

Herbicide and Frost Interactions on the Growth and Seed Production of *Lotus pedunculatus*

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ABSTRACT

Growers of lotus (*Lotus pedunculatus* Cav. syn *uliginosus* Sch) seed crops have often observed that ethofumesate used in combination with propyzamide or carbetamide for winter weed control can severely reduce lotus growth, particularly during frosty weather.

To further study these interactions, two controlled environment experiments examined the timing of frost and propyzamide or carbetamide applications in relation to ethofumesate applications on seedling lotus. Plants were subsequently grown in the field to seed harvest.

Frost played the dominant role in reducing lotus growth irrespective of the cultural temperature regime, and also enhanced activity of all three herbicides. As lotus is only moderately tolerant of ethofumesate, a second potentially detrimental event during the period of ethofumesate activity further reduced growth. Frost, or propyzamide or carbetamide application four weeks after ethofumesate application, depressed growth more than if they occurred at the same time as ethofumesate application.

By seed harvest all effects of frost had disappeared. Propyzamide alone (and to a lesser extent carbetamide) reduced seed yield, while ethofumesate alone or in combination tended to increase seed yield. Contrasting site of herbicide activity is suggested as the mechanism. Seed production is dependent on the degree of branching, and propyzamide and carbetamide, in inhibiting root growth, may have reduced shoot growth and branching potential, whereas ethofumesate inhibits primary shoot meristems, reducing apical dominance and increasing branching.

Additional index words: *Lotus pedunculatus*, frost, temperature, ethofumesate, propyzamide, carbetamide, seed production.

INTRODUCTION

Ethofumesate has proved to be useful in the control of volunteer white clover (*Trifolium repens* L.) and annual grass weeds in lotus (*Lotus pedunculatus* Cav.) seed crops during winter (Brock and Henderson, 1976). Cool temperatures generally enhance the activity of ethofumesate (O'Connor, Logan and Rowe, 1975) and the occurrence of frosty weather has been observed to increase damage to treated lotus stands. This enhancement of herbicidal activity appears to operate through increased reduction of plant growth rather than any direct effects of frost damage at application, especially as duration and timing of frost relative to application had little effect on lotus (Brock, Rolston, Greer and Halligan, 1989).

In order to control a broader spectrum of perennial grass weeds, other herbicides are often required which may be applied in combination with ethofumesate. Two such herbicides are propyzamide and carbetamide, which are both winter active and have been observed to be more damaging under frost conditions. The interaction of combinations of these herbicides and their timing of application in relation to temperature and frost events on

lotus seed crops needed further investigation. A controlled environment study on seedling lotus plants was followed by field evaluation of effects on seed yield.

MATERIALS AND METHODS

Two experiments were conducted on seedling lotus plants in the controlled environment (CE) rooms of the Climate Laboratory at DSIR Fruit and Trees, Palmerston North, New Zealand, for the frost and herbicide treatments, and the plants were then grown on in the field at DSIR Grasslands, Palmerston North. Detailed cultural and environmental procedures are given in Brock *et al.*, (1989).

In early March 1980, 250 3.3 litre pots containing an Opiki loam, were sown with pre-germinated lotus seed and placed in a temperature controlled glasshouse (25°C day/15°C night). After 8 weeks plants were thinned to 4 per pot and the pots allocated to the two experiments.

Experiment 1 commenced in the CE rooms on 5 May 1980. After two weeks acclimatisation, frost and herbicide treatments were applied over the following four

weeks, and the pots removed from the CE rooms two weeks later on 28 June 1980. Pots were placed outside on concrete and the plants scored for growth on 15 July and 1 September 1980, then cut to 1 cm for herbage yield assessment.

Experiment 2 commenced in the CE rooms on 30 June 1980, and frost and herbicide treatments were applied between 2 and 30 July 1980. Pots were removed on 1 September, scored for growth and plants cut to 1 cm for herbage yield measurement.

