TESTS OF CHLORPYRIFOS FOR CONTROL OF THE NORTH AMERICAN ELM BARK BEETLE

(*HYLURGOPINUS RUFIPES* EICHH.)

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ABSTRACT

Chlorpyrifos was found to be effective in controlling overwintering and branch feeding by the North American elm bark beetle, *Hylurgopinus rufipes* Eichh., the principal vector of Dutch elm disease in Canada. Experiments were conducted in New Brunswick, Quebec, Ontario and Manitoba. Development of a method for determining chlorpyrifos residues in elm bark permitted investigation of the chemical's fate and residual activity after spraying of elm tree trunks. It also enabled calculation of the amount of chemical required to prevent beetle feeding, which leads to infection of elm trees with the disease fungus.

RÉSUMÉ

Chlorpyrifos s'est avéré efficace à combattre l'hivernement et le broutage des branches par le Scolyte de l'Orme, *Hylurgopinus rufipes* Eichh., principal agent vecteur de la graphiose au Canada. Des expériences ont été effectuées au Nouveau-Brunswick, au Québec, en Ontario et au Manitoba. La mise au point d'une méthode de détermination de la présence de résidus de chlorpyrifos dans l'écorce de l'Orme a donné lieu à des recherches sur le sort de ce produit chimique et sur son activité résiduelle après sa pulvérisation sur les troncs des ormes. Cela a également permis de calculer la quantité du produit chimique requise pour prévenir le broutage du Scolyte, qui ouvre la voie à l'infection des ormes par le champignon pathogène (*Ceratocystis ulmi* [Buism.] C. Moreau).

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CONTROL OF OVERWINTERING BEETLES

Previous research (Gardiner 1976) has shown that prevention of overwintering by adult beetles in the bark at the base of living elms (*Ulmus* spp.) should be effective in reducing both beetle populations and incidence of Dutch elm disease (*Ceratocystis ulmi* [Buism.] C. Moreau). The most efficient way to do this would seem to be by applying a suitable insecticidal barrier to the trunk. When our search for a suitable insecticide began, the only chemical registered for use against elm bark beetles was methoxychlor. Unfortunately, repeated tests at the Great Lakes Forest Research Centre had shown that this chemical does not provide adequate control of the native elm bark beetle (*Hylurgopinus rufipes* Eichh.), however effective it is reported to be against the European elm bark beetle (*Scolytus multistriatus* [Marsh.]). A preliminary study in 1975 showed that chlorpyrifos emulsifiable concentrate (Dow Chemical Company's Dursban 2E®) was much superior to methoxychlor in preventing the overwintering of beetles in living elms (Gardiner 1976).

In 1976, extensive tests of chlorpyrifos were carried out in Brandon, Selkirk and Winnipeg, Manitoba; Sault Ste. Marie, Ontario; Grand'mère and Shawinigan, Quebec; and Fredericton, New Brunswick. In 1977, the tests were repeated in Fredericton and Sault Ste. Marie.

Methods

In all test areas, the chemical was applied to the lower 2.5 m of the trunks of living trees, at a concentration of 0.5% a.i., until the bark was wet but not running with the liquid. In Sault Ste. Marie and Selkirk backpack mistblowers were used; hand-operated pressure (hydraulic) sprayers were used at all other test sites. All sprays were applied in the latter half of August, just before the beetles begin excavating hibernation sites at the bases of elm trunks.

In Sault Ste. Marie, 606 trees were treated in 1976 in an area bounded by Shannon Road, Wellington Street, Pine Street and St. Mary's River. Since many of the trees were privately owned, permission to treat was required. Accordingly, a letter was sent to owners and tenants of properties that, according to city maps, bore elms (Gardiner 1977). Roughly 82% of addressees responded, all positively. Those who did not reply were approached at home at the time of spraying and all gave consent. Thus all elms over 5 cm DBH in the experimental area were treated.

Effectiveness of treatment was assessed by counting piles of boring dust in bark fissures on a 25-cm band of bark near the base of treated and untreated trees. This does not measure the beetle population since more than one beetle may contribute to a boring-dust pile; however, it appears to give a reliable estimate of beetle activity.

