

Effect of plant density and depth of harvest on the production and quality of licorice (*Glycyrrhiza glabra*) root harvested over 3 years

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Abstract Licorice (*Glycyrrhiza glabra*) root production was investigated in Central Otago, Canterbury, and Waikato regions of New Zealand over 4 years. Increasing plant populations at establishment had a large effect on production. Maximum root and rhizome production was achieved with plant populations above 24 000 plants/ha. Low plant densities favoured rhizome production and high densities favoured root production. Sampling roots and rhizomes to 900 mm showed that the majority of production was in the top 300 mm. Roots harvested

from below 300 mm contained less glycyrrhizin than surface roots. Glycyrrhizin concentration increased each year and surpassed the minimum international standard of 4% in the first harvest in the Waikato but not until the third harvest at South Island sites. By the third harvest both Waikato and Canterbury grown licorice contained similar amounts of glycyrrhizin but with 90% of the glycyrrhizin content in roots in the 0–300 mm soil layer in the Waikato and in the 0–600 mm soil layer in Canterbury. These trials show that licorice can be grown in New Zealand with fresh root and rhizome yields of 17–28 t/ha in the second and third years of production and with a glycyrrhizin content above the minimum international standard.

Keywords licorice; *Glycyrrhiza glabra*; glycyrrhizin; plant density; production

INTRODUCTION

Licorice (*Glycyrrhiza glabra* L.) root (collectively, roots and rhizomes) extract has been widely used for medicinal, flavouring, and confectionary purposes since early civilisation (Nieman 1957; Chandler 1985; Fenwick et al. 1990; Olukoga & Donaldson 1998). The plant itself is a herbaceous, perennial legume native to the Mediterranean, near East, central Asia, and western Siberian regions (Lange 1998). It grows naturally where the mean annual rainfall varies from 400 to 1160 mm, the mean annual temperature varies from 5.7 to 25°C, and the soil pH varies from 5.5 to 8.2, but it grows best in dry, sunny, hot climates with an annual rainfall of 500–650 mm and adequate soil moisture (Duke 1981). Wild licorice populations have been the traditional source of licorice root but unsustainable harvesting has reduced this supply and increased the need for cultivation to meet market demands (Lange 1998). Licorice is considered an easy plant to grow (Whitten 1997; Olukoga & Donaldson 1998) but there is little published information defining the agronomic requirements to optimise root production.

Licorice is normally grown from rhizome cuttings or harvested crowns taken from mature crops and laid out in shallow furrows (Molyneux 1975; Anon. 1982; Singh et al. 1984). Plant spacings to establish the crop have varied from 0.33 m × 0.67 m (45 000 plants/ha) in England (Molyneux 1975), 0.45–0.6 m × 0.9 m (18 500–24 400 plants/ha) in India (Singh et al. 1984), 0.4–0.5 m × 0.6–0.7 m (28 600–41 700 plants/ha) in Italy (Marzi et al. 1993b), and 0.3 m × 0.9–1.2 m (27 800–37 000 plants/ha) in Australia (Whitten 1997). We could find little published evidence to substantiate the choice of these plant spacings, except for research in Italy and Sicily. Marzi et al. (1993b) compared licorice grown in rows spaced from 0.4 m to 1.2 m and within row plant spacings of 0.4 m to 1.2 m to give a plant population range from 6900 to 62 500 plants/ha. Fresh root yields were maximised (20.4 t/ha) at a plant population of 42 000 plants/ha with the low and high plant population extremes producing root yields of 7–9 t/ha. Leto et al. (1996) in Sicily compared licorice grown in 0.7 m spaced rows with intra-row spacings of 0.3–0.9 m to give a plant population range from 15 900 to 47 600 plants/ha. At one site the fresh root yield increased from 6.4 t/ha to 12.2 t/ha as the plant populations were increased to 47 600 plants/ha but at a second site a plant population of 20 400 plants/ha gave a root yield (12.4 t/ha) similar to that from the highest plant population.

