Wasabi yield and isothiocyanate responses to fertiliser type

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Abstract

Wasabi (*Wasabia japonica* (Miq.) Matsum.) is grown to produce the hot paste used extensively in Japanese cuisine. The hotness comes from the isothiocyanate (ITC) content of the paste. Current commercial fertiliser recommendations for growers are expensive to implement. In this trial, commercially recommended applications of lime, manure and nitrogen fertiliser were examined singly and in combination for their effect on yield and ITC content of leaves, petioles, stems and roots. Also, the effects of alternative forms of nitrogen fertiliser with different sulphur contents were examined. The heaviest component of yield was the leaves, but the highest concentrations of ITC were found in the thickened stems and roots. Manure increased leaf and petiole yields, but lime had no effect on yield. Lime increased the ITC content of roots, but manure had no effect on ITC concentrations of any plant component. Fertiliser type had little effect on yield, but increasing the sulphur content of the fertiliser increased ITC content in petioles, stems and roots. Growers could considerably reduce their fertiliser costs by using ammonium sulphate instead of the fertiliser mixtures recommended for contract wasabi growers in Canterbury.

Additional Key Words: *Wasabia japonica*, nitrogen, sulphur, manure, lime, yield components

Introduction

Wasabi (*Wasabia japonica* (Miq.) Matsum.) is a semi-aquatic crucifer grown to produce a hot paste that is used extensively in Japanese cuisine to flavour sushi, raw fish, and some noodle dishes. Details of its botany, morphology, and uses are given in Chadwick *et al.,* (1993). It is traditionally grown in running water under shade in Japan (Follett, 1986). It is also grown in soil in Taiwan (Chadwick *et al.*, 1993) and, since the 1980s, in New Zealand (Martin and Deo, 2000). Different processors use different parts of the wasabi plant according to end use, e.g. either the stems alone, or stems and roots, or stems, leaves and petioles.

The hotness of the paste comes from isothiocyanates, which are found as breakdown products of glucosinolates. Glucosinolates occur in the cells of wasabi and other crucifers and are believed to deter insect pests (Duncan, 1991). When crushing or grating disrupts the cells, the glucosinolates are hydrolyzed into isothiocyanates (ITCs). Most of the ITC is in the form of 2-propenyl-isothiocynate, commonly referred to as allyl isothiocyanate (Sultana *et al.*, 2002), but there are a number of other ITCs that influence the taste and pungency of wasabi (Depree *et al.*, 1999).

Little is known about the fertiliser requirements of wasabi (Follett, 1986; Chadwick *et al.*, 1993). Typical industry contract fertiliser recommendations for soil-grown wasabi in Canterbury are for 400 m^3/ha of pig manure and sawdust and 30 t/ha chicken manure (together costing $4,500/ha), 400 kg/ha of Agroblen ($1,800/ha),and 4000 kg/ha of gypsum ($1000/ha), giving a total cost of $7,300/ha (Craigie, 2002), which adds considerably to the cost of wasabi production. In this study, the effect of recommended manure and lime applications on yield and ITC contents of different plant parts was examined. Nitrogen fertiliser treatments applied were: no fertiliser; the recommended level of slow release fertiliser; the same rate of N applied as other forms of nitrogen fertiliser; half the rate of slow release fertiliser; and split dressings of a conventional nitrogen fertiliser.

Materials and Methods

The trial was carried out at the Lincoln farm of Crop and Food Research. The soil is a deep (>1.6 m) Templeton sandy loam (*Udic Ustochrept*, USDA Soil Taxonomy) (New Zealand Soil Bureau, 1968) with an available water holding capacity of c. 190 mm/m of depth. Soil physical properties on an adjacent site were detailed by Martin *et al.,* (1992). The crop was grown in a 1324 m^2 shade house, which gave about 80 % shade. This trial followed a previous wasabi crop described by Martin and Deo (2000). After that crop had been removed, the trial area was fallowed over winter, then ploughed on 16 October 1997. Soil nutrient status (µg/g in the 0-15 cm layer) at the start of the trial was pH 6.4, Ca 1625, K 280, P 15, Mg 90, Na 35, S 2 and NO_3-N 15, with total N 0.21 g/100g oven dry soil.