Results and Discussion

Table 1 gives the 1976 results from test sites in Manitoba, Fredericton and Sault Ste. Marie. Clearly, the treatment resulted in very good control of overwintering by native elm bark beetles. The somewhat lesser degree of control achieved at the Manitoba sites resulted from a local difference in the beetle's habits. It was observed in the fall of 1976 that, in Manitoba, the native beetle tends to overwinter much lower in the trunk than it does in the Maritimes, Quebec and Ontario--in fact, as close to the ground level as possible. Undoubtedly this represents an adaptation on the part of the population to lower winter temperatures and lesser snow depth. This difference was not known at the time of spraying and particular care was not taken to make sure that, where the trees were surrounded by long grass, the trunk was treated right to the ground level. When the grass was cut short, e.g., at the Legislative Buildings in Winnipeg, this problem did not arise and no beetle activity was detected in the treated trees.

Available time and human resources did not permit making counts of boring dust piles at the Quebec spray sites; however, brief examination of these sites in October, 1976, showed the same, striking difference in beetle activity between treated and untreated trees as recorded in other spray areas.

In 1977, formal tests were restricted to Fredericton and Sault Ste. Marie.

As in 1976, all elm trees over 5 cm DBH in the test area bounded by Shannon Road, Wellington Street, Pine Street and the St. Mary's River were treated. Chlorpyrifos was applied as before in the latter half of August. Trunks were sprayed in early morning when wind was minimal, until the bark was wet. Again, residents in the area cooperated fully.

Efficacy of treatment was again assessed by counting boring dust piles in bark fissures in a 25-cm basal band on trees inside and outside the test area. The results are given in Table 2.

In Fredericton, the chemical was applied to trunks of city-owned trees in the older part of the city. Some trees had been treated in 1976. Again the results showed a marked reduction in overwintering as evidenced by lack of boring dust piles on the treated trees, even in the year after treatment (Table 3).

Table 1. Trunk treatment against overwintering *H. rufipes* 1976.

Treatment	No. of trees	Boring dust piles/m^2							
		Manitoba		Winnipeg		Fredericton			
		Selkirk	Brandon	Legislative Bldg.	Crestwood	Wilmot Park	Southwood Park	Sault Ste. Marie	% Control
Control	10	1991.3							
	15		187.2						
	11			55.7					
	30				230.4				
	20					1.5			
	20						14.4		
	11							207.9	
Hand sprayer	15		31.5						83
Hydraulic	10			0.0					100
	30				26.4				89
	20					0.0			100
	20						0.0		100
Mistblower	10							2.1	99
	11	94.3							95

All 0.5% Dursban 2E

Table 2. Effect of trunk treatment against overwintering *H. rufipes*

Treatment	No. of trees	Boring dust piles/m^2	Control %
Outside area	16	109.6	--
Inside area	20	0.8	99

Table 3. Effect of trunk treatment against overwintering *H. rufipes* in Fredericton, 1977

Location and treatment	No. of plots	No. of trees	Boring dust piles/m^2
Central area			
Trunk spray 1976 - 1977	4	34	0
Trunk spray 1976	2	20	1*
Trunk spray 1977	2	20	0
No spray	5	45	18
Southern area	2	14	86
Northern area	3	30	623
Outside city	4	39	419

* The figure results from the fact that there were beetles in only 2 of 20 trees.

Conclusion

Results show that thorough spraying of the lower 2.5 m of the trunks of living elm with the chlorpyrifos mixture until the bark is wet will virtually eliminate overwintering by native elm bark beetles. This treatment does not protect individual trees except from the rare event of infection through an overwintering niche. It is designed to exclude overwintering beetles from a Dutch elm disease control area.

It is interesting to note that the beetles are not simply repelled by the spray. There was abundant evidence, in the form of dead beetles in fissures and entrance holes, to show that they are killed in attempting to penetrate the bark.

CONTROL OF BRANCH FEEDING

Since, in Canada, most cases of Dutch elm disease arise through infection in feeding niches caused by native elm bark beetles in branches of elm, prevention of such feeding in spring and early summer is desirable in controlling the disease. The use of chlorpyrifos in 1975 and 1976 for preventing beetle penetration into trunk bark suggested that this chemical might be effective in reducing branch feeding.

Methods

In 1977, under experimental permit, whole-tree treatment with chlorpyrifos (Dow Chemical Company's Dursban 2E®) was carried out in a park at Selkirk, Manitoba, and in the Cathedral-Legislature area of Fredericton, New Brunswick. At Selkirk, 40 trees were sprayed on 29 April with 0.5% a.i. and on 12 May another 50 trees were treated with a 1.0% spray, both being applied with a hydraulic sprayer capable of producing a pressure of 3.45×10^3 KPa. In Fredericton 54 trees were treated with 1.0% chlorpyrifos using a mistblower.

