

EFFECTS OF AN ADJUVANT (TWEEN 20) ON EFFICACY OF FOREST HERBICIDES

(A Summary of Greenhouse and Field Results 1986)

by

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INTRODUCTION

Spray adjuvants are chemicals which have potential to modify and facilitate the effectiveness of herbicide active ingredients. In so doing they reduce the cost of application and chemical burden in the environment. Even though considerable research has been carried out with herbicide and adjuvant interactions with agricultural weeds, very little information is available concerning the influence of surfactants on forest weeds. Three reasons are cited: (i) the agricultural market is lucrative to manufacturers of pesticides and hence more research is geared towards agricultural weeds; (ii) most forest weeds are perennial and difficult to control compared to annual agricultural weeds and (iii) use of chemicals (herbicides and adjuvants) for weed management in forestry is more controversial than in agriculture and therefore poses special, economical and environmental considerations.

Herbicides are regarded as one of the most cost-effective tools in the regeneration of forests but new forest herbicides (Garlon, Roundup and Velpar) tend to be more expensive than traditional forest herbicides like 2,4-D. Consequently considerable research is needed to improve the efficacy and cost-effectiveness of these new herbicides under forestry conditions. With this objective in mind, the herbicide project at FPMI initiated some research on herbicide and adjuvant interactions on forests weeds under greenhouse and field conditions. The present report describes the effects of Tween 20 alone and in combination with three forest herbicides (Garlon, Roundup and Velpar) on three forest weeds (aspen, red alder and white birch) under greenhouse and field (small plot) conditions. Data on the effects of herbicide and

adjuvant formulations on crop tolerance of balsam fir, white spruce and black spruce were also collected.

MATERIALS AND METHODS

(A) Greenhouse Experiments

(a) Cultivation of plants: Seeds of alder (*Alnus rubra* L) aspen (*Populus tremuloides* Mich.) and white birch (*Betula papyrifera* L.) were obtained from a certified seed company. After appropriate stratification treatments, the seeds were germinated in a controlled chamber set at 20°C using a sterilized bedding mixture consisting of peat moss and soil (1:1) laid in a polystyrene tray. Regular watering of this tray ensured uniform germination and when the seedlings were one month old, they were transplanted into individual pots (15 cm x 15 cm) filled with the above mixture. Surface irrigation with a standard nutrient solution and frequent watering of these pots, yielded seedling growth of excellent vigour and uniformity. About 4-6 month old seedlings with ca. 16 leaves were employed for screening the effects of various formulations. All plants were grown in the greenhouse under controlled conditions of temperature ($20 \pm 1^\circ\text{C}$), light (2200 lux 16 ± 8 hr) and relative humidity ($70 \pm 15\%$). Light intensity was provided by incandescent and fluorescent bulbs simulating natural day light.

(b) Treatment of plants: When plants of each species were of appropriate stage (16 leaves) they were treated with a range of concentration (0.1, 0.5, 1.0%) of adjuvant to ascertain if the adjuvant would be phytotoxic. Similarly conifer crop species (balsam fir, *Abies*

balsamea (L.) Mill; white spruce, *Picea glauca* (Moench) Voss; black spruce, *Picea mariana* (Mill) BSP; jack pine (*Pinus banksiana*) Lamb. and western species, Douglas fir (*Pseudotsuga menyiesii*) Mirb., were also subjected to the same concentrations of adjuvants. No phytotoxicity at lower concentrations was observed and the recommended concentration Tween 20 (0.1%) was then mixed with the herbicides for testing the efficacy. Initially, a pilot experiment was conducted with each herbicide to determine an acceptable dosage level that could cause 20-50% damage in foliar growth. Accordingly, all herbicides, adjuvants and their combinations were prepared as liquids and sprayed onto foliage of test species in the Institute's spray chamber (Research Instrument Manufacturing Company, Guelph, Ontario). A #8005 flat fan hydraulic nozzle travelling at 4 km/h delivered 80 litres of spray per hectare at 206 kPa to the test plants.

Field rates of each herbicide (Garlon 1.8, Roundup 2.1, Velpar 2.5 a.i. kg/ha) were lethal to greenhouse grown plants and therefore dosages were lowered to about 1/10 for Velpar and 1/20 for Garlon and Roundup in strength. These lowered rates induced levels of phytotoxicity which could be easily attributed to the adjuvant being tested. Immediately after the test plants were sprayed, they were brought to a post-treatment chamber operated under conditions identical to those for pre-treatment plants and then monitored for symptoms of toxicity over a 3-week period.

