Hart et al., 2011). In a public health setting, Se fertilisation has already been adopted at a national scale in Finland, in 1984, following primary legislation. This led to immediate increases in the Se concentrations of Finnish foods and dietary Se intakes (Eurola et al., 1991; Broadley et al., 2006).

The aim of this study was to determine the potential for increasing grain Se concentration in maize in Malawi using fertiliser-based approaches. Malawi was chosen because there is evidence of widespread low Se intakes and status among the population due to the low plant-available Se concentrations of the soils and a lack of diversity within the typical diet (Chilimba et al., 2011). Furthermore, to secure maize yields, Malawi has operated a Farm Input Subsidy Programme (FISP) since 2005/06 (Dorward and Chirwa, 2011), under which fertiliser is distributed to small-scale farmers *via* a voucher system. The FISP involves major commitments of financial and human resources through the national extension service system and represents a potential public health intervention route, as adopted previously in Finland.

2. Materials and methods

2.1. Overview

Three sets of field experiments were conducted in Malawi, in each of the 2008/09 and 2009/10 cropping seasons, to determine the response of maize to three forms of selenate-Se containing fertiliser. These were: (1) a liquid drench of $Na_2SeO_{4(aq)}$ (41.8% Se, Sigma–Aldrich Company Ltd, Dorset, UK), (2) compound fertiliser granules containing NPK + Se, representing a 25:5:5 + Na product marketed under the trade name Top Stock® (Yara UK, Immingham, UK) which contains 0.0012% Se (w/w) as Na_2SeO_4 and (3) calcium ammonium nitrate (CAN + Se; Yara) containing 0.005% Se (w/w), also as Na_2SeO_4.

2.2. Site and crop selection, cultivation and experimental design

In both years, fields were selected at research stations of the Malawi Ministry of Agriculture and Food Security (MoAFS), at Bvumbwe, Chitala, Chitedze, Makoka, Mbawa and Ngabu (Table 1). All sites were rain-fed. As lack of rain and crop failure occurred at Ngabu in 2009/10, a late-sown crop was grown under irrigation at a replacement site at nearby Kasinthula, within the same Shire Valley Agricultural Development Division (ADD). Soils at all sites were Luvisols except for the Shire Valley ADD sites (Eutric Vertisol). Experiments with $Na_2SeO_{4(aq)}$ and NPK + Se were conducted at six sites in 2008/09 and 2009/10 using *Zea mays* L. var. SC627 (a local hybrid). Experiments with CAN + Se were conducted at a subset of

Table 2
Experimental timelines for all sites examinedin Malawi during the 2008/09 and 2009/10 cropping seasons. 'na' denotes not applicable.

Site	2008/09				2009/10			
	Sowing	Base dressing	Top dressing	Harvest	Sowing	Base dressing	Top dressing	Harvest
Bvumbwe	11 December	23 December	14 January	30 April	25 December	15 January	15 February	3 May
Chitala	10 December	27 December	16 January	28 April	23 December	3 January	23 January	26 April
Chitedze	9 December	30 December	22 January	4 May	15 December	1 January	19 January	27 April
Makoka	9 December	22 December	15 January	14 April	15 December	22 December	23 January	27 April
Mbawa	10 December	29 December	27 January	6 May	21 December	4 January	21 January	29 April
Ngabu	11 December	24 December	14 January	15 April	na	na	na	na
Kasinthula	na	na	na	na	16 February	25 February	18 March	6 June

three sites in each year (Bvumbwe, Chitedze and Ngabu/Kasinthula) using maize varieties SC627 and ZM623 (an open pollinated variety).

At each site, the soil was ploughed to 30 cm depth and subsequently harrowed. Ridges 30 cm in height were prepared at 75 cm spacings. Shortly after first rainfall, two maize seeds were sown on the top of each ridge at 25 cm spacings on the dates shown in Table 2. Each experimental plot comprised four ridges 5 m in length. The two outer ridges and three terminal maize plants at each end of the ridge were used as guard rows, giving a net plot size of two ridges by 4 m in length. After approximately 2 weeks, the seedlings were thinned to one plant per planting station and a base NPK fertiliser dressing was applied (see below). Plots were weeded twice during the growing season. Crops harvested from a representative area of 7.5 m^2 after grain had ripened were dried in the field before being separated into cobs and stover. Cobs were weighed in the laboratory. Stovers were weighed in the field using lower-precision balances.

A randomised block design was adopted for each experimental unit. For experiments with $Na_2SeO_{4(aq)}$ and NPK + Se, there

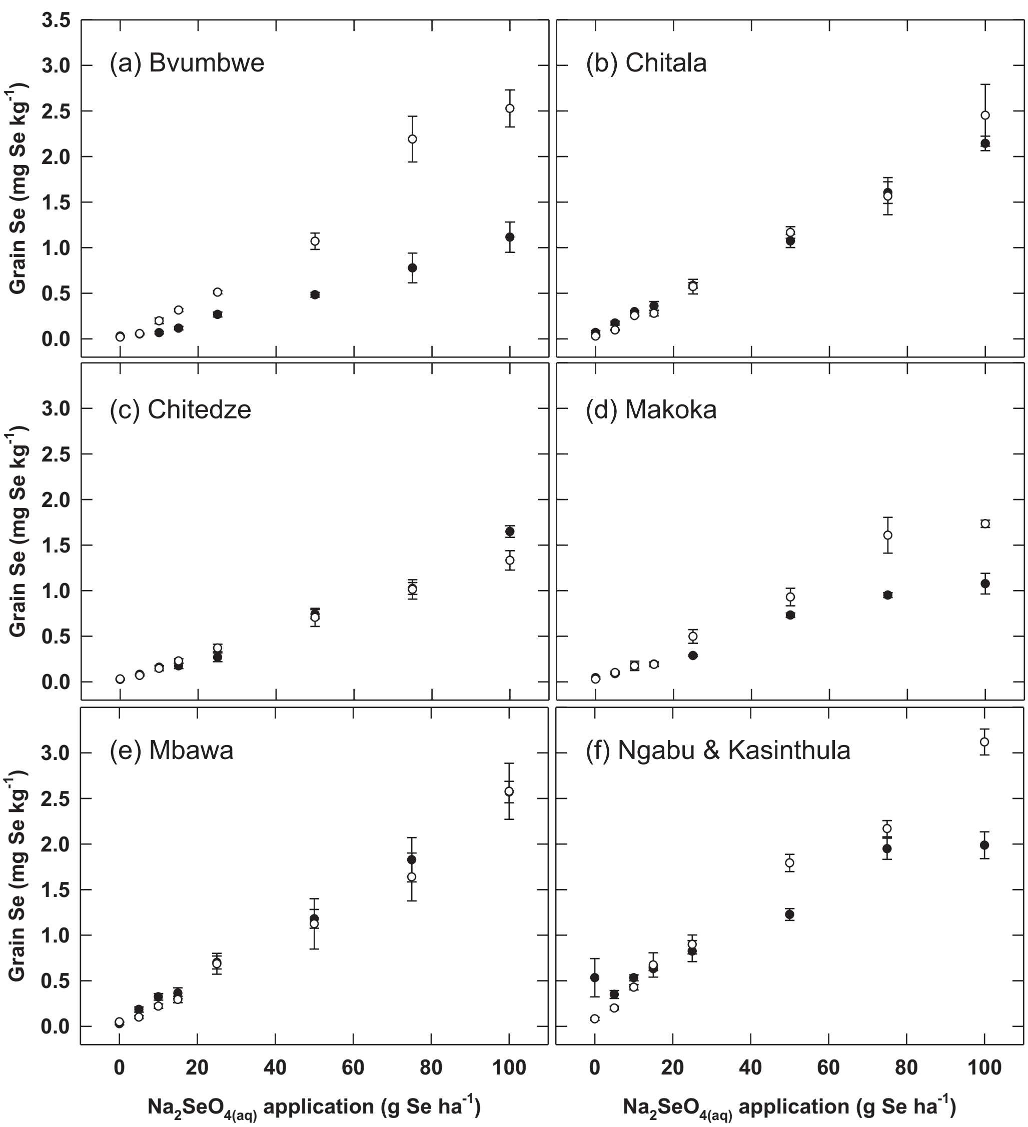

Fig. 1. Influence of fertilisation with $Na_2SeO_{4(aq)}$ on grain Se concentration in maize grown at six sites in Malawi: filled circles 2008/09; open circles 2009/10 (±SEM).