Sample branches were taken from treated trees in May and tested for chemical activity using a bioassay technique developed previously.

In the bioassay technique, individual beetles are confined on the bark of a 25 cm section of sample branch using cages made with 7 mm brass tubing. A 25 mm length of tubing is used and one end is closed with fine brass screen soldered to the tubing. In confining the beetle on the branch sample a circle is cut in the bark with a 7 mm (approx.) cork borer and the cage containing the beetle is forced into the cut where it is held by friction. Three beetles are thus confined on each branch sample. The sample is then held in an incubator at 26°C and 90% RH for 6 days.

Assessment of chemical effect may be measured in several ways at the end of the period: by beetle mortality, beetle penetration into phloem, or beetle penetration through the phloem to *score* the surface of the xylem.

The last criterion is the one generally used in these tests. It is a meaningful standard in that a beetle, contaminated with the Dutch elm disease fungal spores, must penetrate to the xylem of the tree where the spore(s) must germinate in order to produce a new infection.

On 16 May 1978 the crowns of all elms at the Courthouse in Sault Ste. Marie, were sprayed with 0.5% aqueous mixture of chlorpyrifos, with a hydraulic sprayer. Ten times throughout the season five sample

branches were taken from the sprayed trees and submitted to bioassay for protection against penetration by native elm bark beetles to the xylem tissue. Each time, a 10 cm^2 sample of bark was taken from the branches, freeze-dried, pulverized and held in freezer storage for chemical assay of chlorpyrifos residues.

Results

The results of the 1977 bioassays are given in Table 4 and those of 1978 in Table 5. The 1977 data suggest that a 1% spray is more effective than a 0.5% spray; however, these result from only a single bioassay and are, therefore, not so reliable as the data generated in 1978. In the latter year, repeated bioassays show that excellent protection throughout an entire growing season can be achieved with a 0.5% spray (Table 5).

Table 4. Bioassay results of sample branches from 1977 whole-tree treatments with chlorpyrifos

Treatment		N	Into phloem	Scored xylem	Alive	Control (%)[a]
Fredericton	1.0%	45	7	0	2	100
Untreated		45	33	19	35	
Selkirk	0.5%	60	6	3	4	89
Untreated		60	44	28	52	
	1.0%	60	22	2	12	92
Untreated		60	46	26	50	

[a]Measure of prevention of xylem scoring

Table 5. Bioassay results on branch samples from 1978 whole-tree treatment with chlorpyrifos

Date	Control (%)[a]	Beetle mortality (%)
17 May	100	100
19 May	86	90
25 May	100	100
30 May	100	100
2 June	100	100
19 June	100	100
7 July	100	100
9 August	0	66
6 September	100	90
8 November	100	90

[a]Measure of prevention of xylem scoring

With one exception, all tests showed that the treatment had provided good protection against branch feeding by *H. rufipes*.

Figure 1 shows the results of chlorpyrifos residue determination. The curve shows the typical, sharp initial decrease in chemical after application, probably because of volatilization and chemical breakdown. This is followed by a period, also typical and usually lasting the season, when the chemical remains at a fairly constant low, but effective, level. Residue analysis explained the poor bioassay results obtained with the August 9 sample. Most branches in the sample held only trace amounts of chlorpyrifos which were not effective in preventing beetle penetration through the bark. That the choice of these branches was done by chance alone is shown by the two perfectly adequate, subsequent samples taken in September and November. This emphasizes the importance of ensuring thorough coverage during the application.