Plants to be frosted were placed in a special frost room (Robotham, Lloyd and Warrington, 1978) on the appropriate days. Frost damage was assessed after each frost event using an electro-conductivity method (Green and Warrington, 1978) for calculating a conductivity ratio (CR) ranging from 0 (no damage) to 1 (total kill). Pots to be sprayed were removed from their CE rooms to a concrete pad one hour before commencement of reducing temperature for the night period. Herbicide was applied by knapsack boom sprayer operating at 200 kPa and delivering 225 l ha⁻¹. After drying, pots were returned to the appropriate CE rooms.

During September, plants from both experiments were planted in the field at 1 m spacing on a Kairanga silt loam and grown to seed harvest during February 1981. On 26 January 1981, plants were scored for percentage flowering and presence of seed pods. Plants were harvested when 70% of pods were light brown and pod shattering had just commenced (Hare and Lucas, 1984). Harvest dates varied as treatments altered rates of plant maturity. At harvest, plant fresh weight (FW) and seed yield were measured.

Experimental design and statistical treatments

In order to accommodate the range of treatment variables within the CE rooms, two non-replicated split plot factorial designs were used, with growth temperatures and frost treatments as main plots (as CE rooms were not replicated) and two sets of subplots designed to investigate (a) the effects of individual herbicides alone, and (b) combinations of herbicides and their timing of application.

Experiment 1:

Main plot treatments were:

1. Growth temperatures of CE rooms ($\pm 0.5^\circ\text{C}$) selected to represent the range of winter temperatures experienced in New Zealand, i.e. $16/9^\circ\text{C}$ day/night, $13/6^\circ\text{C}$ day/night, $10/3^\circ\text{C}$ day/night.
2. Frost - either nil or three nights at -4°C following each herbicide application.

Subplot treatments were:

- a. Herbicide and rates - nil, ethofumesate (E) at 1.5 and 3.0 kg active ingredient (a.i.) ha⁻¹, propyzamide (P) at 0.75 and 1.5 kg a.i. ha⁻¹, and carbetamide (C) at 1.5 and 3.0 kg a.i. ha⁻¹.
- b. Timing of herbicide combinations* - E + P or C applied together, E alone + P or C applied two weeks later, E alone + P or C applied four weeks later.
(* at the rates given above).

Experiment 2:

Main plot treatments were:

1. Growth temperature of CE rooms - $16/9^\circ\text{C}$ day/night, $10/3^\circ\text{C}$ day/night.
2. Frost - nil, -6°C for three nights at ethofumesate application, -6°C for three nights four weeks after ethofumesate application.

Subplot treatments were:

- a. Herbicides - nil, ethofumesate (E) at 3.0 kg a.i. ha⁻¹, propyzamide (P) at 1.5 kg a.i. ha⁻¹, carbetamide (C) at 3.0 kg a.i. ha⁻¹.
- b. Timing of herbicide combinations - E + P applied together, E + C applied together, E with P applied four weeks later, E with C applied four weeks later.

As a consequence of the split plot design used, analysis of variance could not include the growth temperature by frost interaction as this was used to test temperature and frost main effects. As the experimental design was unbalanced, analyses of variance for each experiment were calculated separately for subplots a and b. LSD_{0,05} values were also calculated for the comparison between a and b.

RESULTS

Frost damage (Conductivity ratio, CR)

There was a significant interaction between growth temperature and time of frost in experiment 1, associated with cold tolerance and the development of frost hardiness (Table 1). Initially, plants growing under $16/9^\circ\text{C}$ had low resistance to frost compared with those grown at $10/3^\circ\text{C}$, but after the first frost event all had increased frost resistance to a similar level. In experiment 2, the heavier frost level of -6°C resulted in more severe damage with frost hardiness evident only in the four week older plants grown at $10/3^\circ\text{C}$.

As growth scores at 15 July and yield/pot and growth scores at 1 September produced similar results,

TABLE 1

The influence of growth temperature and time on the development of frost hardiness of lotus seedlings grown in a controlled environment, as indicated by changes in the conductivity ratio (0 = no damage, 1 = total kill).