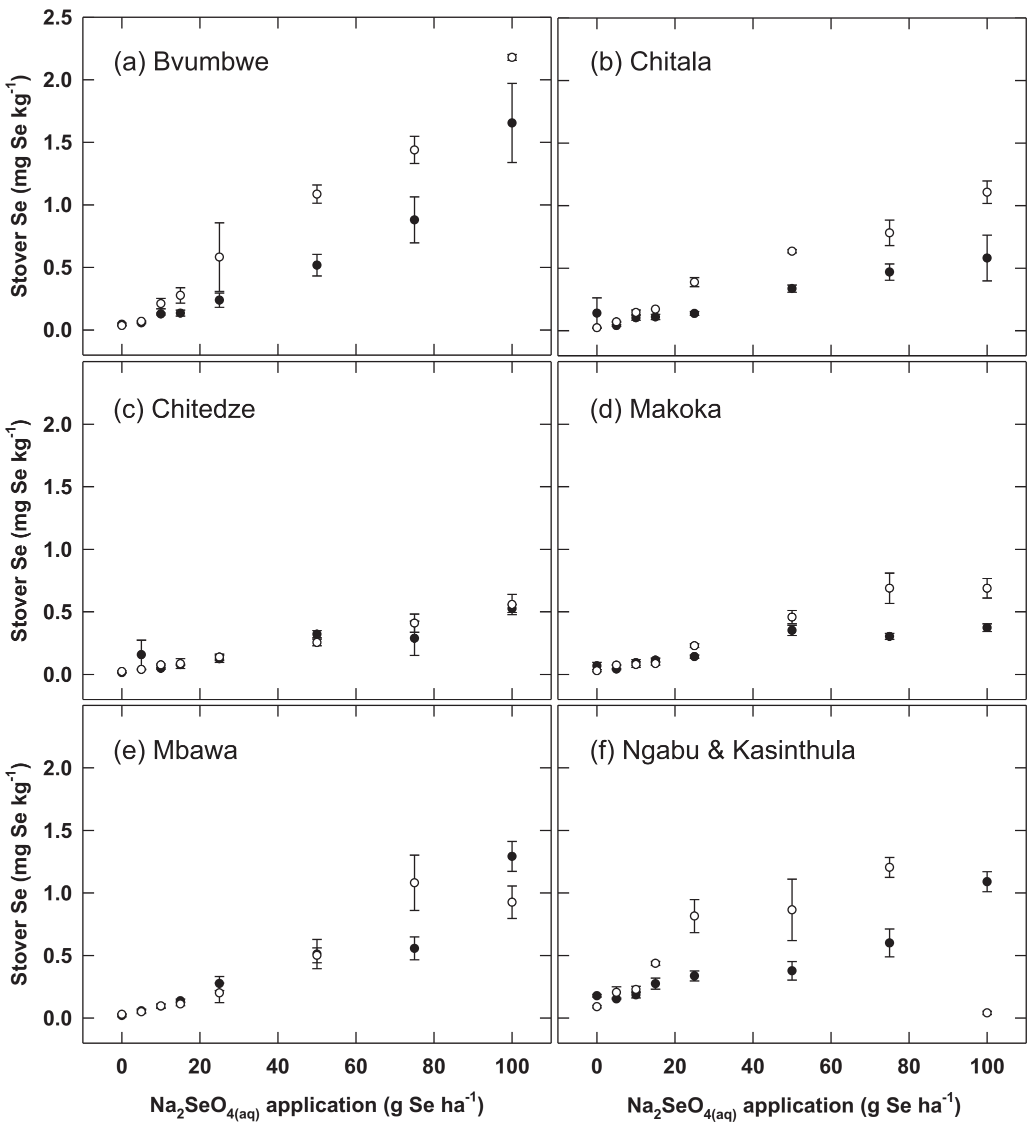

Fig. 2. Influence of fertilisation with $Na_2SeO_{4(aq)}$ on stover Se concentration in maize grown at six sites in Malawi: filled circles 2008/09; open circles 2009/10 (±SEM).

were four replicates per treatment, except at Bvumbwe in 2009/10 where three replicates were used due to space constraints. For experiments involving CAN + Se, there were three replicates per treatment at all sites. All data analyses were conducted in GenStat (V.13.3.0.5165, VSN International, Hemel Hempstead, UK).

2.3. *Fertiliser applications*

For the $Na_2SeO_{4(aq)}$ experiment, eight treatment levels (0, 5, 10, 15, 25, 50, 75 and 100 g Se ha^{-1}) were included at six sites in each of 2 years, representing 376 plots in total. The $Na_2SeO_{4(aq)}$ was applied at early stem extension (~'knee high'; Table 2) during the rainy season. To ensure even application, the $Na_2SeO_{4(aq)}$ was applied as a high-volume drench using a knapsack sprayer, with the operator wearing personal protective equipment of overalls, boots, a face-shield and nitrile gloves (Broadley et al., 2010). A 16 L Berthoud Vermorel 2000Pro knapsack tank (Exel GSA, Villefranche-sur-Saône, France) was connected to a 1 m boom housing three Lurmark 110°, flat-fan spray nozzles (Hypro EU Ltd, Longstanton, Cambridge, UK), spaced equally, with a spray-swath of 1.5 m. A coarse nozzle type "08 white" was used (1180 mL nozzle^{-1} min^{-1}; British Crop Protection Council, 2001) to minimise potential aerosol drift. Ergonomically acceptable drench rates were calibrated to treat four replicate plots from a single tank at appropriate walking speed with two passes (833 L water ha^{-1}). Plots were treated in ascending order of target Se application rates; no water was applied to control plots to minimise any risk of Se-contamination. A base application of N, P_2O_5 and K_2O (46, 20 and 10 kg ha^{-1}, respectively) was made to all plots using a 23:10:5 + 3 S fertiliser (Yara UK) and a top dressing of urea at 46 kg N ha^{-1} was subsequently applied (Table 2). Base dressings were applied shortly after emergence; top-dressings were applied at early stem extension (Table 2). Fertiliser granules were applied *via* calibrated cups to the base of individual plants, using a hand-placement method.

For the NPK + Se experiment, five treatment levels were used (0, 1.5, 3.0, 4.5 and 6.0 g Se ha^{-1}) at six sites in each of 2 years. A split Se treatment was included as a sub-factor, giving nine NPK + Se treatments, representing 423 plots in total. Splits represented base:top applications of Se as: 0 g Se ha^{-1} (0:0), 1.5 g Se ha^{-1} (100:0, 0:100), 3 g Se ha^{-1} (100:0, 50:50, 0:100), 4.5 g Se ha^{-1} (25:75, 75:25) and 6 g Se ha^{-1} (50:50). Fertiliser granules were applied by hand-placement as described previously. To ensure that 50:50 base:top split applications of NPK were identical for all plots, applications were balanced using a 25:5:5 NPK granular product marketed under the trade name Super Grass® (Yara UK), i.e. only Se applications were split. In total, each plot received the equivalent of 100, 20 and 20 kg ha^{-1} of N, P_2O_5 and K_2O, respectively.

For the CAN + Se experiment, five treatment levels were used (0, 5, 10, 15 and 20 g Se ha^{-1}), at three sites in each of 2 years using two varieties of maize, representing 180 plots in total. A base application of N, P_2O_5 and K_2O (46, 20 and 10 kg ha^{-1}, respectively) was made to all plots (Table 2) using a 23:10:5 + 3 S fertiliser (Yara UK). CAN + Se was applied as a top dressing, using the hand-placement method described previously. Nitrogen was balanced using calcium ammonium nitrate (CAN) without Se. In total, each plot received the equivalent of 130, 45 and 23 kg ha^{-1} of N, P_2O_5 and K_2O, respectively.

