# SOLASODINE PRODUCTION FROM SOLANUM LACINIATUM IN THE SOUTH ISLAND OF NEW ZEALAND

D. J. G. Davies and J. D. Mann Crop Research Division and Applied Biochemistry Division DSIR, Lincoln, Canterbury

# ABSTRACT

This paper reviews agronomic studies on the production of solasodine-containing leaves from Solanum laciniatum grown as an annual crop in the South Island. The best yields were below 200 kg ha<sup>-1</sup> of solasodine, and well below commercial feasibility.

# INTRODUCTION

Solasodine, a steroidal alkaloid valuable to the pharmaceutical industry, can be extracted from the leaves of *Solanum aviculare* and *S. laciniatum*, as well as from the fruits of these and numerous other species of *Solanum*. The feasibility of using leaves for commercial extraction from *S. laciniatum*, on an annual basis, is uncertain.

This paper summarises four years of research on the production of solasodine from *S. laciniatum* (poroporo) in the South Island of New Zealand. The problems of establishment (including planting time, method, and density), fertilisation, harvesting and drying are covered; details of the extraction and chemical modification of solasodine are being published separately (Mann, 1978).

In most of the trials described below, the North Island species, S. aviculare, was planted as well as S. laciniatum, but growth of the former species was very restricted.

## METHODS AND RESULTS

## Seed germination and stand establishment

The seed of both species is small and difficult to germinate. Some attempts to improve seed germination have succeeded but each new seed acquisition seems to need a different approach (Fawkner, 1974; Sudiatso and Wilson, 1974; Porter and Gilmore, 1976). Field results did not always agree with glasshouse tests. Pelleted seed of *S. laciniatum* was easier to sow and germinated better if formulated with a calcium/reverted superphosphate coating.

Germination of seed grown in cold frames was accelerated by treatment with hot  $(40^{\circ}C)$  water for 8 hours. An additional five-minute treatment with 1.7% sodium hypochlorite, followed by rinsing with clean water, brought emergence time down to as little as 10 days (H. Geven and N. G. Porter, personal communication).

Careful sowing and irrigation is needed to obtain a good stand from direct-seeding. Most field results showed considerably better yields from the use of transplants that had earlier been germinated in the glasshouse or cold frame.

Weed control is important. Direct seeded plants are disadvantaged when the frequent shallow irrigations that are necessary, stimulate weed growth. Trifluralin (2 1 ha<sup>-1</sup>) was routinely incorporated by discing before direct sowing, but a pre-emergence follow-up spray with paraquat or diquat was also found to be necessary. Timing the latter spray was difficult because of the prolonged germination period of untreated *Solanum* seed. For transplants, metribuzin (0.5 kg ha<sup>-1</sup>) applied before planting gave satisfactory weed control (Betts, 1975).

## Climatic requirements

S. laciniatum grows well in coastal regions that are relatively frostfree and have a uniformly distributed rainfall. Shelter from the prevailing cold easterly or sou'westerly wind is very beneficial. In drier regions of Canterbury and Otago, irrigation is essential. Solanum is very susceptible to late frosts, and the risk increases with altitude. Warm day temperatures (15 to 20°C) are important to sustain rapid vegetative growth.

## Type of soil

Studies were carried out on 12 sites with a range of different soils. *Solanum* grew best on slightly acid to neutral soils that were friable, free draining, and of moderate organic matter content.

## Time of planting

Because direct seeding has not been reliable, and because a full growing season is essential in the South Island, transplanted seedlings were used in most trials. Transplanting was done in late November or early December from seeds sown six to eight weeks earlier in cold frames. Each frame was sown with 5 to 20 grams of treated seed, depending on the germination expected. The 1000-seed weight averaged 1.28 grams.

Unfortunately large scale transplanting is not economic. For instance, if 800 hectares had to be planted at 50 000 plants ha<sup>-1</sup>, assuming 10 g seed was sown to a bed space of 28.6 m<sup>2</sup>, the area of beds required would be 18.3 ha, nearly 6400 cold frames. Solanum growing by transplants would have to be organized along similar lines to tobacco planting.

Somewhat lower yields of *Solanum* might be acceptable if a double-cropping regime were possible, such as growing vining peas first, followed by *Solanum* in mid-January. Table 1, however, shows that any delay in planting reduces yield, and planting after December is not feasible.