Licorice is slow to establish from unrooted cuttings and in both Italy and Pakistan irrigation improved the establishment by 7% to over 90% (Mohammad & Rehman 1985; Marzi et al. 1993b). In the 1–2 year period of slow growth after planting, inter-row cropping may be undertaken to provide income (Molyneux 1975; Whitten 1997) but this practice is not recommended (Singh et al. 1984), and inter-row cropping with cereals and ryegrass have depressed licorice root yields (De Mastro et al. 1993; Marzi et al. 1993b; Bezzi & Aiello 1996). Licorice may develop a root system 2–3.5 m deep (Duke 1981) but most of the root mass occurs in the top 300 mm (Leto et al. 1996; Martin et al. 1996). Normally roots are harvested from only the top 1 m, but where complete root harvests have been made, fresh root yields of 50 t/ha have been reported (Molyneux 1975; Duke 1981; Anon. 1982). Licorice harvests are usually undertaken 3–5 years after establishment (Molyneux 1975; Duke 1981; Singh et al. 1984) and fresh root yields of 14–26 t/ha have been harvested from 3- or 4-year-old experimental crops in Italy (Marzi et al. 1993b; Bezzi & Aiello 1996; Leto et al. 1996). Bezzi & Aiello (1996)

showed that delaying harvest from 3 years to 5 years increased root production from 14 t/ha to 23 t/ha. Irrigation also increased the fresh root yields by 30% to 26.5 t/ha on a sandy-clay soil in Italy (Marzi et al. 1993b) and the dry root yields by 13% to 6.1 t/ha on sand dunes in Pakistan (Mohammad & Rehman 1985). Contrary to these results a rainfed licorice crop grown for 4 years on a deep silty-clay soil in Italy gave a fresh root yield of 24.3 t/ha with irrigation depressing the root yield by 17% (De Mastro et al. 1993).

Fertile soils are considered necessary to grow licorice (Singh et al. 1984; Whitten 1997). Singh et al. (1984) recommended applying 37–49 t/ha of farmyard manure before planting but considered fertiliser was unnecessary on fertile soils. Nevertheless few fertiliser comparisons are evident in the published literature. Marzi et al. (1993b) found a single application of a fertiliser mixture containing 100 kg/ha nitrogen (N), 44 kg/ha phosphorus (P), and 166 kg/ha potassium (K) increased the fresh root yield of a 3-year-old crop by 34% to 26.5 t/ha but obtained no benefit from applying each element separately. In India, Maheshwari et al. (1984) obtained no response to 120 kg/ha N and 120 kg/ha P applied in addition to a basal dressing of 40 t/ha of farmyard manure.

Licorice root for use as a medicinal herb is required to contain at least 4% (40 g/kg) glycyrrhizin (glycyrrhizic acid, glycyrrhizic acid) (American Botanical Council 1998; WHO 1999). Typically, licorice root contains 2–9% glycyrrhizin (WHO 1999) with an average of 7–10% in the Mediterranean region (Nieman 1957) but with concentrations as high as 15%, depending on variety, age, where the licorice was grown, and when it was harvested (Fenwick et al. 1990; Hayashi et al. 1998). Glycyrrhizin concentration increases as roots and rhizomes become older and larger in diameter (Hayashi et al. 1993; Marzi et al. 1993a; Usai et al. 1995). Tap roots had higher glycyrrhizin concentrations than rhizomes in pot-grown plants with the relative differences reduced in plants harvested after 3 years instead of 2 (De Mastro & Circella 1993; Marzi et al. 1993a). In field studies, rhizomes had a higher glycyrrhizin concentration than tap roots harvested after 5 years of growth (Bezzi & Aiello 1996). Maheshwari et al. (1984) found no effect of different rates of N and P fertilisers on glycyrrhizin concentration.

Licorice is not a commercial crop in New Zealand and little information is available on its productive potential and the agronomic requirements to achieve

high quality root production. Preliminary research begun in the early 1980s (Palmer 1987) was not continued but more potential was shown by a commercial trial crop established in 1987 which gave fresh root yields up to 22 t/ha after 7 years of growth (Martin et al. 1996). This current research was begun in 1993 as part of a new crop development programme to investigate the potential of a range of new crops for New Zealand and their agronomic and quality requirements (Douglas 1993). As part of the research on licorice we have investigated weed control strategies (Hartley 1996) and developed an efficient method for glycyrrhizin measurement (Lauren et al. 2001). This paper presents results from agronomic research on licorice production conducted in 3 field trials over a 4-year period in 3 contrasting environments.

MATERIALS AND METHODS

Three field trials were conducted using cutting-grown licorice plants derived from a small commercial planting near Ashburton, Canterbury, established from tissue cultured plants originally sourced from Sicily (Martin et al. 1996).