2.4. *Maize grain Se analysis*

Milled grain (~0.4 g dry weight, DW) was digested under microwave heating for 45 min at a controlled pressure of 2 MPa in 3.0 mL of 70% trace analysis grade (TAG) HNO_3, 2.0 mL H_2O_2 and 3.0 mL Milli-Q water (Fisher Scientific UK Ltd, Loughborough, Leicestershire, UK). The microwave system comprised a Multiwave 3000 platform with a 48-vessel 48MF50 rotor (Anton Paar GmbH, Graz, Austria). Samples were digested in vessels comprising perfluoroalkoxy (PFA) liner material and polyethylethylketone (PEEK) pressure jackets (Anton Paar GmbH). Digested samples were diluted to 20 mL (30% HNO_3) with Milli-Q water (18.2 MΩ cm) and stored at room temperature pending elemental analysis. Immediately prior to analysis, samples were diluted 1-in-10 with Milli-Q water. Selenium (^{78}Se) analysis was undertaken by ICP-MS (X-Series^{II}, Thermo Fisher Scientific Inc., Waltham, MA, USA) using a hydrogen reaction cell. Samples were introduced from an autosampler (Cetac ASX-520, Omaha, NE, USA) with 4 × 60-place sample racks, at 1 mL min^{-1} through a concentric glass venturi nebuliser and Peltier-cooled (3 °C) spray chamber (Thermo Fisher Scientific Inc.). Internal standards introduced to the sample stream *via* a T-piece included Ge and Rh (10 μg L^{-1}) in 2% TAG HNO_3. An external wheat flour standard (NIST 1567a; National Institute of Standards and Technology, Gaithersburg, MD, USA) was used as reference material. Each digestion batch (n = 48) included two blank digestions and two certified reference samples; final Se concentrations were converted to mg kg^{-1} DW.

3. Results

3.1. *$Na_2SeO_{4(aq)}$ experiments*

Selenium concentration in maize grain and stover increased when a single high volume drench of $Na_2SeO_{4(aq)}$ was applied to maize crops at all six sites in each of 2 years (12 experimental units; Figs. 1 and 2; Table 3). The relationship between crop Se concentration and Se fertilisation rate was approximately linear ($R^2 > 0.87$) for both grain and stover in all experimental units except for stover fractions under irrigation at Kasinthula in 2009/10 (Table 3). For each g Se ha^{-1} applied, Se concentration in maize grain increased by 11–29 μg Se kg^{-1} and stover Se concentration increased by 3–21 μg Se kg^{-1} (Table 3). Across all experimental units, crop yield varied from 2112 to 7009 kg grain ha^{-1} and 3169 to 16,458 kg stover ha^{-1}, with a strong effect of site in each year ($P < 0.001$; Table 4). However, there were no significant effects of Se application on grain or stover yield in any of the experimental units ($P > 0.05$).

Table 3
Summary of linear regression analyses for $Na_2SeO_{4(aq)}$, NPK + Se and CAN + Se fertilisation experiments on maize grown at six sites in Malawi in 2008/09 and 2009/10, representing a total of 30 experimental units.

Site	Se source	Number of Se application levels	2008/09						2009/10					
			SLOPE (mg Se kg^{-1} g^{-1} Se ha^{-1})		INTERCEPT (mg Se kg^{-1})		R^2		SLOPE (mg Se kg^{-1} g^{-1} Se ha^{-1})		INTERCEPT (mg Se kg^{-1})		R^2	
			Grain	Stover	Grain	Stover	Grain	Stover	Grain	Stover	Grain	Stover	Grain	Stover
Bvumbwe	Na_2SeO_4	8	0.011	0.015	−0.018	−0.065	0.99	0.94	0.027	0.021	−0.077	−0.001	0.98	0.99
	NPK + Se	5	0.019	0.020	0.025	0.029	0.97	0.99	0.016	0.015	0.006	0.022	0.99	0.98
	CAN + Se	5	0.008	0.006	0.025	0.049	0.99	0.99	0.018	0.016	0.022	−0.006	0.99	0.96
Chitala	Na_2SeO_4	8	0.021	0.005	0.068	0.055	1.00	0.95	0.023	0.011	−0.018	0.044	0.99	0.99
	NPK + Se	5	0.025	0.017	0.063	0.031	0.91	0.99	0.014	0.015	0.048	0.019	0.73	0.94
Chitedze	Na_2SeO_4	8	0.016	0.004	−0.033	0.039	0.98	0.88	0.013	0.005	0.026	0.012	1.00	1.00
	NPK + Se	5	0.021	0.012	0.037	0.025	0.97	0.97	0.020	0.007	0.029	0.015	0.98	0.92
	CAN + Se	5	0.010	0.005	0.024	0.015	1.00	0.92	0.016	0.007	0.006	0.014	0.97	0.98
Makoka	Na_2SeO_4	8	0.011	0.003	0.054	0.066	0.98	0.87	0.019	0.008	0.004	0.029	0.98	0.96
	NPK + Se	5	0.026	0.005	0.052	0.055	0.99	0.58	0.033	0.007	0.000	0.013	0.96	0.97
Mbawa	Na_2SeO_4	8	0.025	0.011	0.036	−0.021	1.00	0.91	0.024	0.011	−0.017	−0.008	0.99	0.91
	NPK + Se	5	0.025	0.013	0.028	0.017	0.99	0.91	0.029	0.019	0.048	0.012	0.92	0.93
Ngabu/Kasinthula	Na_2SeO_4	8	0.017	0.008	0.394	0.113	0.96	0.90	0.029	0.003	0.138	0.366	0.99	0.09
	NPK + Se	5	0.011	0.013	0.276	0.152	0.82	0.71	0.030	0.019	0.029	0.009	0.93	0.92
	CAN + Se	5	0.004	0.001	0.358	0.217	0.17	0.09	0.033	0.021	0.075	0.010	1.00	0.94

Table 4
Summary of yield data and treatment effects for $Na_2SeO_{4(aq)}$ fertilisation experiments on maize grown at six sites in Malawi in 2008/09 and 2009/10. 'na' denotes not applicable, i.e. no trial was conducted.

Experimental site	Grain yield (kg ha^{-1})		Stover yield (kg ha^{-1})	
	2008/09	2009/10	2008/09	2009/10
Bvumbwe	4141	3050	7333	4279
Chitala	6498	5242	15792	14875
Chitedze	6527	9369	5475	7117
Makoka	7009	5560	6542	11192
Mbawa	3906	3058	4758	4408
Ngabu	2764	na	16458	na
Kasinthula	na	2112	na	3169
Site	$F_{5,141}=116$; $P<0.001$	$F_{5,133}=175$; $P<0.001$	$F_{5,141}=306$; $P<0.001$	$F_{5,133}=127$; $P<0.001$
Se treatment	$\mathbf{F_{7,141}=1.54; P=0.159}$	$\mathbf{F_{7,133}=0.64; P=0.719}$	$\mathbf{F_{7,141}=1.02; P=0.423}$	$\mathbf{F_{7,133}=1.02; P=0.421}$
Site/Se treatment	$F_{35,141}=1.01$; $P=0.465$	$F_{35,133}=0.93$; $P=0.590$	$F_{35,141}=1.13$; $P=0.308$	$F_{35,133}=0.59$; $P=0.963$

3.2. NPK + Se experiments

The relationship between crop Se concentration and Se fertilisation rate was approximately linear when a granular NPK + Se compound was applied to maize crops at six sites in both years (12 experimental units; Figs. 3 and 4; Table 3), in a response similar to the liquid drench experiment. For grain, $R^2>0.90$ at all sites and years except for Ngabu in 2008/09 ($R^2=0.82$) and Chitala in 2009/10 ($R^2=0.73$). For stover fractions, $R^2>0.90$ except for Makoka ($R^2=0.58$) and Ngabu ($R^2=0.71$) in 2008/09. For each g Se ha^{-1} applied, grain Se concentration increased by 11–33 μg Se kg^{-1} and stover Se concentration by