(c) Response measurement: For the sake of convenience the phytotoxicity was assessed by scoring the percent damage (0-100%) as set up in the guidelines of the Expert Committee on Weeds

(Prasad 1985). Changes in fresh weight of the plants were also recorded so as to substantiate the percentage data collected by the aforesaid guideline. Because of a large volume of work and restricted space in the greenhouse, a 3-week period of screening was found to be optimal and therefore all assessment was completed in 3 weeks. In some cases where toxicity was high, roots and stems of treated plants were dissected for examination of any internal injury to the tissues.

(d) Procurement of chemicals: Adjuvants were obtained from different manufacturers whose details are listed in Table I. Surfactant (G3780A) was supplied for studies with the glyphosate-C¹⁴ (Table I). Roundup (glyphosate) and Velpar-L (hexazinone) were obtained from herbicide manufacturers (Monsanto and DuPont Chemical Cos., respectively) and these were the commercial formulations relevant for weed control in coniferous forests. Glyphosate-C¹⁴ (sp. act., 1.5 mCi/mM) was obtained from Monsanto Chemical Company, St. Louis, Mo. as parent acid and converted to monoisopropylamine salt by addition of isopropylamine in a 1:1 molecular ratio. Scintillation cocktail chemicals were purchased from Canlab Ltd. (Toronto). Other analytical reagents used for extraction of radioactivity were of pure quality.

Table I. Properties of Adjuvants

Adjuvant	Form	Action	Dosage (v/v)	Manufacturer
Tween 20	Non-Ionic	Wetting	0.1%	Atkemix Inc. Brantford, Ontario
G3780A*	Ionic	Wetting Sticking	0.05%	Monsanto Chemical Co. St. Louis, Missouri

* Used only in uptake studies with glyphosate-C¹⁴.

(e) Uptake of glyphosate-C¹⁴: When absorption and translocation of glyphosate-C¹⁴ in the presence and absence of an adjuvant (G3780A), was investigated, only one leaf of white birch and alder was treated with glyphosate (1200 ppm). A fully expanded third leaf was selected and glyphosate-C¹⁴ alone and in combination with surfactant (G3780A-0.05%) was pipetted into a lanolin-emulsion ring on the lamina of the leaf according to a procedure described by Prasad, Foy and Crafts (1967). The leaf was fed for 2 weeks in a growth chamber set at constant temp. ($22 \pm 1^\circ\text{C}$), light (2200 lux, 18 ± 6 h dark) relative humidity ($70 \pm 15\%$). Radioactivity was monitored by gross autoradiography (Crafts and Yamaguchi 1964) and by the standard scintillation counting technique (Chase and Rabinowitz 1964). The method of extraction of glyphosate-C¹⁴ from woody plants was similar to one prescribed by Sprankle, Meggitt and Penner (1975).

(f) Experimental design and statistical treatment: Because of variation in growth characteristics of each species and because of some inherent variability in the method of treatments, all experiments were designed statistically with 15 replicates in each treatment. Data were treated with analysis of variance and where necessary some data on percentages were transformed to angular arc sin to minimize variability in the treatment (Snedecor 1957).

(B) Field Experiments

(a) Selection of site: A recent cut-over area representing some 10 ha of forest near Thessalon, Blind River, Ont. was selected. The Ministry of Natural Resources (Ontario) is replanting these sites

with red pine, (*Pinus resinosa* Ait.) jack pine, (*P. banksiana* Lamb.) and white spruce. The soil is sandy loam and the weed spectrum is dominated by aspen-poplar, pin cherry (*Prunus pensylvanica* L.) raspberry, (*Rubus strigosus* Michx), birches and grasses (*Agrostis* spp. *Agropyron repens* L. *Bromus* spp.). In low lying areas alder (*Alnus rugosa* (DuRoi) Spreng.) and willows (*Salix nigra* Marsh) are also found. For evaluating the effectiveness of adjuvant with and without 2 herbicides (Roundup and Velpar), an upland site with aspen-poplar was chosen. Only uniform and healthy stands (1-2 m high) were selected and each plot contained 50-70 plants. The plot size was 5 m x 5 m with buffer zones in between each treatment. To minimize variability, all treatments were replicated three times and an average of 120 plants per treatment was assessed for phytotoxicity rating. The design of the experiment was a standard randomized block layout.

(b) Spray application: Based on experiments in the greenhouse, two herbicides (Roundup and Velpar 0.5 kg/180 L/ha) were included for testing the efficacy of Tween 20.