Trial 1: Redbank Research Station, Clyde, Central Otago (Lat. 45°14'S, Long. 169°37'E)

This trial was conducted on a Manuhierikia silt loam with soil quick tests (0–150 mm) of pH 6.9, Ca 1136 ppm, K 164 ppm, P 34 ppm, Mg 91 ppm, S 7 ppm. The experimental design was fully randomised blocks with three plant densities × three harvest dates × three replicates. Plants were established at 0.25 × 0.25 m (166 700 plants/ha), 0.50 × 0.50 m (40 000 plants/ha) and 1 × 1 m (10 000 plants/ha) spacings and harvested after 1, 2, or 3 years of growth. Root cuttings were taken from mature plants in September 1993 and sprouted in propagation trays. These were planted as rooted cuttings in March 1994 with 12 datum plants in each plot surrounded by a single guard row planted at the same spacing and a 1 m gap between plots. Plant establishment was affected by winter frosts in the first year and plant deaths were not replaced. Contact herbicides were used to kill weeds during the winter dormant period after planting and subsequently they were controlled by a 20–30 mm sawdust mulch applied in spring and hand weeded. Plots were topdressed with fertiliser (500 kg/ha Nitrophoska blue TE; 12:5:14:4:1.2:4 % N P K S Mg Ca plus trace elements) at planting and 300 kg/ha 15% potassic superphosphate (8:7:9 % P

K S) was applied in December 1995. Spray irrigation was applied during the October to March period of each year with 382 mm applied in 1994/95, 304 mm in 1995/96, and 396 mm in 1996/97 to augment the rainfall of 210, 387, and 232 mm for the respective periods. The crop was harvested when the plants were dormant to a depth of 0.5 m in June 1995, May 1996, and June 1997. The root mass was washed and cleaned and sorted into tap root and rhizome components with subsamples dried at 65°C to determine the dry matter (DM) content. Apart from the first harvest, additional subsamples for glycyrrhizin analyses were dried at 35°C, ground to a fine powder, and stored at –18°C.

Trial 2: Canterbury Agricultural Centre, Lincoln, Canterbury (Lat. 43°37'S, Long. 172°28'E)

This trial was established on a Templeton silt loam overlying sand with soil quick tests (0–300 mm) of pH 6.0, Ca 1250 ppm, K 220 ppm, P 29 ppm, Mg 90 ppm. Soil physical characteristics on an adjacent area have been given by Martin et al. (1992).

In July 1994, cuttings from dormant licorice plants grown near Ashburton (Martin et al. 1996) were planted into pots and kept in a shaded area outdoors. In December 1994, the young plants were transplanted into rows 0.82 m apart with 0.5 m between plants (24 400 plants/ha). A factorial trial was laid out in November 1995 with two rates of irrigation (none or irrigated) and two rates of N fertiliser as urea (none or 70 kg N/ha), with three replicates. Each plot was 9 m long by 5 rows wide. The trial was run for 3 years with annual harvests with N and irrigation treatments as main plots and year of harvest as subplots. The N fertiliser was hand-broadcast on 9 November 1995, 15 October 1996, and 6 October 1997 at the beginning of spring growth.

Before the trial was planted the area was irrigated to provide moist conditions for transplanting. Subsequent irrigation treatments were applied through a trickle system, with laterals along each row and drippers at each plant. Irrigation was applied from October to March each season with a minimum irrigation application of 15 mm. Between 25 and 95 mm irrigation was applied each month with 335 mm in 1995/96, 245 mm in 1996/97, and 370 mm in 1997/98. The crop was hand weeded during establishment and in subsequent winters when the crop was dormant.

The trial was harvested when the licorice was dormant in August 1996, July 1997, and June 1998.

Six plants in a 2.4 m² quadrat were harvested from each plot. A mini-excavator was used to successively strip the soil in the quadrat to 150, 300, 600, and 900 mm depths. For each depth interval, all crown, tap-root, and rhizome material was removed separately, weighed in the field, and subsampled for DM and glycyrrhizin determinations. DM determinations were made after drying at 35°C in a forced-air oven. A 50 g sample of the dried material was ground to a powder and stored at -18°C before glycyrrhizin analyses.