TABLE 1:	Effect	of	time	of	planting	on	yield,	Lincoln,
_	1976							

Planting Date	Yield, T ha <sup>-1</sup>	% Solasodine	Solasodine, kg ha <sup>-1</sup>
November 20 December 12	12.9 7.5	1.30 1.0	168 75
January 13	Nil	-	-

#### Planting density

Previous work suggested that the optimum planting density for S. laciniatum was 40 000 plants  $ha^{-1}$  (Fryer, 1972). Lash (personal communication, 1974) considered the ideal population to be 80 000 plants  $ha^{-1}$ , spaced 7.5 cm apart in 60 cm rows. Recent studies suggest that even higher densities may give greater yields.

A density trial was conducted at Wanaka, Central Otago, in 1975, using a radial spacing arrangement originated by Nelder (1962). This design gives a large range of plant populations over a relatively small area with a limited number of plants. Figure 1 shows that highest dry matter production occurred at densities above 200 000 plants ha<sup>-1</sup>.

The concentration of solasodine in leaves and in berries was not affected by plant density (Table 2). A useful corollary, therefore, is that dry matter production, which is easily measured, can serve as a good indicator of total solasodine yield in cases where all plants are of similar age.

TABLE 2:	Effect	of	pla	ntin	g (	lens	sity	on	sola	sodi	ne
	concent	ratio	ons	in	leav	es	and	ber	ries	of	S.
	laciniati	um,`	Wan	aka,	197	75/1	976.				

Plant density,	Solasodine percentage						
'000 ha <sup>-1</sup>	Leaves	Berries	Stems				
13	1.96	3.52	-				
37	1.87	lost	-				
106	1.76	4.03	-				
394	1.88	3.53	-				
Means	1.85	3.69	0.16				

#### Soil fertility

Establishment of S. laciniatum was enhanced by 250 kg ha<sup>-1</sup> of superphosphate. Nitrogen and potassium requirements were met by application of 125 kg ha<sup>-1</sup> of 1:1:1 NPK fertiliser as a side-dressing when the plants were making most rapid growth. Responses to added fertiliser have ranged from a marginal improvement up to nearly doubling the yield of solasodine; the better responses were found on soils of moderate fertility.

#### Sowing method

As already indicated, transplanting has been the preferred method for field research. However, during 1970 to 1975, direct-seeded crops were successfully established at CRD using a Stanhay precision seeder with 9 x 40 belts, T chokes and plain bases, applying 234 000 seeds ha<sup>-1</sup>.

Figure 1. Yield of berries and of leaves from *S. laciniatum* at Wanaka, 1975/76.



Density (10<sup>3</sup> plants ha<sup>-1</sup>)

A randomised block experiment with six replicates compared transplanting with direct seeding in 1975. Transplants occupied the ground for 105 days and yielded 9 tonnes dry matter per hectare. Solasodine concentration was 1.1%, equivalent to an alkaloid yield of 90 kg ha<sup>-1</sup>. In contrast, the direct seeded plants occupied the ground for 147 days, yielded 6.4 tonnes dry matter per hectare, with a solasodine concentration of 0.8% and total alkaloid yield of 51 kg ha<sup>-1</sup>. These differences were significant (P  $\leq 0.05$ ). Early growth of seedlings was slow and could not take advantage of a limited growing season.

#### Harvesting

No consistent differences between single and multiple-cut harvests were found. Uramowa (1965) reported that when cuts were made at 33 to 45 day intervals the total alkaloid production was not quite as high as from a single late harvest; on the other hand, higher solasodine concentration (i.e., a less woody stem) resulted from multiple cuts. In practical terms, this suggests that fields of *Solanum* could be cut according to the scheduling needed to keep a drying or extraction factory fully utilised, without much effect on total yield of solasodine.

Although agronomic manipulations of density, fertiliser and hormone treatments have produced relatively small improvements in solasodine yields, major amounts of solasodine can be lost during harvesting. For example, in one harvest, leaves were fine-chopped by a forage harvester and blown into lorries driven alongside. The combination of tissue damage and self-heating in these deep layers resulted in loss of solasodine. Small samples of the crop, taken just prior to harvest and dried in an 80°C forced-air oven, contained 1.2% solasodine (dry wt basis). Comparable samples of the chopped material, taken from the lorries reaching the drying plant, contained only 0.6% solasodine. The bulk of the chopped material was dried in a lucerne triple-effect dryer with excessive inlet air temperatures; the resultant dried product contained only 0.2% solasodine.

Drying would be necessary if leaves were to be processed in a distant factory. The energy requirements for drying are important economically. An alternative possibility is to process fresh leaves; this would require careful siting of the processing facility close to the farm.