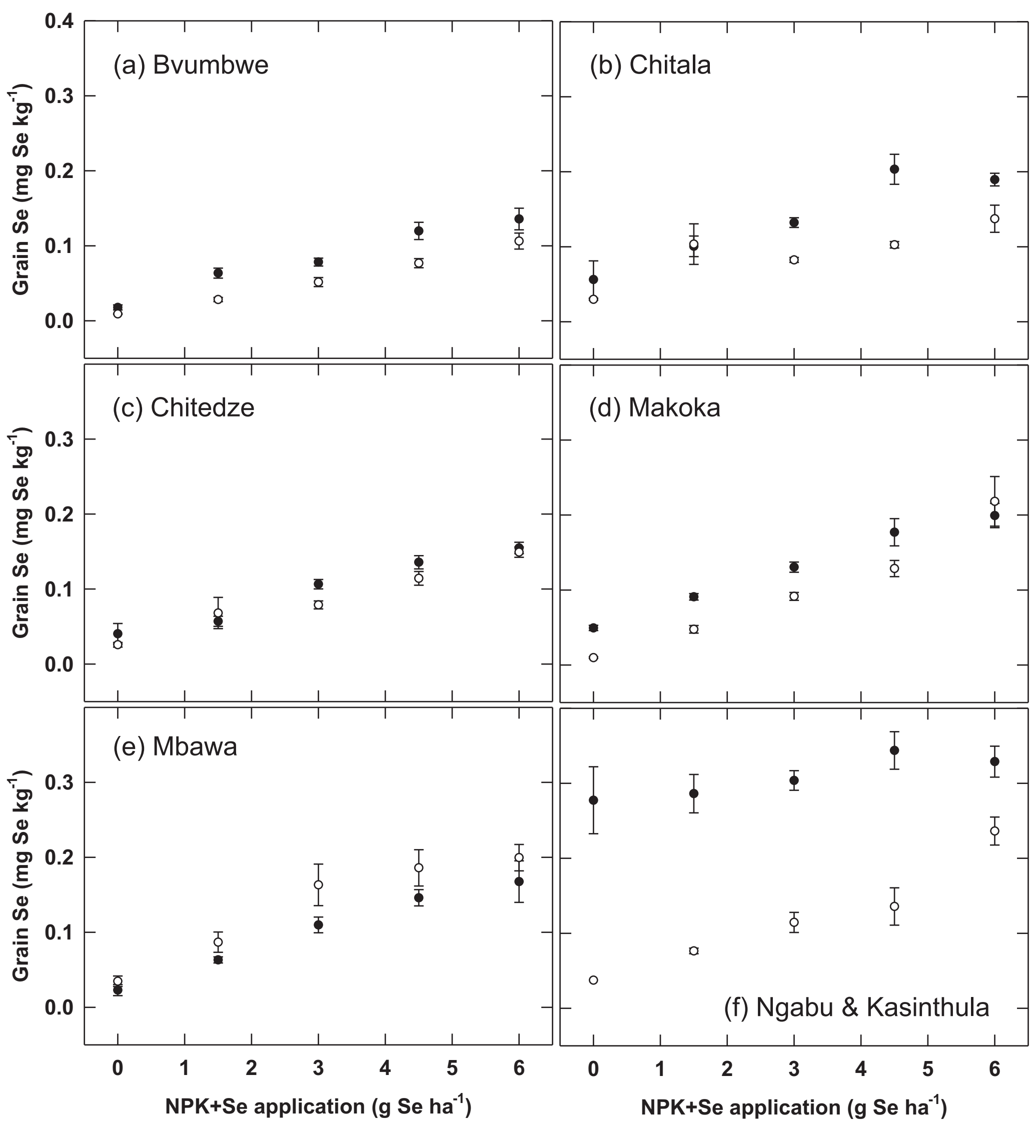

Fig. 3. Influence of fertilisation with granular NPK + Se on grain Se concentration in maize grown at six sites in Malawi: filled circles 2008/09; open circles 2009/10 (±SEM).

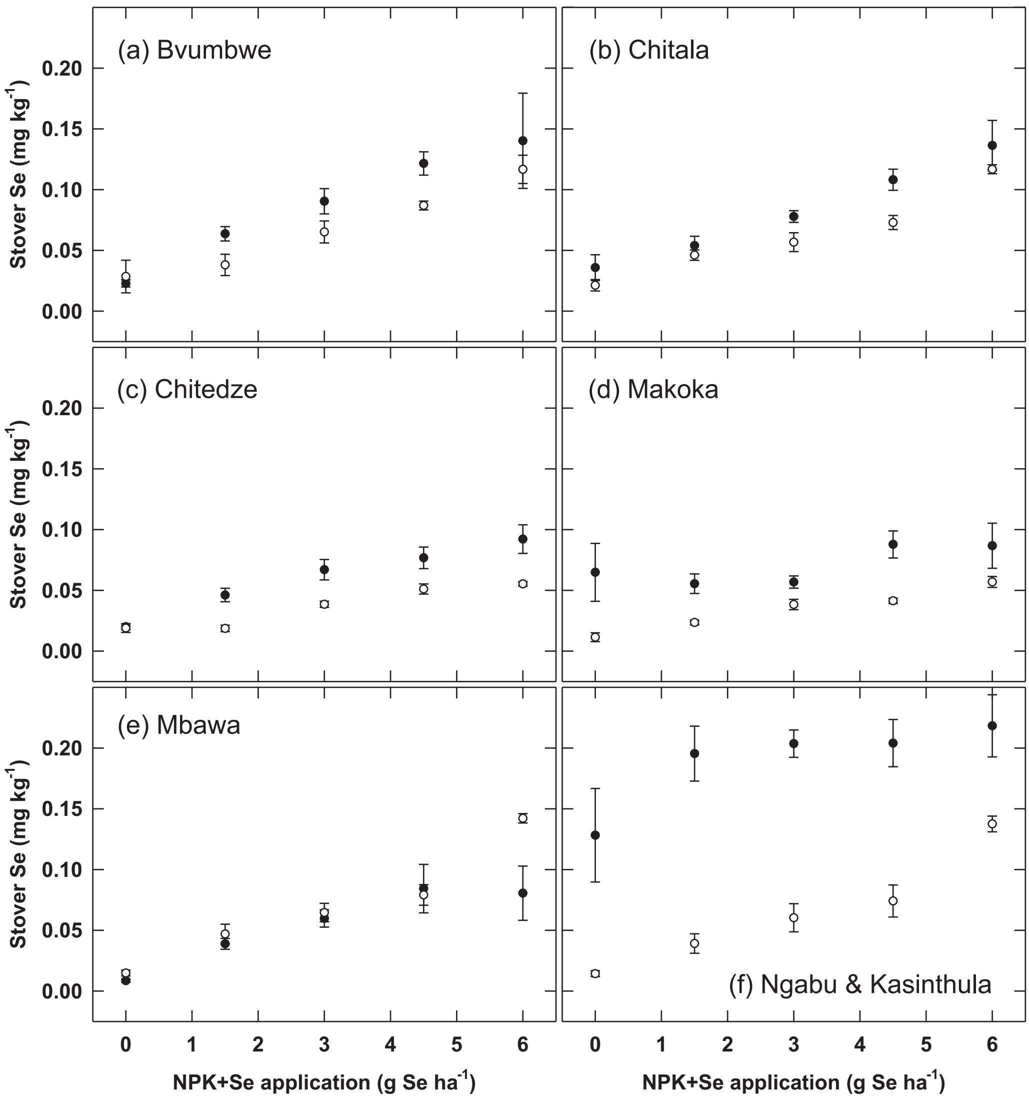

Fig. 4. Influence of fertilisation with granular NPK + Se on stover Se concentration in maize grown at six sites in Malawi: filled circles 2008/09; open circles 2009/10 (±SEM).

5–20 μg Se kg^{-1} (Table 3). Across all experimental units, crop yield varied from 2598 to 7637 kg grain ha^{-1} and 3961 to 18,807 kg stover ha^{-1}, with a strong effect of site in each year ($P < 0.001$; Table 5). Again, there were no significant effects of Se application on grain or stover yield in any of the experimental units ($P > 0.05$).

Across all experimental units and fertiliser application rates, the timing of application affected grain Se concentration (Fig. 5). Although the significance of this effect was marginal in 2008/09 ($P = 0.06$), grain Se concentration at five of the six sites was higher in the late (top dressing) Se application treatment than in the (base) application plots, with an overall difference of 13%. The effect of

Table 5
Summary of yield data and treatment effects for NPK + Se fertilisation experiments on maize grown at six sites in Malawi in 2008/09 and 2009/10.