Trial 3: Waikato Research Orchard, Rukuhia, Waikato (Lat. 37°52'S, Long. 175°18'E)

This trial was established on a Horotui sandy loam with soil quick tests (0–150 mm) of pH 5.4, Ca 454 ppm, K 127 ppm, P 68 ppm, Mg 41 ppm, S 69, without irrigation. In October 1994, 200–300-mm-long dormant root cuttings, supplied from Canterbury, were planted in 100 mm furrows with 0.8 m between rows and 0.5 m between plants within the rows to give a plant population of 25 000 plants/ha. Root production from 1, 2, or 3-year growth periods was compared in a randomised block design with 5 replicates. Before planting, the area was fertilised with 1.5 t/ha 30% potassic serpentine superphosphate and 0.5 t/ha diammonium phosphate. Weeds were controlled by annual, early spring applications of Gesatop[®] before the licorice emerged, followed by Fusilade[®] to control summer grasses. Weeds were also controlled by a sawdust mulch applied in May 1995. Dead top growth was mown and removed each winter when the licorice was dormant and any weeds controlled by the use of contact herbicides. The total root mass was harvested by hand from an area of 0.8 × 2 m in each plot when the licorice was dormant in June 1996, May 1997, and May/June 1998 with the roots collected separately from the 0–300, 300–600, and 600–900 mm soil depths. The roots were washed and air dried before weighing and a sample oven-dried at 65°C was used to calculate the DM percentage. Additional subsamples were taken and dried at 35°C, ground to a fine powder and stored at -18°C for glycyrrhizin analyses.

Glycyrrhizin analyses

Determination of the glycyrrhizin content was carried out on ground root samples using the method of Lauren et al. (2001) in which glycyrrhizin is first extracted and then hydrolysed to the aglycone, 18β-glycyrrhetic acid, for analysis. Briefly, a subsample (1 g) was subjected to hot soxhlet

extraction using 20:80 ethanol-water mixture for 4 h. An aliquot of the extract was hydrolysed by diluting with 10% sulphuric acid and heating in a water bath at 92°C for 2 h. The cooled, hydrolysed sample was then partitioned against chloroform and the mixture separated by centrifuging at 2000 g. The chloroform layer was filtered and dried by passing through anhydrous sodium sulfate, and an aliquot was evaporated to dryness then dissolved in methanol for high performance liquid chromatography (HPLC) analyses. Quantitative determination was by comparison with an external standard of 18β-glycyrrhetic acid. Analyses undertaken as part of this method development showed that increasing the drying temperature of licorice roots from 35°C to 65°C did not reduce the glycyrrhizin concentration (Lauren et al. 2001).

Biometrical analysis

Data were analysed by analysis of variance using Genstat statistical packages (Genstat 5 Committee 1997). Where necessary to satisfy the assumptions for the analysis of variance the data were log transformed. For presentation of the treatment means the data was back transformed with bias correction.

RESULTS

The mean monthly rainfall and temperatures recorded at the trial sites over the trial period are shown in Table 1. The Clyde site had a low, summer dominant, annual rainfall of 454 mm and cold winter conditions. At Lincoln the winter temperatures were less severe with a winter dominant annual rainfall of 506 mm. Rukuhia had a winter dominant annual rainfall (1315 mm) almost 3 times greater than that at Clyde with the mean annual temperature (13.9°C) 2.1–3.5°C warmer than the other two sites. All sites experienced winter frosts (data not shown) with winter conditions most severe at Clyde where surface soil freezing occurs.

Trial 1: Clyde

Effect of plant population on root production

The plant populations established in the individual plots varied from 5400 to 8200 plants/ha at the low plant density (mean 6300 plants/ha), 22 200 to 32 200 plants/ha at the medium plant density (mean 25 800 plants/ha), and 66 800 to 109 900 plants/ha at the high plant density (mean 87 600 plants/ha). Frost kill in the first winter reduced the established

plant numbers. High plant population at establishment was associated with increased root yields in each year of harvest (Fig. 1). At the first harvest the individual plant yield at the lowest plant population (5500 plants/ha) was 142 g and this decreased to 104 g (–27%) at the highest population (91 600 plants/ha). The mean yield increment was 1270 kg/10 000 plants up to the mid-population of 32 200 plants/ha and 924 kg/10 000 plants above this population up to 91 600 plants/ha. At the second harvest the individual plant yield at the low population (5400 plants/ha) was 1264 g and this decreased to 149 g (–88%) when the population was lifted to 109 900 plants/ha. The mean yield increment was 3230 kg/10 000 plants up to 22 200 plants/ha and 473 kg/10 000 plants above this population up to 109 900 plants/ha. At the third harvest the individual plant yield at the lowest population (8200 plants/ha) was 1643 g and this decreased to 304 g (–82%) when the population was lifted to 66 800 plants/ha. The mean yield increment was 2764 kg/10 000 plants up to 24 100 plants/ha and 567 kg/10 000 plants above this population up to 66 800 plants/ha.