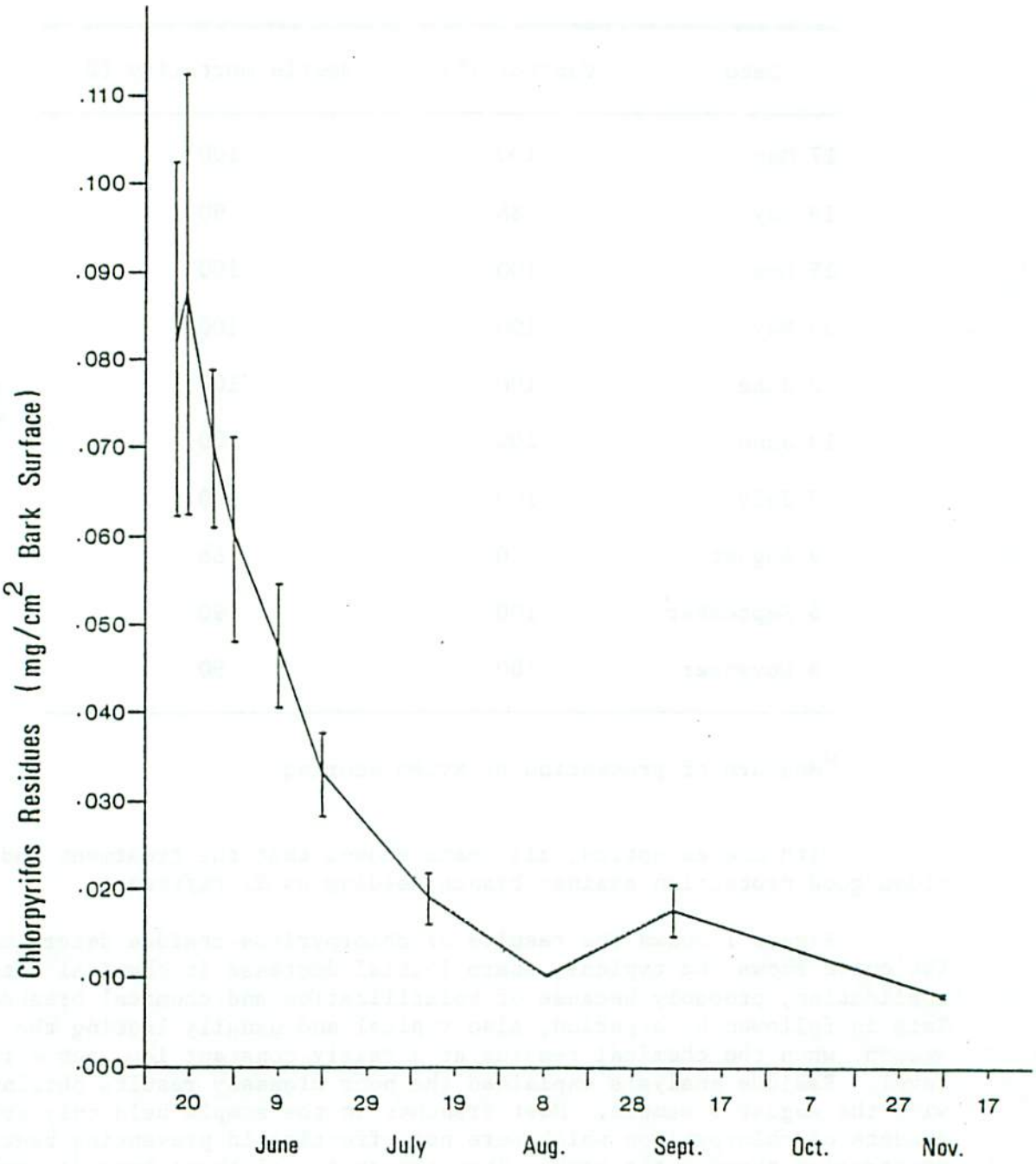


Figure 1. Chlorpyrifos residues on branches of trees sprayed with 0.5% mixture on 16 May, 1978.

Conclusion

These experiments show that a 0.5% chlorpyrifos spray is effective throughout the growing season for preventing branch feeding by *H. rufipes*, and should be useful in giving maximum protection to high-value elm trees. However, this seems to be the minimum concentration, or close to it, to do the job. Because of the reduction in chemical residue by the end of the year, one would not expect that adequate protection would carry over into the following spring. This was confirmed by bioassay in the spring of 1979. It appears, therefore, that unlike the trunk spray, crown spray must be applied each year.

DETERMINATION OF RESIDUES OF O, O- DIETHYL O-(3,5,6 - TRICHLORO-2 - PYRIDYL) PHOSPHOROTHIOATE (CHLORPYRIFOS) IN WHITE ELM (*ULMUS AMERICANA* L.) BARK BY GAS LIQUID CHROMATOGRAPHY

Scope

This method is designed for determination of residues of chlorpyrifos in elm bark treated at operational control levels of 0.1 to 1.0% Dursban 2E®. The method is applicable for routine determination of residues at levels as low as 10 ppm. Determinations at 0.1 ppm are possible with minor modification of dilution; however, routine analysis at these levels will require additional cleanup procedures.