Experimental site	Grain yield (kg ha^{-1})		Stover yield (kg ha^{-1})	
	2008/09	2009/10	2008/09	2009/10
Bvumbwe	4206	3208	8870	4591
Chitala	7068	4759	16037	13667
Chitedze	5802	7637	5230	5670
Makoka	6955	7520	7000	18807
Mbawa	5684	2641	7328	3961
Ngabu	2598	na	15285	na
Kasinthula	na	3890	na	5835
Site	$F_{5,159} = 72.2$; $P < 0.001$	$F_{5,150} = 103$; $P < 0.001$	$F_{5,159} = 208$; $P < 0.001$	$F_{5,150} = 293$; $P < 0.001$
Se treatment	$\mathbf{F_{4,159} = 2.14; P = 0.079}$	$\mathbf{F_{4,150} = 1.16; P = 0.331}$	$\mathbf{F_{4,159} = 0.61; P = 0.659}$	$\mathbf{F_{4,150} = 2.24; P = 0.067}$
Site/Se treatment	$F_{20,159} = 0.68$; $P = 0.840$	$F_{20,150} = 0.65$; $P = 0.865$	$F_{20,159} = 0.36$; $P = 0.995$	$F_{20,150} = 0.70$; $P = 0.825$
Se treatment/split	$F_{4,159} = 1.76$; $P = 0.140$	$F_{4,150} = 1.17$; $P = 0.325$	$F_{4,159} = 0.62$; $P = 0.65$	$F_{4,150} = 2.25$; $P = 0.066$
Site/Se treatment/split	$F_{20,159} = 1.79$; $P = 0.026$	$F_{20,150} = 1.01$; $P = 0.454$	$F_{20,159} = 1.17$; $P = 0.286$	$F_{20,150} = 0.64$; $P = 0.874$

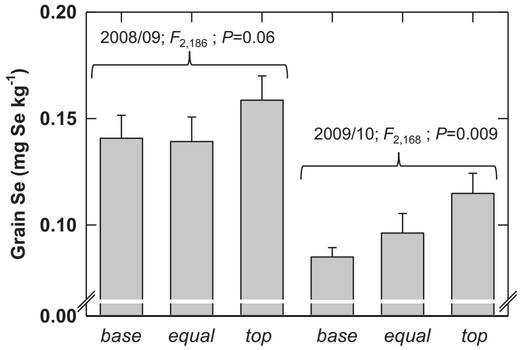

Fig. 5. Influence of split applications of granular NPK + Se on mean grain Se concentration in maize grown at six sites in Malawi. Each bar represents a mean application of 3 g Se ha^{-1}. Base dressings include 1.5 and 3 g Se ha^{-1} treatments at 100:0 and 4.5 g Se ha^{-1} treatments at 75:25 splits. Equal split dressings were 0 g Se ha^{-1} and 3 and 6 g Se ha^{-1} treatments with 50:50 splits. Top dressings include 1.5 and 3 g Se ha^{-1} treatments at 0:100 and 4.5 g Se ha^{-1} treatments with 75:25 splits. n = 69 and 63 for 2008/09 and 2009/10, respectively, for each bar (±SEM).

timing was highly significant in 2009/10 (P = 0.009). Grain Se concentration at all six sites was higher following late Se application compared to early Se application, with an overall difference of 33% (Fig. 5).

3.3. CAN + Se experiments

The relationship between crop Se concentration and Se fertilisation rate was again approximately linear when a granular CAN + Se compound was applied to two maize genotypes, a local hybrid (SC627) and an open pollinated variety (ZM623), at three sites in both years (six experimental units; Figs. 6 and 7; Table 3). As there was no significant effect of variety on grain or stover Se concentration, data for both varieties were combined for subsequent analyses. For grain, R^2 > 0.97 for all sites and years except Ngabu in 2008/09 (R^2 = 0.17). For stover fractions, R^2 > 0.92 except for Ngabu (R^2 = 0.09) in 2008/09. For each g Se ha^{-1} applied, maize grain Se concentration increased by 4–33 μg Se kg^{-1}, and stover Se concentration increased by 1–21 μg Se kg^{-1} (Table 3). Across all experimental units, crop yield varied from 2638 to 8311 kg grain ha^{-1} and 4773 to 15,200 kg stover ha^{-1}, with a strong effect of site in each year (P < 0.001; Table 6). As observed with the other forms of Se, there were no significant effects of Se application on grain or stover yields in any of the experimental units (P > 0.05). There were significant variety × site interaction terms for grain and stover yields in 2008/09 but not in 2009/10 (Table 6).

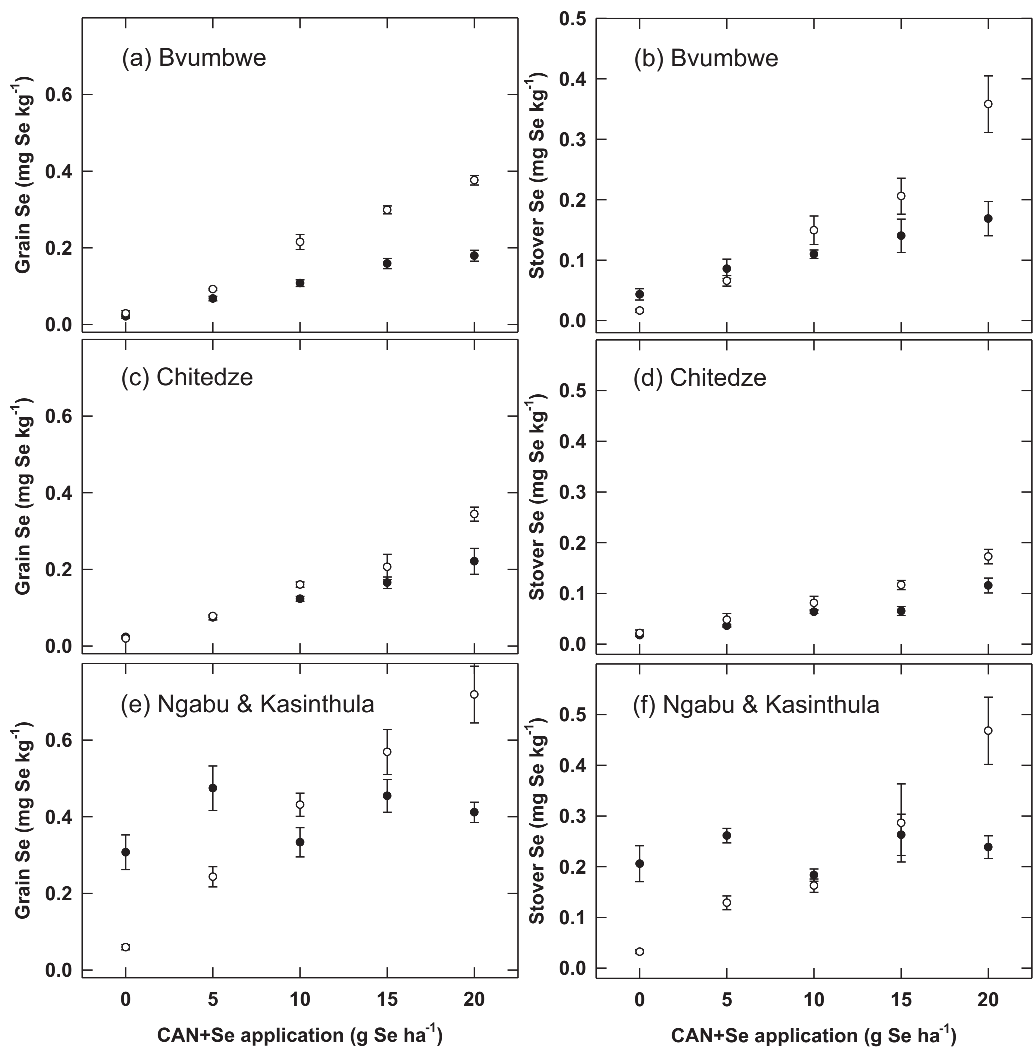

Fig. 6. Influence of fertilisation with CAN + Se on grain and stover Se concentration in maize grown at six sites in Malawi: filled circles 2008/09; open circles 2009/10 (±SEM).