Plots were treated with herbicides alone and in combination with the adjuvant by using a "Solo" back pack sprayer supplied by Canadian Forestry Equipment Ltd. Sprays were deposited on the foliage during the month of August (12/86) when conifers were completely "hardened-off". To minimize drift, spraying was carried out on a calm and clear day and Kromekote cards were placed on the foliage and ground level to ascertain the pattern and quantity of deposit of each formulation. On a sunny and warm day, droplets of herbicides and adjuvant mixtures dried out on foliar surfaces within 30-120 min. Evaluation of efficacy was carried

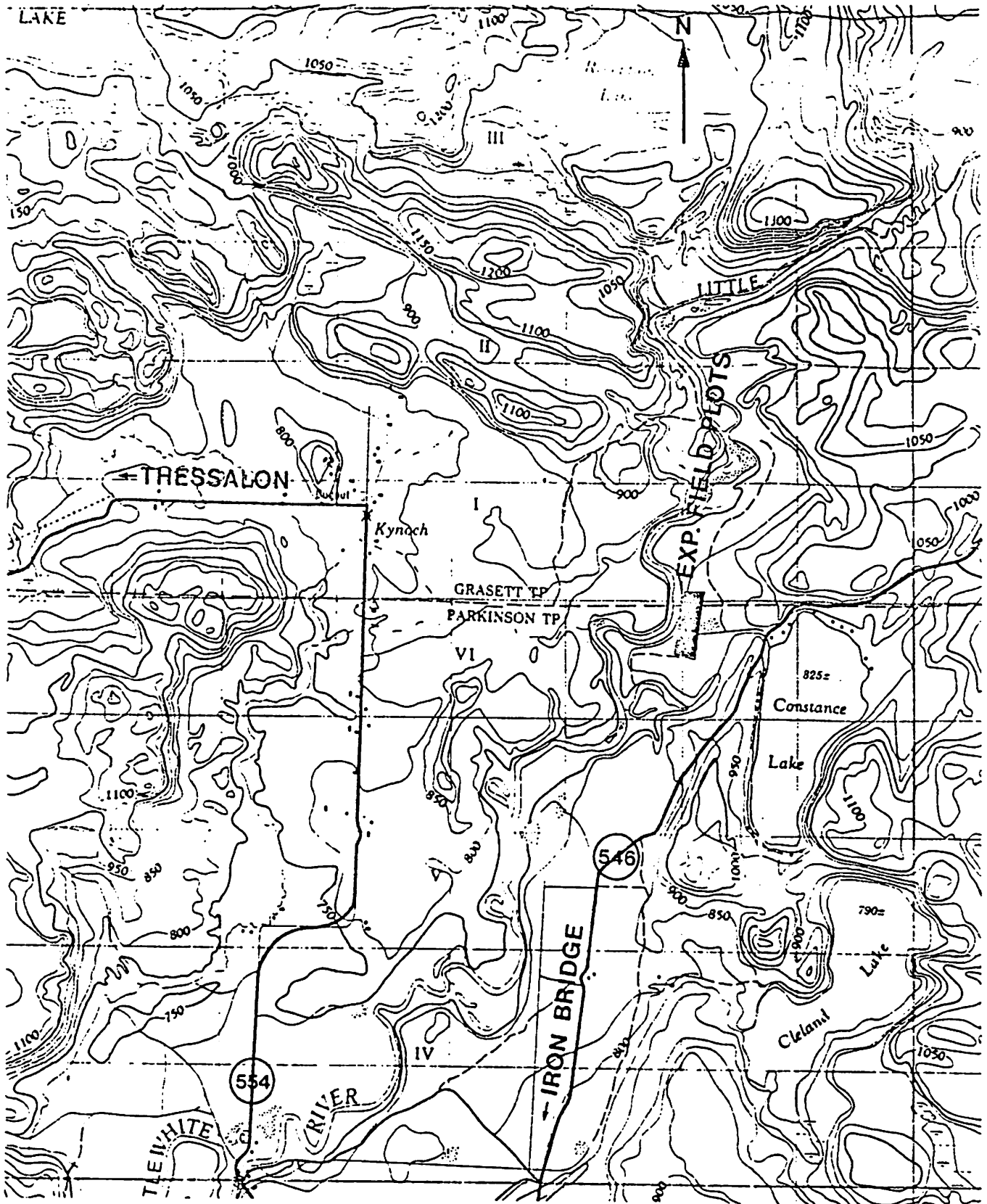


Fig. A. Location of field plots

out after a month (12 Sept. 86) by employing the E.C.W. guidelines as described earlier.