The best drying conditions are with forced-air giving leaf temperatures under  $100^{\circ}$ C. Short five-minute periods initially of  $120^{\circ}$ C or even  $160^{\circ}$ C caused no detectable loss of solasodine, but 15 minutes at the higher temperature resulted in 24% loss. The initial water loss from leaves kept them cooler, but later the drier leaves became as hot as the air. If drying was then stopped, the stems were found to retain considerable moisture, which slowly migrated out to the leaves during storage, resulting in fungal attack on the entire mass. Therefore, low temperature drying had to be continued until the total sample was dry.

Alternatively, field-wilting of the leaves might reduce the cost of drying, and this was investigated. A McCormick-Deering reaper-binder was used to tie sheaves of leafy stems, which were then hung to dry on fences. As the outer leaves dried, they began to blow off, so the sheaves were then transferred to a bin drier using forced hot air at a temperature of 30°C. The centres of the sheaves were very wet, and the small savings in drying time did not compensate for the extra labour of handling the sheaves. However, in contrast to the very large solasodine losses described previously, this technique produced a dry product with 0.8% solasodine relative to 1.1% solasodine for laboratory-dried samples of this crop in the field. It was also found that, after drying, leaves could be mechanically separated on a wire mesh screen by relatively gentle agitation. These leaves were baled in polypropylene sacks, using a conventional wool press, and were successfully shipped overseas without any loss of alkaloid.

Other field-drying methods tried, such as windrowing, led to excessive loss of leaves. Chemical defoliation was tested to see if it offered any advantages. Paraquat and diquat caused rapid leaf dessication, with accompanying fragility, so that leaves blew away in the slightest breeze. A combination of 100 to 1000 ppm ethephon and 20 to ppm 100 cycloheximide (acti-dione) caused abscission of mature leaves. Ethephon alone caused leaf epinasty but retarded spontaneous abscission; cycloheximide by itself caused localised necrosis with minimal abscission. The combination resulted in most leaves abscising, still turgid, within 3 to 10 days, depending on ambient temperature. These leaves turned yellow before or soon after abscission. Unfortunately, the ethephon-cycloheximide treatment also caused severe losses of solasodine, to a final level of 0.79% compared with 1.74% in the controls.

## Yields

Trials were located in Central Otago, Canterbury, Marlborough, and Nelson. Yields averaged 150 kg solasodine per ha in Central Otago, and less than 100 kg ha<sup>-1</sup> in Canterbury. Results from Marlborough and Nelson were less comprehensive but approximated 150 kg ha<sup>-1</sup>. Industrial advisors have indicated that minimum yields of 230 kg solasodine per ha are essential for this crop to be economically viable.

#### Conclusions

Using techniques similar to those used for tobacco,

it is feasible to produce crops of *S. laciniatum* in the South Island, but at present these annual leaf crops cannot compete with *S. aviculare*, which can be grown as a perennial crop in the North Island.

# ACKNOWLEDGEMENTS

Assistance from Dr H. C. Smith, Mrs J. Lancaster, Dr N. Porter, Mr A. R. Wallace, and Miss H. F. Geven is gratefully acknowledged.

## REFERENCES

- Betts, M. V. 1975: Chemical week control in Solanum laciniatum. Proceedings New Zealand Weed and Pest Control Conference 28: 39-41.
- Fawkner, J. J. 1974: Establishment of Solanum aviculare Forst. as a field crop for the production of pharmaceutical raw materials. International Plant Propagation Society Proceedings 24: 331-334.
- Fryer, R. F. 1972: Cultivation of Solanum in Canterbury. Proceedings Agronomy Society of New Zealand 2: 73-84.
  Mann, J. D. 1978. Production of solasodine for the
- Mann, J. D. 1978. Production of solasodine for the pharmaceutical industry. Advances in Agronomy 30: 207-245.
- Nelder, A. 1962: Systematic designs for spacing experiments. *Biometrics* 18: 283-307.
- Porter, N. G. and Gilmore, H. M. 1976: Germination studies of the seed of Solanum laciniatum Ait, and S. aviculare Forst. New Zealand Journal of Experimental Agriculture 4: 343-345.
- Sudiatso, I. S. and Wilson, D. R. 1974: Seed germination in Solanum laciniatum Ait. New Zealand Journal of Agricultural Research 17: 455-458.
- Uramowa, B. 1965: Results of work on the cultivation of Solanum laciniatum in Wiekkopolska. Herba Polonica 11: 162-169 (Horticultural Abstracts 37: 1583).