Table 6
Summary of yield data and treatment effects for CAN + Se fertilisation experiments on maize grown at three sites in Malawi in 2008/09 and 2009/10.

Experimental site	Grain yield ($kg\,ha^{-1}$)				Stover yield ($kg\,ha^{-1}$)			
	2008/09		2009/10		2008/09		2009/10	
	SC627	ZM623	SC627	ZM623	SC627	ZM623	SC627	ZM623
Bvumbwe	5930	4332	4720	3964	10044	7422	6947	5947
Chitedze	5209	5498	8098	8311	4773	5324	7040	6258
Ngabu	2638	3648	na	na	15200	11911	na	na
Kasinthula	na	na	4013	3996	na	na	6021	5994
Site	$F_{2,58} = 24.9$; $P < 0.001$		$F_{2,58} = 86.6$; $P < 0.001$		$F_{2,58} = 72.4$; $P < 0.001$		$F_{2,58} = 1.13$; $P = 0.329$	
Se treatment	$\boldsymbol{F_{4,58} = 1.03}$; $\boldsymbol{P = 0.401}$		$\boldsymbol{F_{4,58} = 0.59}$; $\boldsymbol{P = 0.668}$		$\boldsymbol{F_{4,58} = 0.90}$; $\boldsymbol{P = 0.470}$		$\boldsymbol{F_{4,58} = 0.53}$; $\boldsymbol{P = 0.716}$	
Variety	$F_{1,58} = 0.12$; $P = 0.725$		$F_{1,58} = 0.42$; $P = 0.522$		$F_{1,58} = 9.52$; $P = 0.003$		$F_{1,58} = 2.87$; $P = 0.095$	
Site/Se treatment	$F_{8,58} = 0.60$; $P = 0.777$		$F_{8,58} = 0.53$; $P = 0.832$		$F_{8,58} = 1.04$; $P = 0.420$		$F_{8,58} = 0.61$; $P = 0.766$	
Site/variety	$F_{2,58} = 7.61$; $P = 0.001$		$F_{2,58} = 1.02$; $P = 0.368$		$F_{2,58} = 4.19$; $P = 0.020$		$F_{2,58} = 0.69$; $P = 0.508$	
Se treatment/variety	$F_{4,58} = 1.41$; $P = 0.243$		$F_{4,58} = 0.07$; $P = 0.990$		$F_{4,58} = 0.37$; $P = 0.828$		$F_{4,58} = 0.05$; $P = 0.994$	
Site/Se treatment/variety	$F_{8,58} = 1.16$; $P = 0.336$		$F_{8,58} = 0.37$; $P = 0.935$		$F_{8,58} = 1.14$; $P = 0.354$		$F_{8,58} = 0.47$; $P = 0.870$	

Table 7
Proportional recovery of Se in maize, calculated from the linear response of crop Se concentration to Se-fertilisation across all Se application levels (Table 3) multiplied by mean yields of grain and stover fractions (Tables 4–6).

Site	Se source	Efficiency 2008/09			Efficiency 2009/10		
		Grain (%)	Stover (%)	Total efficiency (%)	Grain (%)	Stover (%)	Total efficiency (%)
Bvumbwe	Na_2SeO_4	5	11	**16**	8	9	**17**
	NPK + Se	8	18	**26**	5	7	**12**
	CAN + Se	4	5	**9**	8	10	**18**
Chitala	Na_2SeO_4	14	8	**22**	12	16	**28**
	NPK + Se	18	27	**45**	7	21	**27**
Chitedze	Na_2SeO_4	10	2	**13**	12	4	**16**
	NPK + Se	12	6	**18**	15	4	**19**
	CAN + Se	5	3	**8**	13	5	**18**
Makoka	Na_2SeO_4	8	2	**10**	11	9	**20**
	NPK + Se	18	4	**22**	25	13	**38**
		0	0		0	0	
Mbawa	Na_2SeO_4	10	5	**15**	7	5	**12**
	NPK + Se	14	10	**24**	8	8	**15**
Ngabu/Kasinthula	Na_2SeO_4	5	13	**18**	6	1	**7**
	NPK + Se	3	20	**23**	12	11	**23**
	CAN + Se	1	1	**3**	13	13	**26**

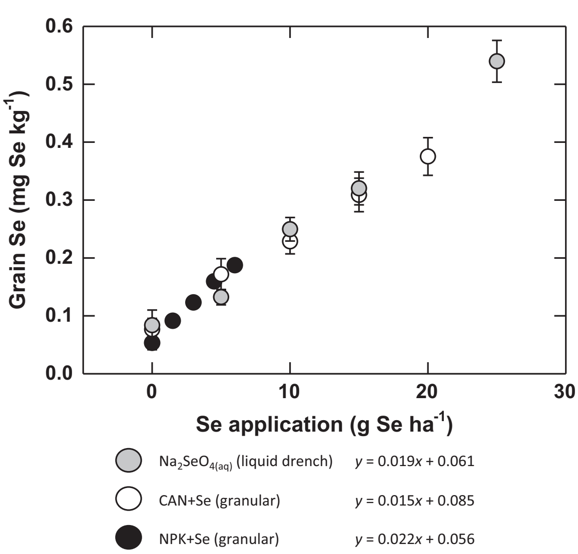

Fig. 7. Summary of response of maize grain Se concentration to three forms of selenate-Se fertiliser. Data are for all sites and both years at application rates <25 $g\,Se\,ha^{-1}$ (±SEM). Further details are given on the inset legend.

3.4. Overall efficiency of the fertilisation process

The mean proportional recovery of Se in maize applied in exogenous forms averaged 18% and 20% in the whole crop in 2008/09 and 2009/10, respectively (Table 7). The recovery was split approximately equally between grain and stover fractions. Crop recoveries of Se were calculated from the linear response of crop Se concentration to Se-fertilisation across all Se application levels (Table 3), multiplied by mean yields of grain and stover fractions (Tables 4–6). However, there was considerable variation in overall recovery of Se, ranging from 3% for CAN + Se applied at Ngabu, up to 45% for NPK + Se at Chitala, both in 2008/09 (Table 7).

4. Discussion

Agronomic biofortification of maize with Se appears to be a feasible option for increasing dietary Se intake in Malawi as grain Se increased by 19.7, 20.7 and 14.8 $\mu g\,Se\,kg^{-1}$ grain for each $g\,Se\,ha^{-1}$ applied as $Na_2SeO_{4(aq)}$, NPK + Se and CAN + Se, respectively (Fig. 7). However, if agronomic biofortification is to be adopted, the process must be reliable and cost-effective, in terms of health benefits and efficiency of resource-use, compared to alternative strategies such as the use of mineral supplements.

Selenium intake from maize sources in Malawi is estimated to be <6 $\mu g\,Se\,person^{-1}\,d^{-1}$ for 50% of the population and <7.5 $\mu g\,Se\,person^{-1}\,d^{-1}$ for 90% of the population (Chilimba et al., 2011). These intake data are based on extrapolated soil and maize grain Se concentration data from a preliminary survey, combined with average *per capita* maize consumption. Based on limited published data for Malawi, average Se intake from non-maize sources is likely to range between 15 and 22 μg Se person^{-1} d^{-1} (Donovan et al., 1992; Eick et al., 2009). However, many individuals will obtain a much larger proportion of their dietary energy from maize than average *per capita* maize consumption patterns suggest, and suboptimal Se intake is clearly very widespread. From the present study, an application of 5 g Se ha^{-1} to maize crops would increase average dietary Se intake in Malawi by 26.3–36.6 μg Se person^{-1} d^{-1}. Such levels would increase dietary Se intake to accepted reference values of ~50–70 μg Se person^{-1} d^{-1} (Fairweather-Tait et al., 2011). The risk of overdose, based on a current safe upper limit of 400 μg Se person^{-1} d^{-1} intake (Department of Health, 1991; Institute of Medicine, 2000), would appear to be minimal at these application levels, even for individuals with diverse diets. However, any public health intervention involving widespread agronomic biofortification with Se would clearly require careful monitoring to ensure beneficial health outcomes. Whilst it is widely accepted that Se intake < 30 μg Se d^{-1} is suboptimal for most adults, there remain considerable gaps in our knowledge of the relationships between Se intake, plasma Se concentrations and selenoenzyme activities, and definitive health outcomes (e.g. immune functioning), especially among individuals of very low-Se status in SSA. This situation must now be addressed *via* controlled intervention experiments as a matter of urgency.