RESULTS AND DISCUSSION

(A) Greenhouse Studies

(a) Effects of Tween 20 on phytotoxicity: At the outset an experiment was conducted on the effect of adjuvant *per se* on toxicity to three weed and five conifer species. A range of low concentrations from 0.1 to 0.5% gave no apparent injury symptoms (1.0% was slightly phytotoxic). Results from such a trial are shown in Table II (a & b). Similarly a dose-response curve for each herbicide was also established. Greenhouse-grown plants at dosages 10-20 times less than the field rates gave 30-50% phytotoxic response. These were considered acceptable for investigating the influences of adjuvants. The results are shown in Table III.

(b) Effects of adjuvant + herbicide combinations on phytotoxicity:

A recommended concentration (0.1%) of the adjuvant was selected and this was used in combination with herbicides, Garlon, Roundup and Velpar (0.01 to 0.5 kg/80 L/ha) to screen the effects on efficacy and crop tolerance (Table IV and V).

Because a low concentration (0.1%) of Tween 20 combined with the three herbicides did not induce any phytotoxicity on conifer species, the next logical step was to investigate the affects of this formulation on phytotoxicity to weed species. As can be seen from Table V, Tween 20 is indeed effective in increasing the efficacy of the herbicides tested.

Table IIa. Effects of Varying Concentrations of Tween 20 Alone on Weed Species After Three Weeks

Conc. % (v/v)	Phytotoxicity Rating (%)*		
	Alder	Aspen	W. Birch
0	0	0	0
0.10	0	0	0
0.50	0	0	0
1.00	2.0	1.3	3.0

*No phytotoxicity = 0%

Complete phytotoxicity (kill) = 100%

Table IIb. Effects of Varying Concentrations of Tween 20 Alone on Crop Species After Three Weeks

Conc. % (v/v)	Phytotoxicity Rating (%)*				
	Balsam Fir	Jack Pine	White Spruce	Black Spruce	Douglas Fir
0	0	0	0	0	0
0.10	0	0	0	0	0
0.50	0	0	0	0	0
1.00	1.6	1.4	0.2	0.2	4.4

* No phytotoxicity = 0%

Complete phytotoxicity (kill) = 100%

Even though Roundup and Velpar produce different degrees of response in each species, the augmented effects of Tween 20 plus herbicide formulations are clearly marked. Generally Velpar and Velpar + Tween 20 produce quicker and greater effects than Roundup and Roundup + Tween 20 combinations. This is partly because of the fact that Roundup is a systemic herbicide and takes longer to produce phytotoxicity than Velpar which behaves like a contact herbicide under greenhouse conditions. Also all herbicides and their formulations with Tween 20 produce qualitatively different symptoms on the foliage of these weeds: Velpar

Table III. Effects of Application of Low Rates of Herbicides on Phytotoxicity, After Three Weeks, to Forest Weeds Under the Greenhouse Conditions

Herbicide*	Rate A.I. (kg/ha)	Phytotoxicity (%)		
		Aspen	Alder	White birch
Garlon	0.01	19	37	23
	0.05	71	97	94
Roundup	0.5	70	81	90
	0.25	60	36	80
	0.12	21	20	29
Velpar	0.5	60	75	88
	0.25	52	60	34

*Recommended maximum field dosage: Velpar 2.5 kg/ha; Roundup 2.1 kg/ha. Garlon 1.8 kg/ha

and Velpar + Tween 20 tend to burn up and dessicate the leaves while Roundup and Roundup + Tween 20 and Garlon, Garlon + Tween 20 formulations bring about a systemic injury - necrosis, bleaching, chlorosis and gradual death of leaves. These effects are illustrated in the attached photographs (Figs. 1-6).

(c) Effects of Adjuvant + Herbicide Mixture on Changes in Fresh

Weight of Leaves: So far the effects of surfactant and herbicide mixtures were measured on foliar symptoms only. To gain some insight as to whether water relations and photosynthetic processes of these treated leaves were also affected, changes in fresh weight of treated and untreated leaves of aspen, white birch and alder were also recorded. Analysis of the data (Table V) revealed significant effects of adjuvant + herbicide combination on the reduction in weight i.e.,

leaves were being killed and photosynthetic processes were completely inhibited. The inhibitory effects were more marked when Tween 20 was used in conjunction with Garlon and Velpar than with Roundup.