Principle

Chlorpyrifos is extracted from ground elm bark in acetone in a Soxhlet extractor. The extract is made to volume and an aliquot is transferred into benzene for dilution and gas chromatography analysis.

Equipment

1. Gas-liquid chromatograph, Hewlett Packard Model 7623A or equivalent fitted with Ni^{63} electron capture detector and a Hewlett Packard Model 3380A digital integrator.

2. Gas chromatography column, 1.8 m, 4 mm I.D. coiled glass column packed with 3% OV-17 on Chromosorb W "HP" 80/100 mesh.

3. Wiley Mill, 1.0 mm screen.

4. Soxhlet extractors and 33 mm x 80 mm Whatman cellulose thimbles.

5. Volumetric flasks, 250 ml and 10 ml.

6. Erlenmeyer flasks, 125 ml with 24/40 ground glass joints, Pyrex brand.

7. Snyder evaporation columns, size 121, 150 mm long, three sections with 24/40 ground glass joints.

Reagents and Materials

1. Acetone and benzene, reagent grade or better (redistilled in glass). Note: Because of the relatively high levels of chlorpyrifos determined by this method, little concentration of solvents is required and as such nanograde or pesticide grade solvents are not normally required but are recommended.

2. Chlorpyrifos standard. Obtained from Sampling Coordinator, Agorganics Department, Dow Chemical, U.S.A., P.O. Box 1706, Midland, Michigan, 48640.

3. Calibration standards 0.1 µg/ml chlorpyrifos in benzene. Wrap in aluminum foil to exclude U.V. light.

4. Argon, 5% Methane carrier gas.

Procedure

1. Elm bark is ground to pass a 1.0 mm screen in a Wiley mill and stored in sealed glass containers at approximately -40°C to -60°C.

2. Two replicate, 1.0 samples are accurately weighed out in separate Soxhlet thimbles.

3. The thimble containing the sample is placed in a Soxhlet extractor with approximately 150 ml of acetone and allowed to extract for 2 hours or approximately 12 wash cycles.

4. The extract is transferred to a 250 ml volumetric flask and allowed to stand for 1 hr at room temperature before making up to final volume.

5. A 5.0 ml aliquot (or an appropriate aliquot depending on dilution required) is removed and transferred to a 125 ml Erlenmeyer flask along with 25 ml of benzene.

6. The flask is connected to a Snyder column and the volume reduced to approximately 5.0 ml on a hot plate.
Note: Do not allow extract to dry out as loss of chlorpyrifos will result.

7. The extract is quantitatively transferred to a 10 ml volumetric flask by use of disposable pipettes and allowed to cool to room temperature before bringing to volume with benzene.

Gas Chromatography

A. Typical Operating Conditions

(a) Column temperature, isothermal at 250°C.

(b) Injection port temperature 270°C.

(c) Electron capture detector temperature 290°C.

(d) Pulse interval 50 μ sec.

(e) Carrier gas (Argon 5% Methane) flow rate 60 ml/min.

(f) Electrometer range X 10 and digital integrator attenuation X 256.

B. Gas Chromatography Procedure

1.0 μl aliquot of prepared extract is injected with a 10 μl syringe. Sample injections are bracketed by 1 μl injections of appropriate chlorpyrifos standards. Peak area of sample is compared to mean peak area for standards injected before and after sample and the amount of chlorpyrifos present is calculated by the following formula:

$$\frac{\text{Dilution factor} \times \text{Unknown peak area} \times \text{Amount of chlorpyrifos standard injected (ng)}}{\text{Chlorpyrifos standard peak area} \times \text{Original sample weight (g)}} \times \mu g/ng = \mu g/g$$

Preparation of Standard Curve to Determine Linearity Range of Electron Capture Detector

Inject 1 μl aliquots of the chlorpyrifos standards covering the concentration range of 0.1 to 10 ng/μl into the chromatograph and record peak areas. Plot peak areas on the ordinate versus nanograms of chlorpyrifos on the abscissa. A typical standard curve is presented in Figure 2. The response is linear over the range from 0.1 to 8.0 ng.