In terms of reliability, the linear response of crop Se concentration to all forms and application rates of Se was striking and consistent at most sites. This is consistent with many previous studies dating back to the 1970s (reviewed by Lyons et al., 2003). For grain Se concentration, $R^2 > 0.90$ for the linear response in 27 of the 30 experimental units. For stover Se concentration, $R^2 > 0.87$ for the linear response in 26 of the experimental units. For those instances where the linear response was less strong, four still had highly significant R^2 values of 0.58–0.82. The three non-significant linear responses were at the Ngabu or Kasinthula sites. In addition to low rainfall at Ngabu in 2008/09, both sites have soils classified as calcareous Eutric Vertisols (FAO system) with $pH_{(water)}$ values of 7.4 and 7.9 (Green and Nanthambwe, 1992; Chilimba et al., 2011). At these pH levels, soil-to-grain transfer of native Se is up to 10-fold greater than under the normal acid conditions seen at Luvisol sites (Chilimba et al., 2011). This is likely to be due to a decrease in sorption strength of $Se^{(IV)}$ in the pH range 6–8 (Duc et al., 2006) and the potential oxidation of $Se^{(IV)}$ to $Se^{(VI)}$ at high pH, which is more available for crop uptake (Vuori et al., 1989; Masscheleyn et al., 1990; Séby et al., 2001). It is noteworthy that Eutric Vertisols comprise just ~0.5% of the land area of Malawi (Chilimba et al., 2011). However, soil types representing a further 23% have not yet been sampled and, given the critical role of soil properties in determining grain Se concentration, there is a pressing need for structured geochemical sampling of soils and grain in Malawi before agronomic biofortification strategies are implemented. Geochemical data should be combined with information on other factors including rainfall, soil management and crop yield. Within this geochemical context, the overall agronomic efficiency of the process must also be carefully monitored and optimised, to ensure the sustainable use of global Se reserves (Haug et al., 2007; Broadley et al., 2010). Clearly, further work is required to understand the variation in overall crop recovery of Se (Table 7) and to determine the fate of unrecovered Se in subsequent cropping years.

As observed previously for field-grown wheat (Broadley et al., 2010), maize grain and stover yields were unaffected by Se applications up to 100 g Se ha^{-1}. These observations are consistent with other field studies of wheat (Ducsay and Ložek, 2006; Grant et al., 2007; Curtin et al., 2008), despite evidence that plant growth may be stimulated by increased Se supply in controlled environment conditions (Hartikainen and Xue, 1999; Xue and Hartikainen, 2000; Turakainen et al., 2004; White et al., 2004; Lyons et al., 2009; Ríos et al., 2009). Selenium-induced growth stimulation in plants has been attributed to increased resistance to oxidative stress and the stimulation of sulphur transport and assimilation pathways. Further studies are needed to assess these phenomena in a wider field context.

In terms of input-costs, the distribution of fertilisers to smallholder farmers and villages and the cost of exogenous Se supplies must be weighed against the projected health benefits at an individual and population level. The distribution and use of fertilisers at the smallholder farmer level is widespread in Malawi. In 2005, following poor maize yields, the Malawi Government introduced an Agricultural Input Subsidy Programme (AISP, since renamed FISP). Under the FISP, small-scale farmers are provided with vouchers for mineral fertilisers and hybrid maize seed *via* national extension services on an annual basis (Denning et al., 2009; Dorward and Chirwa, 2011). The FISP imports ~0.2 Mt yr^{-1} of fertilisers and distributes these according to economic need. The FISP represents a major commitment of financial and human resources, costing 6.6% of GDP in 2008/9, i.e. an annual spend of >$250 m. At a household level, a fertiliser ‘coupon’ is worth >10% of annual income for up to half of the population. An independent review of the FISP recently concluded that it has led to a doubling of maize production and to wider economic growth and poverty reduction (Dorward and Chirwa, 2011). The opportunity to distribute Se-enriched fertilisers *via* the FISP is analogous to the precedent set when the Finnish Government passed primary legislation in 1983 to incorporate Se in compound fertilisers from 1984. The fact that the fertiliser sector was largely under state control facilitated this initiative and led to rapid increases in the Se concentrations of all foodstuffs, dietary Se intakes and the Se status of individuals (Eurola et al., 1991; Broadley et al., 2006). The Finnish programme has continued to the present day.

In terms of exogenous Se, the mean annual price of commercial-grade Se over the 5 year period 2005–2009 has ranged from ~50 to 110 USD kg^{-1} (USGS, 2011). If an application level of 5 g Se ha^{-1} is deemed to be a suitable target for all Se imported under the FISP and assuming that a 25% N-containing product was applied at rate of 50 kg N ha^{-1}, each metric tonne of fertiliser would require incorporation of sufficient Se to treat 5 ha, i.e. 25 g Se t^{-1} fertiliser. This equates to 5000 kg Se to enrich all fertiliser used in the FISP at an additional cost of ~$250–550 k yr^{-1} (or ~1.6–3.5 US cents person^{-1} yr^{-1}). Clearly there are additional technical and compliance costs associated with the incorporation of Se into granular fertiliser. Furthermore, Se-enriched fertilisers distributed under the FISP may not reach all individual farmers. However, a distribution method based on fertilisers is likely to be more equitable than a supplementation programme which targets certain demographic groups (e.g. children), especially given that most individuals in Malawi are likely to be vulnerable to Se malnutrition. It is difficult to envisage a more cost-effective, equitable, or immediate method to alleviate Se malnutrition among the population of Malawi than one based on agronomic biofortification.

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References

Benemariya, H., Robberecht, H., Deelstra, H., 1993. Daily dietary intake of copper, zinc and selenium by different population groups in Burundi, Africa. Sci. Total Environ. 136, 49–76.

British Crop Protection Council, 2001. Hand-held and Amenity Sprayers Handbook. BCPC, Alton, UK.

Broadley, M.R., Alcock, J., Alford, J., Cartwright, P., Fairweather-Tait, S.J., Foot, I., Hart, D.J., Hurst, R., Knott, P., McGrath, S.P., Meacham, M.C., Norman, K., Mowat, H., Scott, P., Stroud, J.L., Tovey, M., Tucker, M., White, P.J., Young, S.D., Zhao, F.J., 2010. Selenium biofortification of high-yielding winter wheat (*Triticum aestivum* L.) by liquid or granular Se fertilisation. Plant Soil 332, 5–18.

Broadley, M.R., White, P.J., Bryson, R.J., Meacham, M.C., Bowen, H.C., Johnson, S.E., Hawkesford, M.J., McGrath, S.P., Zhao, F.-J., Breward, N., Harriman, M., Tucker, M., 2006. Biofortification of U.K. food crops with selenium (Se). Proc. Nutr. Soc. 65, 169–181.

Chilimba, A.D.C., Young, S.D., Black, C.R., Rogerson, K.B., Ander, E.L., Watts, M., Lammel, J., Broadley, M.R., 2011. Maize grain and soil surveys reveal sub-optimal dietary selenium intake is widespread in Malawi. Sci. Rep. 1, 72, doi:10.1038/srep00072.

Curtin, D., Hanson, R., van der Weerden, T.J., 2008. Effect of selenium fertiliser formulation and rate of application on selenium concentrations in irrigated and dryland wheat (*Triticum aestivum*). N. Z. J. Crop Hortic. Sci. 36, 1–7.

Denning, G., Kabambe, P., Sanchez, P., Malik, A., Flor, R., Harawa, R., Nkhoma, P., Zamba, C., Banda, C., Magombo, C., Keating, M., Wangila, J., Sachs, J., 2009. Input subsidies to improve smallholder maize productivity in Malawi: toward an African green revolution. PLoS Biol. 7, e1000023.