Table IV. Effects After Three Weeks of Tween 20 (0.1%) in Combination with Roundup (0.5 kg/ha) and Velpar (0.25 kg/ha) on Crop Tolerance

Treatment	Phytotoxicity Rating (%)		
	B. fir	W. spruce	B. spruce
Control	0	0	0
Roundup alone	0	0	0
R + Tween 20	0	0	0
Velpar alone	0	0	0
V + Tween 20	0	0	0

Table V. Effects After Three Weeks of Tween 20 (0.1%) + Herbicide Formulations (Garlon 0.05 kg/ha, Roundup, 0.12 kg/ha and Velpar 0.25 kg/ha) on Efficacy of Forest Weeds

Treatment	Phytotoxicity Rating (%)		
	Alder	Aspen	White Birch
Control	0	0	0
Garlon alone	37.5	19.0	23.0
G + Tween 20	55.0	10.5	36.0
Roundup alone	19.2	10.3	28.0
R + Tween 20	46.7	16.7	51.0
Velpar alone	75.1	83.3	63.5
V + Tween 20	80.8	87.5	76.1



Fig. 1. Effect of Roundup and Roundup + Tween 20 on white birch seedlings under greenhouse conditions.

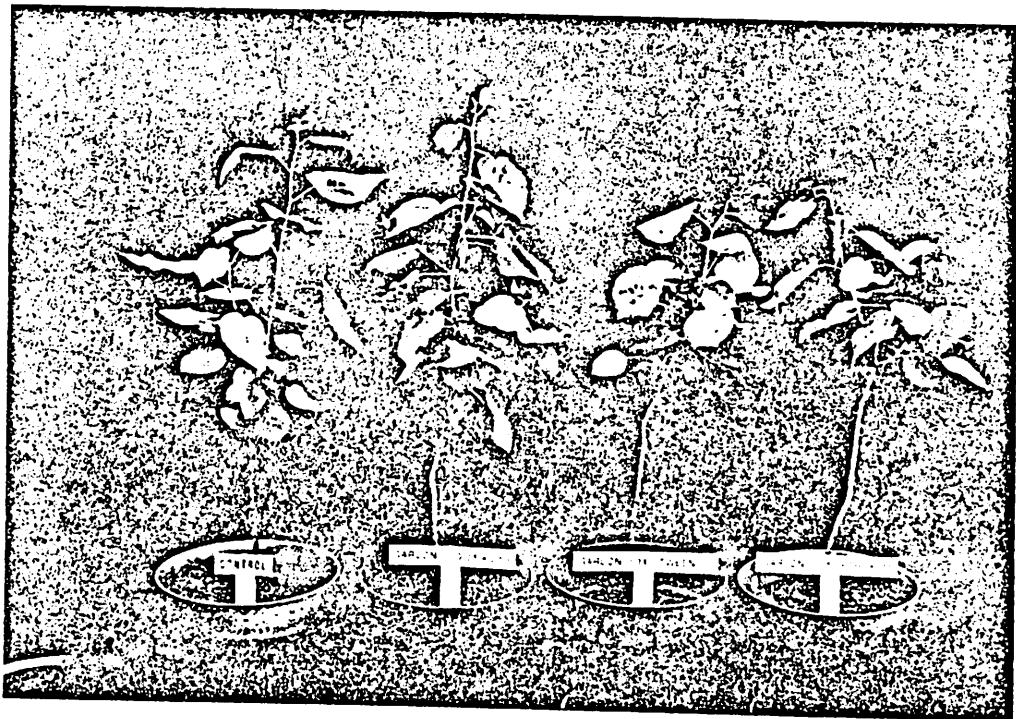


Fig. 2. Effect of Garlon and Garlon + Tween 20 on poplar seedlings under greenhouse conditions.