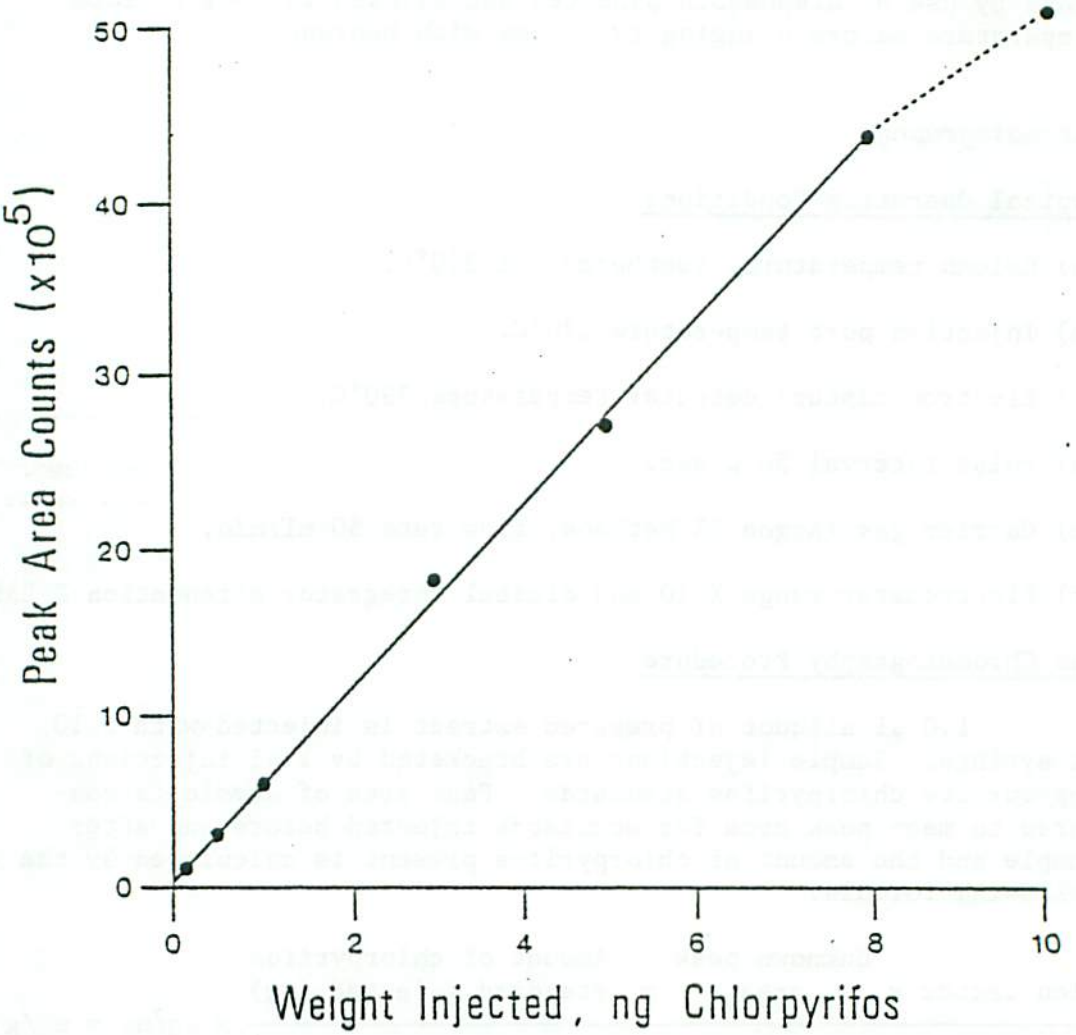


Figure 2. Typical standard curve to determine linearity range of electron capture detector.

Recovery of Chlorpyrifos from Spiked Elm Bark Samples

Six replicate 1.0 g samples of ground elm bark to which 500 µg of chlorpyrifos standard had been added were extracted and analyzed by GLC along with appropriate controls. Analysis indicated a mean of 0.4928 mg ± 0.0120 standard error of chlorpyrifos present in the samples. This corresponds to a recovery of 98.56%.

Efficiency of Extraction Method

Samples from treated (i.e., dipped) elm bark in 0.5% chlorpyrifos were analyzed by the procedures outlined above. Five replicate samples, including one sample of untreated bark, were subjected to three repeated 2-hr Soxhlet extractions. Analysis of chlorpyrifos was performed on each of the three extractions by the methods outlined and results are presented in Table 6. Extraction was complete after a 2-hr Soxhlet extraction time.