Department of Health, 1991. Dietary Reference Values for Food Energy and Nutrients for the UK. Her Majesty's Stationery Office (HMSO), London, UK.

Donovan, U.M., Gibson, R.S., Ferguson, E.L., Ounpuu, S., Heywood, P., 1992. Selenium intakes of children from Malawi and Papua New Guinea consuming plant-based diets. J. Trace Elem. Electrolytes Health Dis. 6, 39–43.

Dorward, A., Chirwa, E., 2011. The Malawi agricultural input subsidy programme: 2005–6 to 2008–9. Int. J. Agric. Sustain. 9, 232–237.

Duc, M., Lefèvre, G., Fédoroff, M., 2006. Sorption of selenite ions on hematite. J. Colloid Interface Sci. 298, 556–563.

Ducsay, L., Ložek, O., 2006. Effect of selenium foliar application on its content in winter wheat grain. Plant Soil Environ. 52, 78–82.

Eick, F., Maleta, K., Govasmark, E., Duttaroy, A.K., Bjune, A.G., 2009. Food intake of selenium and sulphur amino acids in tuberculosis patients and healthy adults in Malawi. Int. J. Tuberc. Lung Dis. 13, 1313–1315.

Eurola, M.H., Ekholm, P.I., Ylinen, M.E., Koivistoinen, P.E., Varo, P.T., 1991. Selenium in Finnish foods after beginning the use of selenate supplemented fertilizers. J. Sci. Food Agric. 56, 57–70.

Fairweather-Tait, S.J., Bao, Y., Broadley, M.R., Collings, R., Ford, D., Hesketh, J., Hurst, R., 2011. Selenium in human health and disease. Antioxid. Redox Signal. 14, 1337–1383.

FAO (Food and Agriculture Organization of the United Nations), 2011. Food Security Statistics (Online), Available at http://faostat.fao.org/site/609/default.aspx#ancor (accessed 3 May 2011).

Garvin, D.F., Welch, R.M., Finley, J.W., 2006. Historical shifts in the seed mineral micronutrient concentration of US hard red winter wheat germplasm. J. Sci. Food Agric. 86, 2213–2220.

Grant, C.A., Buckley, W.T., Wu, R., 2007. Effect of selenium fertilizer source and rate on grain yield and selenium and cadmium concentration of durum wheat. Can. J. Plant Sci. 87, 703–708.

Green, R., Nanthambwe, S., 1992. Land resources appraisal of the Agricultural Development Divisions. Ministry of Agriculture/United Nations Development Programme/Food and Agriculture Organization MLW/85/011. Field Document No. 32, Lilongwe, Malawi.

Hart, D.J., Fairweather-Tait, S.J., Broadley, M.R., Dickinson, S.J., Foot, I., Knott, P., McGrath, S.P., Mowat, H., Norman, K., Scott, P.R., Stroud, J.L., Tucker, M., White, P.J., Zhao, F.J., Hurst, R., 2011. Selenium concentration and speciation in biofortified flour and bread: retention of selenium during grain biofortification, processing and production of Se-enriched food. Food Chem. 126, 1771–1778.

Hartikainen, H., Xue, T.L., 1999. The promotive effect of selenium on plant growth as triggered by ultraviolet irradiation. J. Environ. Qual. 28, 1372–1375.

Haug, A., Graham, R.D., Christophersen, O.A., Lyons, G.H., 2007. How to use the world's scarce selenium resources efficiently to increase the selenium concentration in food. Microb. Ecol. Health Dis. 19, 209–228.

Institute of Medicine, 2000. Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium and Carotenoids. National Academy Press, Washington, DC.

Johnson, C.C., Fordyce, F.M., Rayman, M.P., 2010. Factors controlling the distribution of selenium in the environment and their impact on health and nutrition. Proc. Nutr. Soc. 69, 119–132.

Lyons, G.H., Genc, Y., Soole, K., Stangoulis, J.C.R., Liu, F., Graham, R.D., 2009. Selenium increases seed production in Brassica. Plant Soil 318, 73–80.

Lyons, G., Ortiz-Monasterio, I., Stangoulis, J., Graham, R., 2005. Selenium concentration in wheat grain: is there sufficient genotypic variation to use in breeding? Plant Soil 269, 269–380.

Lyons, G.H., Stangoulis, J.C.R., Graham, R.D., 2003. High-selenium wheat: biofortification for better health. Nutr. Res. Rev. 16, 45–60.

Masscheleyn, P.H., Delaune, R.D., Patrick Jr., W.H., 1990. Transformations of selenium as affected by sediment oxidation-reduction potential and pH. Environ. Sci. Technol. 24, 91–96.

Rayman, M.P., 2004. The use of high-selenium yeast to raise selenium status: how does it measure up? Br. J. Nutr. 92, 557–573.

Rayman, M.P., 2008. Food-chain selenium and human health: emphasis on intake. Br. J. Nutr. 100, 254–268.

Ríos, J.J., Blasco, B., Cervilla, L.M., Rosales, M.A., Sanchez-Rodriguez, E., Romero, L., Ruiz, J.M., 2009. Production and detoxification of H_2O_2 in lettuce plants exposed to selenium. Ann. Appl. Biol. 154, 107–116.

Séby, F., Potin-Gautier, M., Giffaut, E., Borge, G., Donard, O.F.X., 2001. A critical review of thermodynamic data for selenium species at 25°C. Chem. Geol. 171, 173–194.

Thomson, C.D., 2004. Selenium and iodine intakes and status in New Zealand and Australia. Br. J. Nutr. 91, 661–672.

Turakainen, M., Hartikainen, H., Seppänen, M.M., 2004. Effects of selenium treatments on potato (*Solanum tuberosum* L.) growth and concentrations of soluble sugars and starch. J. Agric. Food Chem. 52, 5378–5382.

USGS, 2011. U.S. Geological Survey, Mineral Yearbook 2009, Selenium (online), Available at: http://minerals.usgs.gov/minerals/pubs/commodity/selenium/ (Accessed 4 July 2011).

van Lettow, M., Harries, A.D., Kumwenda, J.J., Zijlstra, E.E., Clark, T.D., Taha, T.E., Semba, R.D., 2004. Micronutrient malnutrition and wasting in adults with pulmonary tuberculosis with and without HIV co-infection in Malawi. BMC Infect. Dis. 4, 61.

Vuori, E., Vääriskoski, J., Hartikainen, H., Vakkilainen, P., Kumpulainen, J., Niinivaara, K., 1989. Sorption of selenate by Finnish agricultural soils. Agric. Ecosyst. Environ. 25, 111–118.

White, P.J., Bowen, H.C., Parmaguru, P., Fritz, M., Spracklen, W.P., Spiby, R.E., Meacham, M.C., Harriman, M., Trueman, L.J., Smith, B.M., Thomas, B., Broadley, M.R., 2004. Interactions between selenium and sulphur nutrition in *Arabidopsis thaliana*. J. Exp. Bot. 55, 1927–1937.

White, P.J., Broadley, M.R., 2009. Biofortification of crops with seven mineral elements often lacking in human diets—iron, zinc, copper, calcium, magnesium, selenium and iodine. New Phytol. 182, 49–84.

Williams, P.N., Lombi, E., Sun, G.-X., Scheckel, K., Zhu, Y.-G., Feng, X., Zhu, J., Carey, A.-M., Adomako, E., Lawgali, Y., Deacon, C., Meharg, A.A., 2009. Selenium characterization in the global rice supply chain. Environ. Sci. Technol. 44, 6024–6030.

Xue, T.L., Hartikainen, H., 2000. Association of antioxidative enzymes with the synergistic effect of selenium and UV irradiation in enhancing plant growth. Agric. Food Sci. Finland 9, 177–186.

Zhao, F.J., Su, Y.H., Dunham, S.J., Rakszegi, M., Bedo, Z., McGrath, S.P., Shewry, P.R., 2009. Variation in mineral micronutrient concentrations in grain of wheat lines of diverse origin. J. Cereal Sci. 49, 290–295.