The trial area had four beds with each bed ridged up into three planting beds 30m long by 1m wide and 0.3m high. A split plot trial with four replicates, one per bed, was applied to the site with four main plot treatments and six sub-plot treatments.

The main plot (lime and manure) treatments were:
a. Control (no lime or manure);
b. Lime (10 t/ha calcium carbonate);
c. Manure (a mixture of poultry manure (3.86 g N/100 g on dry basis, 2088 µg S/g) at 30 t/ha and pig manure (0.8 g N/100g on dry basis, 8 µg S/g) at 400m^3/ha);
d. Lime and manure together (at same rates as in b and c).

Sub-plot fertiliser treatments were:
1. Control, no fertiliser;
2. One application of calcium ammonium nitrate (CAN, 27 % N) at 200 kg N/ha applied at planting (25 November 1997);
3. Four applications of calcium ammonium nitrate (CAN, 27 % N), at 50 kg N/ha at planting, 31 March 1996, 7 September 1996 and 9 February 1997;
4. One application of Agroblen™ (Scotts Australia Pty Ltd) (4.4 % S, 22 % N), at 100 kg N/ha applied at planting as slow release S fertiliser;
5. One application of Agroblen™ (Scotts Australia Pty Ltd) (8.8 % S, 44 % N), at 200 kg N/ha applied at planting;
6. One application of ammonium sulphate (AS, 24 % S, 21 % N), at 200 kg N/ha applied at planting.

The main plots were 4.5 m long by 3 beds wide, while each sub plot was 2.25 m long by 1 m wide. The lime and manure treatments were applied to the beds and rotovated in on 29 October. The beds were then raised using a ridger, and covered with 3 to 5 cm of sawdust. All the beds were irrigated using mini sprinklers, and then fertiliser treatments were applied by hand. On 25 November 1997, 10-week-old seedlings of wasabi cv.Daruma, the current commercial cultivar of wasabi grown in Canterbury, were transplanted into beds at 0.3 m by 0.3 m spacing with three rows per bed. The beds were irrigated again after planting. Subsequent fertiliser applications were broadcast over the appropriate plots and irrigated in. All the plots were irrigated, weeded and sprayed to avoid any other limitation to growth.

The trial was harvested in May 1999. Five plants from each of the three rows in each plot were harvested and weighed. From this 15-plant sample, two plants were selected at random, and divided into leaves, petioles, stems (rhizomes), and roots. A 50-100 g subsample of each of these plant components rhizomes from the two plants was deep-frozen at -18°C for chemical analysis.

The frozen sample was homogenized in a domestic juicer and 2 g of the resulting paste was mixed with 20 g light paraffin oil. Samples were incubated at room temperature for one hour with occasional shaking. The solids were removed by centrifugation and the paraffin oil extract stored in a cold room (0°C) to assay for ITCs. The remainder of the wasabi paste was used for dry matter analysis.

The extracted paraffin oil (6 g) was added with methanol (5 ml) and ammonia solution (1 ml) and incubated at 45 °C with continual mixing by inversion for 5 hours. The ITC was converted to allyl thiourea using a modification of the Daun and Haugen (1976) method. After separation, an aliquot (0.05 ml) of the methanol phase was diluted to 4 ml with methanol for measurement of UV absorbance by spectrophotometer. A standard curve was established with allyl thiourea in methanol, and standards and extracts were measured against methanol blanks.

The results were analyzed by analysis of variance using the GenStat statistical package (Genstat Committee, 2000). There were some missing plots due to lost samples.

Results

The crop produced an overall average of 93 t/ha of fresh material; 54 % of the fresh weight of the wasabi crop was in the petioles, 19 % in the leaves, 18 % in the stems and 9 % in the roots (Table 1).