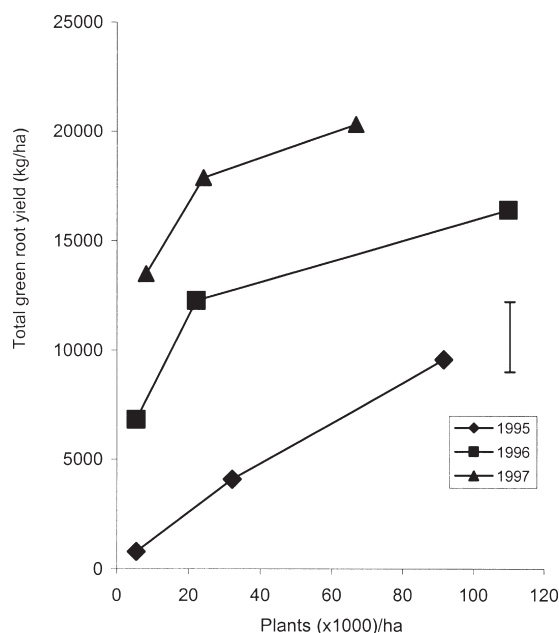


Fig. 1 Effect of plant density on the green root yield of licorice (*Glycyrrhiza glabra*) harvested annually for 3 years (error bar = SED).

Table 1 Mean monthly rainfall and temperature at the trial sites over the trial period 1994/98, New Zealand.

	Rainfall (mm)			Temperature (°C)		
	Clyde	Lincoln	Rukuhia	Clyde	Lincoln	Rukuhia
Jan	60.3	41.8	56.9	17.4	17.2	18.8
Feb	47.3	27.3	67.0	17.4	17.5	19.6
Mar	40.0	52.2	110.1	13.6	14.8	16.8
Apr	44.5	31.6	118.9	10.4	12.7	15.2
May	31.2	43.0	126.0	7.0	9.6	11.8
Jun	27.5*	75.6	137.1	3.5*	6.2	9.5
Jul	25.7	58.9	182.5	2.3	6.0	9.5
Aug	17.9	44.1	116.2	5.3	7.3	9.3
Sep	28.8	40.3	122.5	8.7	9.7	11.5
Oct	38.5	38.2	103.6	10.9	11.5	13.1
Nov	35.4	23.0	88.1	12.5	13.0	14.5
Dec	57.2	30.5	86.7	15.5	16.0	17.1
Total	454.4	506.5	1315.6	10.4†	11.8†	13.9†

*Average of 4 years.

†Mean annual temperature.

Effect of plant population on tap root and rhizome production

Dissection of the fresh root mass showed that the vertical root production dominated the underground production at the first harvest but by the third harvest the horizontal rhizomes were more prevalent (Table 2). Low plant densities were associated with increased rhizome production; high densities were associated with increased root production (Table 2). The DM content of the roots and rhizomes in the first two harvests was 46% and at the third harvest, when they were measured separately, DM content was 46% in the roots and 43% in the rhizomes.

Table 2 Main effect of year of harvest and plant density on the root and rhizome fresh weights harvested at Clyde, New Zealand to a depth of 500 mm (kg/ha).

	Root	Rhizome	Total
Fresh weight			
1995	3260	1550	4810
1996	6020	5810	11830
1997	7850	9360	17210
Mean plant density			
6300 plants/ha	2260	4770	7030
25 800 plants/ha	5460	5940	11400
87 600 plants/ha	9410	6010	15420
LSD (5%)	2145	1429	3373
Significance			
Year	$P < 0.001$	$P < 0.001$	$P < 0.001$
Density	$P < 0.001$	$P = 0.08$	$P < 0.001$
Year \times Density	$P = 0.96$	$P = 0.05$	$P = 0.91$

Glycyrrhizin concentration in the roots and rhizomes

The glycyrrhizin contents of the roots and rhizomes were not measured at the first harvest. At the second harvest the glycyrrhizin content of both the roots and rhizomes was 33.3 g/kg and this increased to 40.5 g/kg (+22%) in the roots and to 45.5 g/kg (+37%) in the rhizomes by the third harvest (Table 3). In the third year the rhizomes grown at the medium density had a higher glycyrrhizin content than those grown at low or high densities, but plant density had no effect on the glycyrrhizin content of the roots. Statistical analysis of the ratio of the glycyrrhizin content in the root and rhizomes revealed no significant differences.