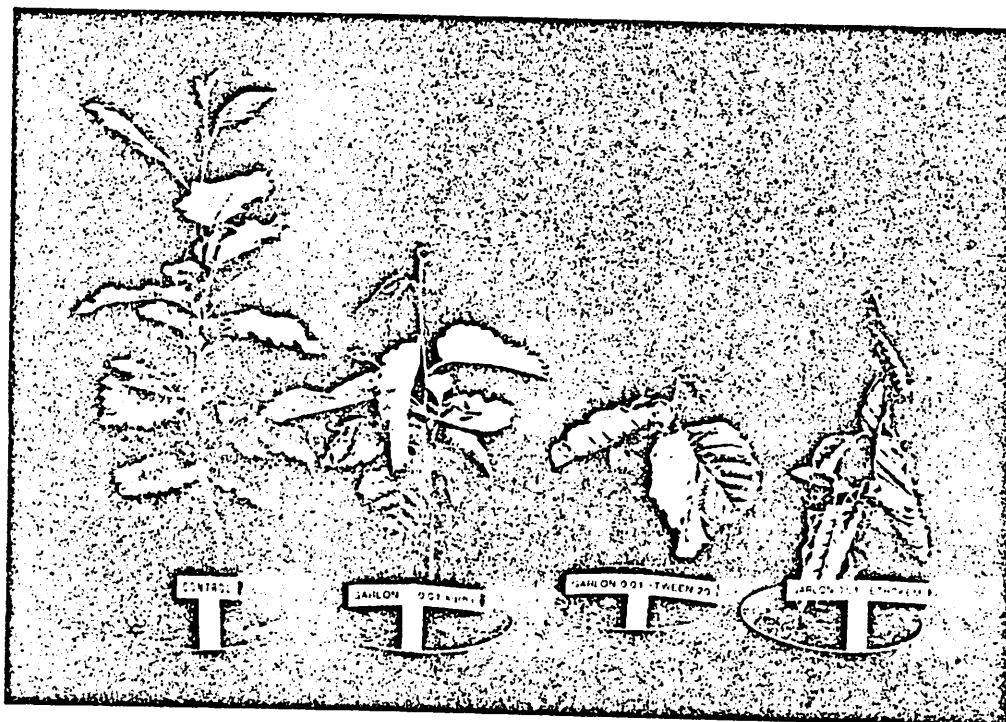


Fig. 3. Effect of Garlon and Garlon + Tween 20 on alder seedlings under greenhouse conditions.

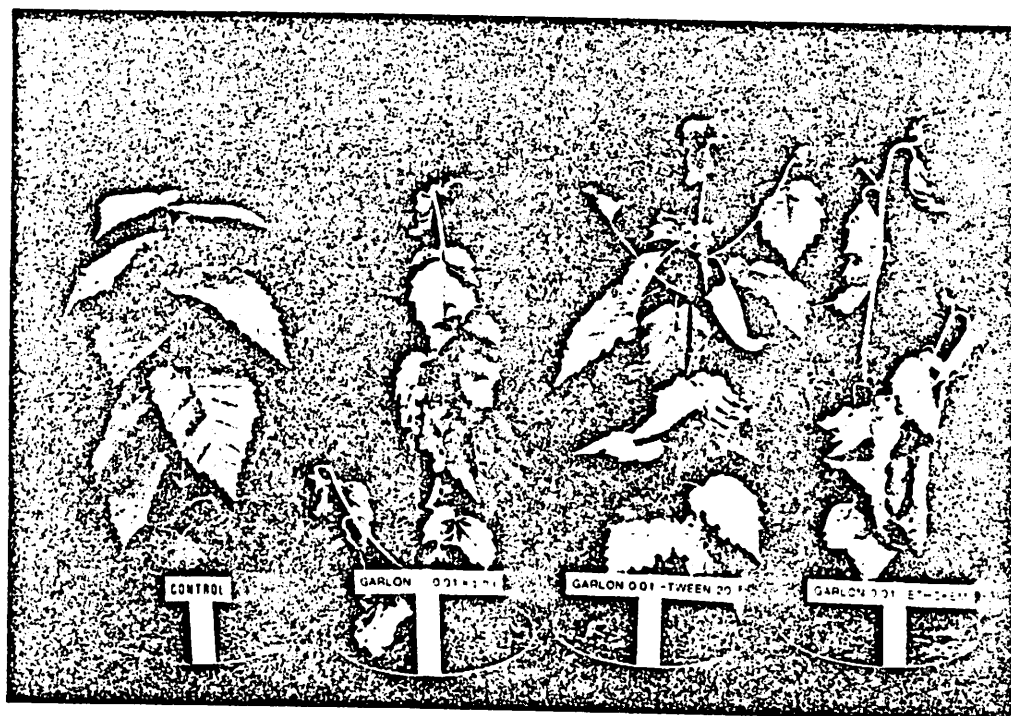


Fig. 4. Effect of Garlon and Garlon + Tween 20 on white birch seedlings under greenhouse conditions.