Growth Temperature	No frost	Frost	Frost at 2 weeks	Frost at 4 weeks
Experiment 1			(3 nights at -4°C for all 3 events)	
16/9°C	0.07 f	0.61 a*	0.15 de	0.16 de
13/6°C	0.07 f	0.45 b	0.13 de	0.10 de
10/3°C	0.06 f	0.26 c	0.19 d	0.08 e
Experiment 2			(3 nights at -6°C at one event only)	
16/9°C	0.07 f	0.76 a	—	0.70 a
10/3°C	0.07 f	0.41 b	—	0.28 c

* Values followed by different letters are significantly different at P < 0.05.

TABLE 2

Experiment 1. The mean effect of frost (-4°C) on the activity of herbicides applied alone, or in combinations, on the growth (g/pot) of lotus seedlings 15 weeks after application.

Frost	Herbicide	Ethofumesate (kg a.i. ha ⁻¹)		
		0	1.5	3.0
a) Herbicide alone				
Nil	Nil	8.33	5.13	3.17
Frost		5.76	2.47	1.13
b) Herbicide combinations				
Nil	P or C*	7.69	4.01	2.73
Frost		4.76	1.53	1.12

* Mean of Propyzamide and Carbetamide

LSD_{0.05} for herbicides alone 2.39

LSD_{0.05} for herbicide combinations 0.48

LSD_{0.05} for comparisons between herbicides alone and in combination 1.26.

TABLE 3

Experiment 2. The interaction of the herbicides ethofumesate (E), propyzamide (P) and carbetamide (C), alone or in combination, with the timing of frost, on the growth of lotus seedlings (g/pot) 9 weeks after initial application.

Herbicide	Frost (3 nights at -6°C)		
	No frost	At ethofumesate application	or 4 weeks later
a) Herbicides alone			
None	19.55	5.15	5.20
Ethofumesate (E)	8.75	3.40	1.60
Propyzamide (P)	9.35	5.20	4.55
Carbetamide (C)	14.95	3.20	3.85
b) Herbicides in combinations			
E + P or C together	8.40	4.93	0.88
E + P or C 4 weeks later	3.03	1.23	1.20

LSD_{0.05} for herbicides alone 4.84

LSD_{0.05} for herbicide combinations 3.38

LSD_{0.05} for comparisons between herbicides alone and in combination 3.81.

TABLE 4

The effect of applying ethofumesate alone, or in combination with propyzamide or carbetamide to lotus seedlings in winter, on their subsequent seed yield (g/plant).

Experiment	Herbicide and rate (kg ha ⁻¹)					
	None	Ethofumesate 3.0	Propyzamide 1.5	Carbetamide 3.0	E3 + P1.5	E3 + C3
1	39.9	48.6	21.4	37.2	39.8	32.9
2	17.3	20.9	11.8	13.1	26.8	25.3

LSD_{0.05} (Expt 1) 10.4

LSD_{0.05} (Expt 2) 8.5

only results for yield/pot will be presented.

In experiment 1 (Table 2), ethofumesate significantly reduced lotus growth at both rates and was more severe in the presence of frost than its absence (70 and 50% reduction respectively, averaged over both rates). Neither propyzamide nor carbetamide greatly affected lotus alone or in combination with ethofumesate. Delaying the application of propyzamide and carbetamide two or four weeks after applying ethofumesate reduced growth by around 25% (no delay 2.79, vs two week delay 2.04, and four week delay 2.21 g/pot). In experiment 2 (Table 3), both ethofumesate and propyzamide in the absence of frost reduced lotus growth, whereas carbetamide did not. Frost reduced growth and eliminated any differences between herbicides. Applying propyzamide or carbetamide four weeks after ethofumesate in the absence of frost reduced growth by 85%, to levels similar to that of applying them together with frost. Frost four weeks after application of ethofumesate was most severe (90% reduction) irrespective of when propyzamide or carbetamide was applied. In neither experiment did growth temperature influence this pattern of herbicidal activity.