Trial 2: Lincoln

The total fresh root and rhizome yield over the 3-year period averaged 25 020 kg/ha without irrigation and 26 108 kg/ha with irrigation (LSD (5%) 2177.6; $P = 0.27$) and 25 504 kg/ha without N and 25 625 with N (LSD (5%) 2177.6; $P = 0.99$). Consequently these treatment results were combined into an analysis examining the distribution of the roots and rhizomes through the soil profile over the 3-year harvest period. At the first harvest in 1996, 20 months after transplanting, the total fresh root and rhizome production to 0.9 m was 15 740 kg/ha with a further 2330 kg/ha in the plant crown (Table 4). Of this total production, 60.6% of the roots and rhizomes were in the 0–300 mm layer with 21.8% as rhizome production, which was dominantly in the

Table 3 Glycyrrhizin concentration of roots and rhizomes at Clyde, New Zealand in 1996 and 1997 at three plant densities (g/kg).

	1996	1997	Mean
Root			
Low density	32.2	39.1	35.6
Medium density	33.6	42.2	37.9
High density	34.2	40.2	37.2
Mean	33.3	40.5	
LSD(5%) Density 2.9, $P = 0.26$			
Year 2.4, $P < 0.001$			
Density \times Year, $P = 0.61$			
Rhizome			
Low density	30.5	42.4	36.4
Medium density	34.4	53.1	43.8
High density	34.9	40.9	37.9
Mean	33.3	45.5	
LSD(5%) Density 6.0, $P < 0.05$			
Year 4.9, $P < 0.001$			
Density \times Year, $P = 0.11$			

0–150 mm layer (Table 4). The total underground biomass increased by 36% each year with the rhizome component in the 0–150 mm layer increasing more than the rest of the roots and rhizomes put together. By the third harvest there was little change in the % of total biomass in the 0–300 mm layer (62.5%). However, whereas the % as rhizomes had risen from 21.8 to 35.3% the % as roots had decreased from 38.8 to 27.2%. Deducting the crown biomass, 75% of the roots and rhizomes were in the 0–300 mm layer by the third harvest. The crown of the plant more than doubled in size from the first to the third harvest but showed no significant change as a portion of the underground biomass (Table 4).

The %DM of the roots and rhizomes all increased from the first to the third harvest without any change in the %DM of the crown (Table 4). The average %DM (36.3%) of the rhizomes at the first two harvests was 11% lower than roots in the same depth profile but by the third harvest this difference had reduced to 3.7% with the rhizome %DM increasing

to 48.7% (Table 4). Glycyrrhizin content increased from the second to the third harvests (from 29.17 to 43.37 g/kg in the roots and rhizomes), but it did not increase in the crowns and remained below 25 g/kg (Table 4). The glycyrrhizin content was higher in the roots (33.7 g/kg) than the rhizomes (26.6 g/kg) in the same depth layer at the second harvest (1997), but this difference had disappeared by the third harvest (1998). Glycyrrhizin concentration was highest in the roots near the surface and progressively declined in concentration in roots harvested from the deeper soil layers in 1997. This effect was still apparent, but not significant ($P < 0.05$), in 1998 (Table 4).

Trial 3: Waikato

The total fresh root and rhizome production at the first harvest was 25 030 kg/ha 20 months after planting with 93% of the production in the top 300 mm of soil (Table 5). There was a 13% increase in total production at the second harvest but no further yield increase at the third harvest with 89%

Table 4 Lincoln trial, New Zealand: licorice (*Glycyrrhiza glabra*) fresh weight, % dry matter (DM), and glycyrrhizin concentration in the crown, tap root, and rhizome components and their percentage of the fresh weight of the total root, mass at different depths over 3 years.

	Crown	Rhizome 0–150 mm	Root 0–150 mm	Rhizome 150–300 mm	Root 150–300 mm	Root 300–600 mm	Root 600–900 mm	Total
Fresh weight (kg/ha)								
1996	2330	3790	3820	220	3190	2860	1860	18070
1997	3090	7820	3950	1200	3570	3200	1920	24750
1998	5290	10670	4870	1410	4350	4440	2760	33790
LSD (5%)	971	2945	1080	687	892	734	542	3859
	$P < 0.001$	$P < 0.001$	$P = 0.11$	$P < 0.01$	$P < 0.05$	$P < 0.001$	$P < 0.001$	$P < 0.001$
Fresh weight % of root mass								
1996	13.2	20.6	21.1	1.2	17.7	15.9	10.4	–
1997	12.8	31.0	16.1	4.6	14.5	13.1	7.8	–
1998	15.9	31.1	14.3	4.2	12.9	13.4	8.2	–
LSD (5%)	4.11	7.73	3.59	2.21	3.33	3.10	1.74	
	$P = 0.23$	$P < 0.05$	$P < 0.01$	$P < 0.01$	$P < 0.05$	$P = 0.14$	$P < 0.05$	
%DM								
1996	50.5	39.7	49.1	33.3	47.6	47.9	47.5	–
1997	49.1	36.8	48.9	35.4	45.0	66.4	58.6	–
1998	48.9	48.3	54.4	49.2	50.6	52.9	52.8	–
LSD (5%)	3.70	4.83	4.83	7.88	1.57	7.45	8.03	
	$P = 0.63$	$P < 0.001$	$P < 0.05$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.05$	
Glycyrrhizin concentration (g/kg)								
1997	25.4 [†]	27.6	35.1	25.6	32.3	28.8	25.6	29.2 [*]
1998	22.2 [†]	43.2	45.8	44.4	44.8	41.8	40.2	43.4 [*]
1997	Rhizome × Depth $P = 0.58$ Root × Depth LSD (5%) = 5.0, $P < 0.01$							
1998	Rhizome × Depth $P = 0.59$ Root × Depth $P = 0.14$							