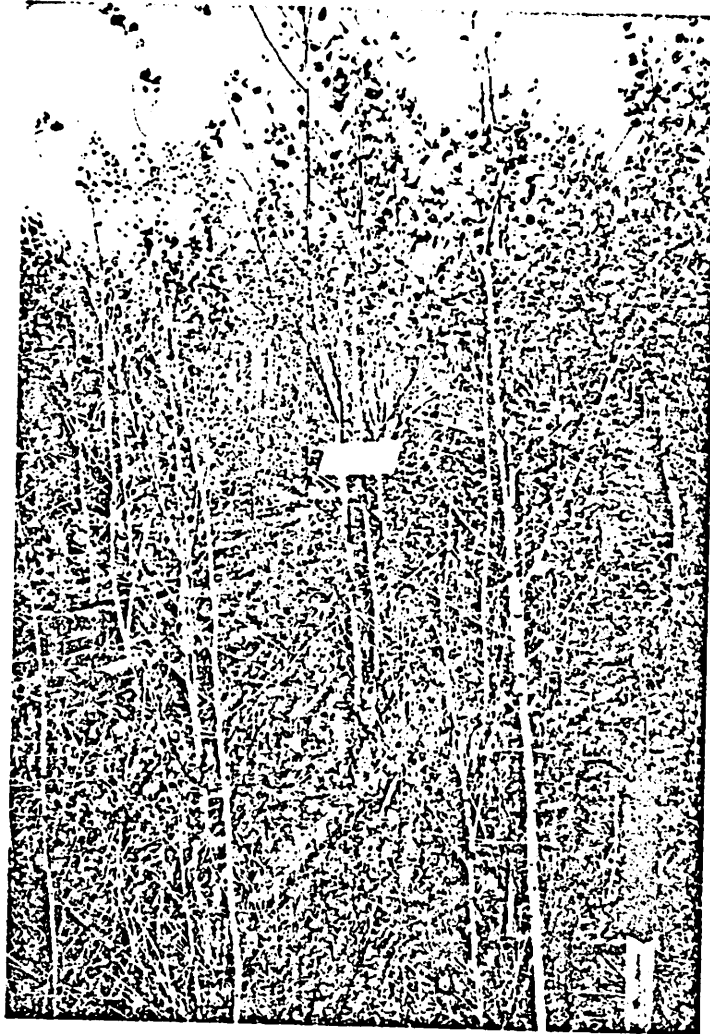


Fig. 5. Influence of Velpar on aspen under field conditions.

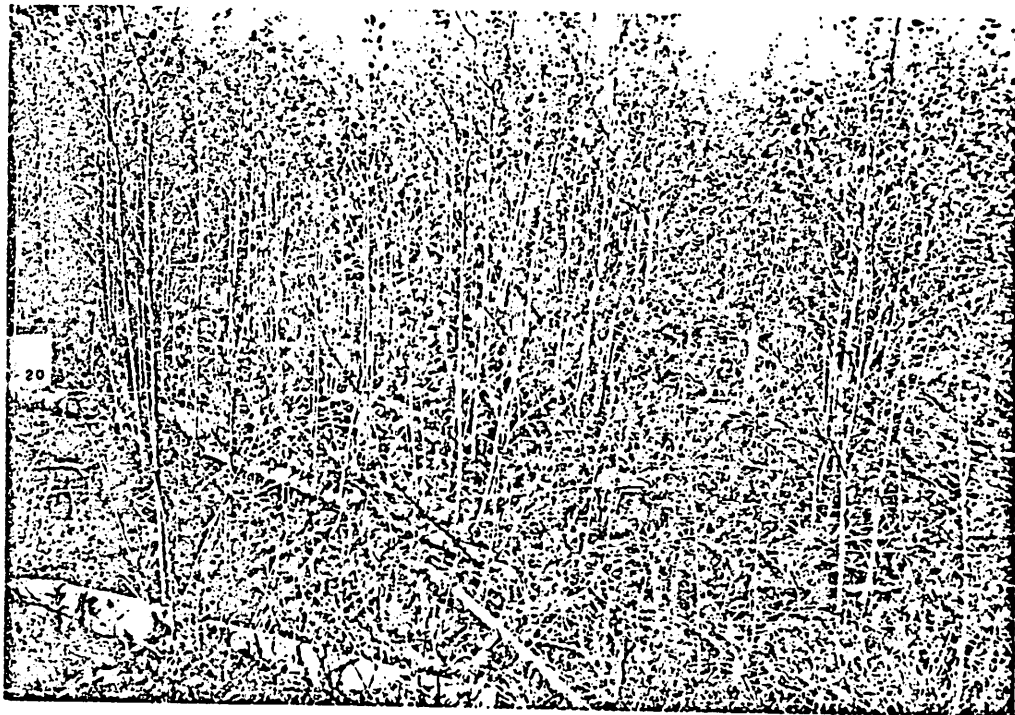


Fig. 6. Influence of Velpar + Tween 20 on aspen under field conditions.