Recovery growth and seed production

By January 1981, herbicidal symptoms apparent at planting in September had disappeared. At this time, only 17% of plants not treated with herbicide had seed pods, whereas 49% of treated plants had pods. By seed harvest this effect was manifested only in a range of harvesting dates spanning three to four weeks.

Differences in seed yield per plant were variable, but in general there was a higher yield from plants treated with the high rate of ethofumesate alone, and lower yields when treated with propyzamide alone (Table 4). Combining propyzamide or carbetamide with ethofumesate gave variable results, increasing yields in experiment 2, but having little effect in experiment 1.

DISCUSSION

Cultural growth temperature was important in the degree of frost hardiness developed at the time of the first frost event only, and had no effect at subsequent frost events. Eleven weeks after frost treatments had ceased in experiment 1, plant dry weights in all frosted treatments were similar, regardless of growth temperature. This further supports the contention that frost severity, not general temperature regime, plays the dominant role in controlling winter growth rates (Brock *et al.*, 1989).

All three herbicides used are most effective when applied in damp frosty conditions in winter (Perrot, Sarjas and Laude, 1969; Carriere, 1974; O'Connor *et al.*,

1975) before the resumption of active growth. Adequate soil moisture is required to facilitate root absorption, the most effective mode of entry of these herbicides into the plant (Carlson, Lignowski and Hopen, 1975a; Hartley, 1975). The action of low temperature is to reduce microbial degradation of the herbicide (Upritchard and Allott, 1970; Hoogstraten, Baker and Horne, 1974), thus enhancing herbicidal activity while plant growth rates are low, particularly during frosty weather.

Because lotus is only moderately tolerant of ethofumesate (Brock *et al.*, 1989), the most severe effects developed when a second potentially detrimental event occurred within the period of ethofumesate activity, either as frost or the application of a second herbicide at a later date. In this way, propyzamide, and even carbetamide (to which lotus is normally tolerant), severely depressed growth when applied four weeks after ethofumesate. Frost or the second herbicide application occurring at the same time as ethofumesate application was less severe in effect.

By the time of seed harvest most of the effects of frost and herbicide treatments had disappeared, although there was a suggestion of a pattern between the two experiments. Lowest yields were associated with propyzamide alone (and carbetamide alone to a lesser extent), and the highest with ethofumesate alone or in combination. Mitotic inhibition of meristem cell division is the mode of action of all three herbicides, ethofumesate of shoot meristems (Duncan, Meggitt and Penner, 1981) and propyzamide and carbetamide of root meristems (Carlson *et al.*, 1975b). Seed yield depends on numbers of inflorescences produced, which in lotus is related to number of branches. Propyzamide and carbetamide, by reducing root production, may have resulted in long term stunting of shoot growth and the production of fewer branches. Ethofumesate, on the other hand, appeared to have the opposite effect, in that inhibition of the primary meristem may have reduced apical dominance and released a higher number of axillary meristems, resulting in increased branching, more inflorescences and increased seed production. Similar effects were reported with the mirid *Calocoris norvegicus* Gmel., which also weakens the primary apical meristem, causing a proliferation of lateral branches and increased seed production, provided the mirid is controlled at the correct time (Clifford, Wightman and Whitford, 1983). Hare (1984, 1987) has shown that the reaction of lotus to treatments that reduce stand density, such as atrazine applied in late spring, is to increase reproductive stem formation from axillary buds, rather than produce new branches from the crown.

These results confirm the observation of growers that combinations of ethofumesate with other winter active herbicides can have detrimental effects on lotus in

the short term, particularly if associated with frost during the period of herbicidal activity. However, the effects may not be all negative in the long term, as the results suggest that ethofumesate, by affecting apical dominance, may increase seed yields. Judicious use of combinations of herbicides during winter may be a further tool for lotus seed producers.

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