* Average of roots and rhizomes.

† Not included in depth analysis.

of the production in the 0–300 mm layer. The %DM of the roots and rhizomes averaged 37.8% with little change over the three harvests, but %DM decreased down the soil profile (Table 5). The glycyrrhizin concentration in the roots and rhizomes increased in the 0–300 mm soil zone from 42.9 to 56.5 g/kg from the first to last harvests with the concentration decreasing down the profile at the final harvest (Table 5).

DISCUSSION

Licorice was grown successfully from southern New Zealand in a cold winter, semi-arid environment (Clyde site) through to the humid, warm-temperate environment of northern New Zealand (Rukuhia site), indicating that licorice can be grown in most regions of New Zealand. Previous results were confined to Canterbury (Palmer 1987; Martin et al. 1996). All cropping regions of New Zealand fall within the mean annual temperature range (5.7–25°C) of endemic licorice (Duke 1981). Irrigation was used in the semi-arid environment to establish and grow the licorice, but in the subhumid environment at Lincoln there was no response to irrigation once the crop was established. The mean annual rainfall (506 mm) at Lincoln falls within the range of 500–650 mm to grow licorice successfully (Duke 1981). Licorice was also grown successfully under

higher rainfall conditions (1315 mm) than the upper limits reported by Duke (1981). Under these conditions some occasional low level infection of black spot (*Phoma medicaginis* var. *pinodella*) on leaves was recorded in summer but it was insufficient to warrant control.

The green root and rhizome yields of up to 28 t/ha in the fourth year of production were similar to the best Italian production (De Mastro et al. 1993; Marzi et al. 1993b) but considerably less than the yields of 50 t/ha reported by Molyneux (1975) and Duke (1981).

Plant density had a major influence on the root and rhizome yields with yields continuing to rise as plant densities increased. There was no indication that yields declined at high plant populations, as found by Marzi et al. (1993b), but insufficient data points were collected to define the optimum plant density with any accuracy. The strong yield gains achieved up to the medium densities tested suggest that the optimum density lies between the medium and high density points. For the third year harvest (a recommended time to harvest the crop) the plant densities were 24 000 to 66 000 plants/ha. This recommended density range is similar to earlier recommendations (Molyneux 1975; Marzi et al. 1993b; Whitten 1997). Plants established at low populations grew at a faster rate than those at higher populations and consequently the population effect

Table 5 Rukuhia trial, New Zealand: licorice (*Glycyrrhiza glabra*) total root-mass fresh weight, % dry matter (DM), and glycyrrhizin concentration measured at three soil depths over 3 years. (nr, not recorded.)

	Depth of root-mass sample (cm)			Total
	0–30	30–60	60–90	
Fresh root mass (kg/ha)				
1996	23 420	1360	250	25 030
1997	25 610	1880	940	28 430
1998	24 430	1980	1120	27 530
LSR (5%) between depths within year = 1.31 $P < 0.001$				
%DM				
1996	40.0	40.1	38.2	
1997	38.7	35.7	30.5	
1998	42.7	39.5	35.3	
LSD (5%) between depths within year = 2.56 $P < 0.01$				
Glycyrrhizin concentration (g/kg)				
1996	42.9	nr	nr	
1997	46.2	nr	nr	
1998	56.5	37.2	27.3	
LSD 5% between years = 9.2 $P < 0.05$				
LSD 5% between depths = 6.8 $P < 0.001$				

on yield would be expected to lessen as the time to harvest was extended. High density planting (91 600 to 109 000 plants/ha) provided a large gain in root production in the first and second years from establishment and indicates that higher plant densities would enhance production in licorice crops grown for less than 3 years. Such a strategy would be dependent on the roots and rhizomes being of acceptable quality.