The application of manure significantly ($P<0.05$) increased yields of both petioles and leaves, compared to the lime treatment resulting in a significant overall increase in total yields (Table 1).

In contrast, the fertiliser treatments had no significant effect ($P<0.05$) on the yields of any of the wasabi components, and there were no significant interactions.

Table 1: Effect of lime, fertiliser and manure on fresh weight (t/ha) of wasabi leaves, petioles, stems and roots.

Treatment	Leaf	Petiole	Stem	Root	Total
Nil	16.43	44.8	16.19	7.95	85.4
Lime	14.94	42.4	13.88	6.87	78.1
Manure	19.33	57.7	17.94	7.97	103.0
Lime & manure	19.98	58.0	19.29	9.28	106.6
LSD (P<0.05, d.f. 9)	3.806	10.10	4.571	2.345	18.59
Fertiliser					
Nil	19.04	49.7	15.45	7.55	91.8
200 CAN[1] single	17.82	50.6	18.54	7.82	94.8
200 CAN split	16.91	49.4	17.67	7.50	91.5
100 Agroblen	18.32	49.9	15.98	8.02	92.3
200 Agroblen	16.37	47.9	15.01	7.89	87.2
200 AS[2]	17.55	56.8	18.32	9.31	101.9
LSD (P<0.05, d.f. 60)	4.307	12.62	3.372	2.289	19.24

[1]CAN= Calcium ammonium nitrate, [2]AS = Ammonium sulphate.

Table 2: Effect of lime, fertiliser and manure on isothiocyanate concentration (mg/kg) of wasabi leaves, petioles, stems and roots.

Treatment	Leaf	Petiole	Stem	Root
Nil	375	198	2296	1674
Lime	379	215	2633	2043
Manure	499	302	2429	1706
Lime & manure	417	238	2094	1264
LSD (P<0.05, d.f. 9)	133.2	90.5	386.7	302.6
Fertiliser:				
Nil	411	192	2278	1710
200 CAN single	370	171	1638	1126
200 CAN split	368	212	1606	1215
100 Agroblen	425	246	2475	1581
200 Agroblen	434	287	2700	1691
200 AS	498	320	3481	2708
LSD (P<0.05, d.f. 60)	105.5	92.8	423.8	323.5

The highest concentration of ITC (overall mean = 2363 mg/kg) was in the stems (Table 2), ten times higher than in the petioles (238 mg/kg). The roots also had a high concentration of ITCs, (1672 mg/kg), but this was only 70 % of that in the stems. Leaf ITC concentration was also low (418 mg/kg), although 1.8 times that in the petioles.

N fertiliser form or rate had no effect on leaf ITC concentration (Table 2). For petioles, the ammonium sulphate and Agroblen at 200 kg N/ha treatments produced significantly ($P<0.05$) higher ITC concentrations than the nil or both CAN treatments. For stems and roots, the ammonium sulphate treatment produced significantly ($P<0.05$) higher ITC concentrations than the other treatments, whereas the two CAN treatments produced significantly lower ITC concentrations. There were no significant interactions between the lime and manure treatments and the fertiliser treatments for leaves, petioles and stems. However, in the roots, ITC concentrations were significantly ($P<0.05$) higher with lime than with manure or lime plus manure for the ammonium sulphate and Agroblen (200 kg N/ha rate) treatments but not for the other fertiliser treatments (data not presented).

Overall, 54 % of the ITC was in the stems, with 18 % in the petioles and roots, and 10 % in the leaves (Table 3). Addition of manure significantly ($P<0.05$) increased leaf and stem ITC, but adding lime had no effect. Lime and manure had no significant ($P<0.05$) effect on stem, root or total ITC yield.

The fertiliser treatments had no effect on leaf ITC yield. For petioles, the ammonium sulphate treatment produced significantly higher ITC yields than all other fertiliser treatments except Agroblen at the 200 kg N/ha rate. For stems and roots, the ammonium sulphate treatment produced significantly higher ITC yields than the other fertiliser treatments, which were not significantly different to each other.