Table 6. Effect of repeated Soxhlet extractions on chlorpyrifos in elm bark samples

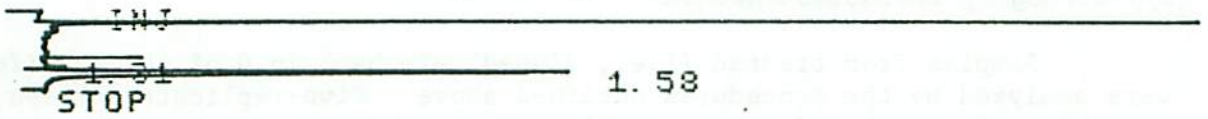
	Chlorpyrifos (mg/g dry wt)		
	Extraction number		
Treatment	1	2	3
A Control	N.D.[a]	N.D.	N.D.
B_1	0.16	N.D.	N.D.
B_2	0.17	N.D.	N.D.
B_3	0.18	N.D.	N.D.
B_4	0.17	N.D.	N.D.
B_5	0.16	N.D.	N.D.
Mean	0.167		
SE	0.003		

[a] N.D. = not detected.

Routine Analysis of Chlorpyrifos

The method has proven efficient and accurate for the routine analysis of chlorpyrifos in elm bark samples collected from sprayed trees. Typical chromatograms are presented in Figure 3.

1.0 ng chlorpyrifos standard

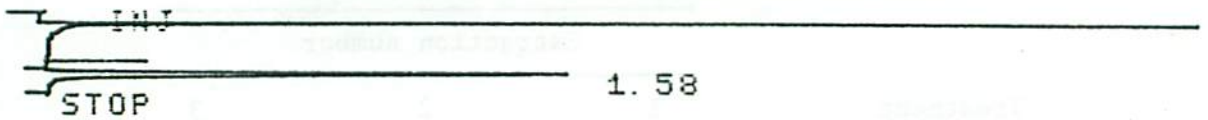


AREA %

RT	TYPE	AREA	
1.58	T	622643	100

HP 3380A
DLY 1. STOP 5 REJECT 10000
MV/M .10 ATTN 256

Sample extract with appropriate dilution

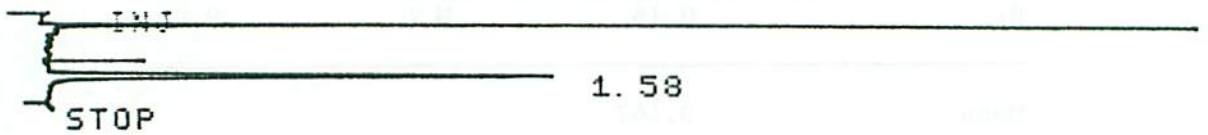


AREA %

RT	TYPE	AREA	
1.58	T	648471	100.

HP 3380A
DLY 1. STOP 5 REJECT 10000
MV/M .10 ATTN 256

1.0 ng chlorpyrifos standard



AREA %

RT	TYPE	AREA	
1.58	TM	673992	100.

HP 3380A
DLY 1. STOP 5 REJECT 10000
MV/M .10 ATTN 256

Figure 3. Typical chromatograms of routine analysis of chlorpyrifos in white elm bark samples.

Identification of Chlorpyrifos in Elm Bark Samples

The chlorpyrifos in elm bark samples was identified by retention time and co-injection of authentic chlorpyrifos standard. Identification was confirmed by gas-liquid chromatography and mass spectrometry.

RESIDUAL BIOLOGICAL ACTIVITY AND AMOUNT OF CHLORPYRIFOS ON ELM BARK RELATIVE TO OVERWINTERING *H. RUFIPES*

At the time of assessing the August 1976 trunk spray in Sault Ste. Marie, counts of boring dust piles were also made on trees that had been sprayed 14 months before. These trees had been treated in August 1975 with chlorpyrifos (Dursban 2E®) at 0.5% and 1% and sumithion plus ethylene dibromide (Sumibark E 40®) at 1%. The treatment of August 1976 was with Dursban 2E® at 0.5%. All sprays were applied to the lower 2 m of trunks of living elm trees with a Holder Supra 40 backpack mistblower. The mixture was applied until the bark was wet but not running with the liquid. Counts of beetle boring dust piles in bark fissures were made on 1976-treated trees 2 months after spraying. The results are given in Table 7.