The dominant portion (75–89%) of the roots and rhizomes in the top 300 mm of soil at Lincoln and Rukuhia was similar to previous results for root and rhizome distribution (Leto et al. 1996; Martin et al. 1996) whereas the high percentage (54%) in the 0–150 mm depth at Lincoln was higher than previously reported (44%) by Martin et al. (1996). This latter result may be an effect of age as it was obtained from a 7-year-old crop whereas the crop reported in this present work was 3 years old. The very high proportion of the root mass in the 0–300 mm layer in the first harvest was considerably higher at Rukuhia (93%) than at Lincoln (70%). This difference may be a result of soil type or the different planting material used in each trial. The Rukuhia trial was established by dormant root cuttings and the Lincoln trial by transplanting rooted plants.

The traditional way to harvest licorice is by hand following deep trenching or ploughing to 0.9–1.2 m alongside the rows (Anon. 1982; Singh et al. 1984). With the dominant portion of the root mass in our trials in the top 300 mm, the need to harvest roots from deeper depths is questionable. At Rukuhia 11% of the roots were below 300 mm and 4% below 600 mm and at Lincoln 25% of roots (excluding crowns) were below 300 mm and 10% below 600 mm after 4 years of growth. Previous results at a second Canterbury site from an 8-year-old crop found 42% of the root mass was below 300 mm with 22% below 600 mm (Martin et al. 1996). This suggests that the proportion of roots increases at greater depths with crop age and this may justify harvesting older crops to 1–1.2 m.

The glycyrrhizin content of licorice roots and rhizomes increased as they aged (Hayashi et al. 1993; Marzi et al. 1993a; Usai et al. 1995). The higher glycyrrhizin concentration in the roots than the rhizomes in the 1997 harvest in the Lincoln trial confirms previously published pot trial results (De Mastro & Circella 1993; Marzi et al. 1993a) and is consistent with the growth of younger rhizomes on an older root structure. By the 1998 harvest at Lincoln the marked differences in glycyrrhizin content between roots and rhizomes had disappeared. At

Clyde there was no difference between the glycyrrhizin content of the roots and rhizomes in the second harvest (1996) but by the third harvest (1997) rhizomes in the medium and high densities had a higher glycyrrhizin content than in the roots. Deeper roots further away from the plant crown and hence younger also had lower glycyrrhizin concentrations than those nearer the surface in the Lincoln and Rukuhia trials. This effect was particularly strong in the latter trial where the glycyrrhizin concentration dropped from 56.5 g/kg in the 0–300 mm layer to below the international standard of 40 g/kg below 300 mm. At Lincoln these glycyrrhizin differences with depth diminished between the 1997 and 1998 harvests as the roots aged. By the third harvest the glycyrrhizin yield at Lincoln (630 kg/ha) and Rukuhia (628 kg/ha) were similar but at Lincoln only 75% of the glycyrrhizin content was in the 0–300 mm soil layer compared with 94% at Rukuhia. At Lincoln 90% of the glycyrrhizin content occurred in the 0–600 mm soil layer. Plant density had no effect on the glycyrrhizin content of the roots but the rhizomes had lower glycyrrhizin concentrations under the low plant density than under the two higher densities. An explanation for this is that the greater inter-plant spaces allowed more unrestricted new horizontal rhizome growth than vertical root growth. At the low plant density, rhizomes dominated the underground biomass by 2.1:1 but at the highest plant densities this ratio had dropped to 0.6:1 with the roots more dominant.

These results show that licorice can be grown successfully in New Zealand to a quality standard of glycyrrhizin concentration above the international standard of 4% (American Botanical Council 1998; WHO 1999). The licorice grown in Central Otago and Canterbury reached this quality threshold in the fourth year of production but in the Waikato the threshold was exceeded at the first harvest 20 months after crop establishment. Whether or not the more rapid glycyrrhizin build-up in the Waikato is solely related to the hotter environment is unclear. The effect of temperature on glycyrrhizin production requires further study since more information may help determine the best location to grow licorice in New Zealand. Harvesting licorice within 2 years rather than 4 would provide greater land-use flexibility and earlier financial returns, but whether such a strategy is possible or profitable remains uncertain. The high percentage of roots in the top 300 mm, at Rukuhia in particular, suggests that mechanised harvesting should be investigated.

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