Table VI. Influence of Adjuvant and Herbicide Combinations Measured After Three Weeks on Changes in Fresh Weight of Leaves Under the Greenhouse Conditions

Treatment	(Fresh weight g/pot)		
	Alder	Aspen	W. Birch
Control	19.1	6.5	10.9
Garlon alone	8.2	5.8	7.4
G + Tween 20	3.7	6.5	4.7**
Control	41.7	-	15.7
Roundup alone	22.8	-	14.5
R + Tween 20	21.1	-	12.7
Control	14.2	-	16.5
Velpar alone	5.9	-	11.3
V + Tween 20	4.5	-	8.3**

Tukey's test, significant at $P = 0.05$.

B. Field Plot Evaluations

As described earlier, after screening the effects of Tween 20 and mixtures of Tween 20 with herbicides on weed and crop species under greenhouse conditions, the next step was to evaluate effects under field conditions. Employing the small field plot technique (Prasad 1986) the influence of Roundup and Velpar at reduced dosages (0.5 kg/180 L/ha) on aspen was first ascertained. It was found that this dosage was appropriate for measuring the additive effects of surfactants and accordingly Tween 20 (0.1%) was mixed with these two herbicides at 0.5 kg/ha in 180 litres of water and sprayed onto foliage of aspen. Quantitative and qualitative assessments were conducted one month after the treatment (Table VII). Tween 20 increased the efficacy of both herbicides under field conditions tested.

Table VII. Effects After Four Weeks of Tween 20 (0.1%) on Efficacy of Roundup and Velpar (0.5 kg/ha) Under Small Field Plot Conditions in Northern Ontario

Treatment	Phytotoxicity Rating of Aspen (%)
Untreated (Control)	0
Roundup alone	50.5
R + Tween 20	62.1
Velpar alone	41.6
V + Tween 20	49.8

No crop injury to conifers (red pine) planted in the cut over site was apparent. These results are preliminary and the long term impact of the surfactant and herbicide formulation need to be monitored next year to obtain conclusive evidence. Nevertheless, results from greenhouse and field trials substantiate the hypothesis that adjuvants can increase the activity of forest herbicides and that forest weeds also respond, like agricultural weeds, to addition of adjuvants in herbicide formulations. Considerable economy in the cost of application can be expected especially from the commercial Roundup formulation which is about 4-5 times more expensive than the 2,4-D herbicide. The *modus operandi* of Tween 20 in promoting the efficacy of these herbicides remains unknown. Some corroborative evidence collected with another adjuvant G3780A (Table VIII) suggests that penetration and translocation of the active material (glyphosate-C¹⁴) is greatly facilitated by these adjuvants. Further work is needed with Tween 20 to elucidate its mechanism of action on uptake of herbicides by forest weeds.

Table VIII. Effects After Two Weeks of Adjuvant (G3870A at 0.05%) on Foliar Penetration and Translocation of Glyphosate-C¹⁴ in Alder and White Birch.

Weed species	Treatment	Content of radioactivity (cpm/g)			
		Leaves	Stem	Roots	Total
Alder	Glyphosate alone	601	817	225	1643
	Glyphosate + Adjuvant	883	1367	330	2580
White birch	Glyphosate alone	1617	10	375	2002
	Glyphosate + Adjuvant	2008	167	450	2625

It is of some interest to note from these results that different weed species react differently in their quantitative response to addition of adjuvants with the herbicides. This is possible because the leaf morphology, anatomy and physiology of each weed species are different and adjuvants are known to engender variable responses in weed species (Hodgson 1983; Prasad et al. 1967; Sherrick et al. 1986; Wyrill et al. 1977). Combinations of adjuvants and herbicides promoting efficacy in forest weeds were tested on crop species (balsam and Douglas firs, white and black spruces) but none were found to cause phytotoxicity to these conifers.

In conclusion, the present study with Tween 20, three herbicides and three forest weeds under greenhouse conditions and with preliminary field experiments demonstrates that Tween 20 is beneficial in forest spray applications and that considerable economy in the use of the active ingredient might be accomplished by judicious use of surfactants.

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