Note that because there were some missing data, the ITC yields in Table 3 are not the sum of the data in Tables 1 and 2.

Table 3: Effect of lime, fertiliser and manure on isothiocyanate yield (g/ha) of wasabi leaves, petioles, stems and roots.

Treatment	Leaf	Petiole	Stem	Root	Total
Nil	588	939	3568	1355	6400
Lime	567	955	3526	1344	6392
Manure	946	1691	4342	1313	8164
Lime & manure	876	1407	3879	1144	7855
LSD ($P<0.05$, d.f. 9)	299.4	309.7	1054	341.1	1575.5
Fertiliser:					
Nil	851	1031	3266	1238	6530
200 CAN single	726	925	3050	843	6003
200 CAN split	558	979	2638	884	5563
100 Agroblen	760	1219	3791	1143	6912
200 Agroblen	718	1406	3998	1225	7079
200 AS	857	1929	6230	2401	11402
LSD ($P<0.05$ d.f. 60)	273.8	582.3	1053.2	413.1	1606.9

Discussion

Highest ITC concentrations and yields were produced by the ammonium sulphate treatment. This fertiliser also supplied the most sulphur to the crop. In trials carried out at the same time as this trial, Craigie (2002) found that applying manure had no effect on yield, but that yields increased with increasing N fertiliser up to 400 kg N/ha of fertiliser. He suggested that using a fertiliser regime of CAN, potassic super and gypsum would save growers

$5,500/ha in fertiliser costs compared to using current commercial rates. This study shows that using ammonium sulphate at his suggested rate of 560 kg S/ha would only cost growers around $500/ha. In Japan, the commercially recommended nitrogen fertiliser rate is 270 kg/ha (Adachi, 1987, quoted in Craigie 2002), which, if applied as ammonium sulphate, would only cost around $300/ha. No studies have been done on the phosphate and potassium requirements of wasabi, but long standing brassica fertiliser recommendations have been for 20 kg P/ha and little or no K (Claridge, 1972), which would cost around another $50/ha, with a similar cost for lime. However, applications of these and other nutrients should be determined on the basis of soil tests or plant analyses, rather than on the current blanket recommendation for all sites.

The increase in root ITC concentrations with lime and high rates of S fertiliser suggests that root zone pH is important for high ITC levels in wasabi. The soil pH in this trial was slightly below the 6.5-7 recommended in Japan (Follett, 1986). The lack of opportunity for rotation (because of the use of fixed structures to provide the shade required by wasabi) means that lime could also be important in reducing the impact of soil-borne diseases, such as clubroot, which can affect wasabi (Follett, 1986; Martin and Deo, 2000).

The only case where organic matter would be useful for wasabi production would be where soil physical problems, such as soil structure or drainage, could impede crop growth or make the crop more susceptible to some of the diseases that can severely affect wasabi production (Martin and Deo, 2000).

Commercial buyers aim to buy stems with a minimum ITC concentration of 2000 ppm. The nil and sulphur containing fertiliser treatments exceeded this level, with the ammonium sulphate treatment reaching 3481 ppm. The nitrogen only fertiliser treatments had less than 2000 ppm ITC in the stems, further reinforcing the need to achieve the correct balance between nitrogen and sulphur in the nutrient supply. Duncan (1991) found that glucosinolate concentrations in other crucifers were increased by increased sulphate concentration in the soil solution, and decreased by increased nitrogen concentration.

Wasabi stems, traditionally called rhizomes in the literature (Chadwick *et al.*, 1993), produced a much higher yield of ITC than leaves, petioles and roots. The lower yield of ITC in these other components resulted from either low fresh weights in the case of roots, or very low concentrations of ITC in the case of leaves and petioles. The stems can be sold to the high priced restaurant trade, whereas the lower yielding components are only suitable for processing. Growers, buyers and researchers should therefore aim to maximize the yield and ITC concentration of wasabi stems to maximize quality and returns to the industry.

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