Table 7. Control of overwintering achieved with Dursban® and Sumibark® in three areas of Sault Ste. Marie

Treatment	Time lapse (months)	No. of trees	Boring dust piles/m^2			Control (%)
			Strathclair	Pim Hill	East End	
Untreated		4	277.9			
		5		143.0		
		11			207.9	
Sumibark 1%	6	5		82.9		41.9
Dursban 0.5%	14	6	7.0			97.5
Dursban 1.0%	6	5		2.3		98.4
Dursban 1.0%	14	12	3.6			98.7
Dursban 0.5%	2	10			2.1	99.0

Obviously, chlorpyrifos on elm bark, applied as described, demonstrated very effective residual activity over a year after application, whereas the activity of Sumibark was reduced to less than half after 6 months. In addition, although counts of boring dust piles were not made, inspection in the fall of 1977 of trees sprayed in the spring of 1976 with 1% Dursban 2E showed that these trees still enjoyed very good protection from overwintering beetles 18 months after chemical application.

Chlorpyrifos residues in bark treated with 0.5% Dursban 2E were determined 2, 4, 6 and 8 weeks and 14 months after spraying in Sault Ste. Marie.

Materials and Methods

Samples were obtained from bark plates and bark on the north and south sides of trees by scraping with knife or chisel. Unfortunately, considerable variation is introduced into this method because of the impossibility of controlling amounts of bark scales, lichen, dust, etc., in the samples. The resulting variability affects the determinations of amounts of chemical residues because these are expressed in relation to the weight of the sample (mg/g). Under existing circumstances, however, any more consistent sampling method, such as recovering all the chemical in a certain area of bark, would have caused unacceptable damage to valuable shade trees.

The samples were ground in a Wiley mill and held in the dark in aluminum foil bags at -60°C until the determinations could be made. Duplicate determinations were made on each sample. The latter were done by D.P. Webb, Great Lakes Forest Research Centre, using a method designed by him and described in this report.

Results and Discussion

The mean amounts (mg/g), with standard errors, of residual chlorpyrifos in samples of bark scrapings are given in Table 8.

The reason for sampling bark plates and fissures separately lies in the fact that chlorpyrifos breaks down under the influence of ultraviolet radiation. It was therefore hypothesized that the chemical might remain at effective levels in the fissures, where the beetles enter in search of overwintering sites, while breaking down more rapidly on the exposed plates. Inspection of the data does not show any consistent significant differences in residues developing during the first 8 weeks after spray application. On the other hand, the residues determined after 14 months do indicate greater persistence of chlorpyrifos in the fissures than on the plates. The inconclusive results obtained in this experiment are attributable mainly to the sampling method employed.

Table 8. Chlorpyrifos residues (mg/g) in bark scrapings taken from sprayed elm trunks

Time after spraying	N	Plates north		Plates south		Fissures north		Fissures south	
		$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE
2 weeks	5	0.289	.042	0.221	.034	0.195	.021	0.205	.026
4 weeks	5	0.375	.039	0.268	.019	0.297	.026	0.226	.017
6 weeks	5	0.348	.043	0.302	.043	0.343	.043	0.295	.061
8 weeks	5	0.305	.009	0.233	.013	0.260	.030	0.180	.020
14 months	2	0.160	.004	0.150	.004	0.255	.024	0.395	.037

The most desirable sampling method would enable residues to be expressed on an area basis. Some limited, sequential samples of bark, 6 x 6 cm, from two trees in Winnipeg were available for residue determinations. The results are shown in Figure 4 and indicate that after the usual initial sharp drop in amount of chemical present, the amount apparently remained fairly static at slightly below 0.2 mg/cm^2 throughout the winter of 1976-1977. The boring dust pile counts show that this level of chlorpyrifos is effective in barring beetles from overwintering in treated bark.

AMOUNT OF CHLORPYRIFOS REQUIRED TO PREVENT BEETLE FEEDING IN BARK OF ELM BRANCHES

To indicate when trees require chemical protection but, at the same time, to prevent application of more chemical than is necessary, it would be useful to have a minimum standard (in mg of chlorpyrifos/ cm^2 bark surface) that would afford protection from beetle feeding. Accordingly, an experiment was designed and carried out to furnish such a standard.

Methods

In August and September, 1977, ten branch samples, 25 cm long, were dipped in aqueous mixtures of Dursban 2E® of the following concentrations of chlorpyrifos (%): 0.5, 0.1, 0.05, 0.01, 0.005, 0.001 and

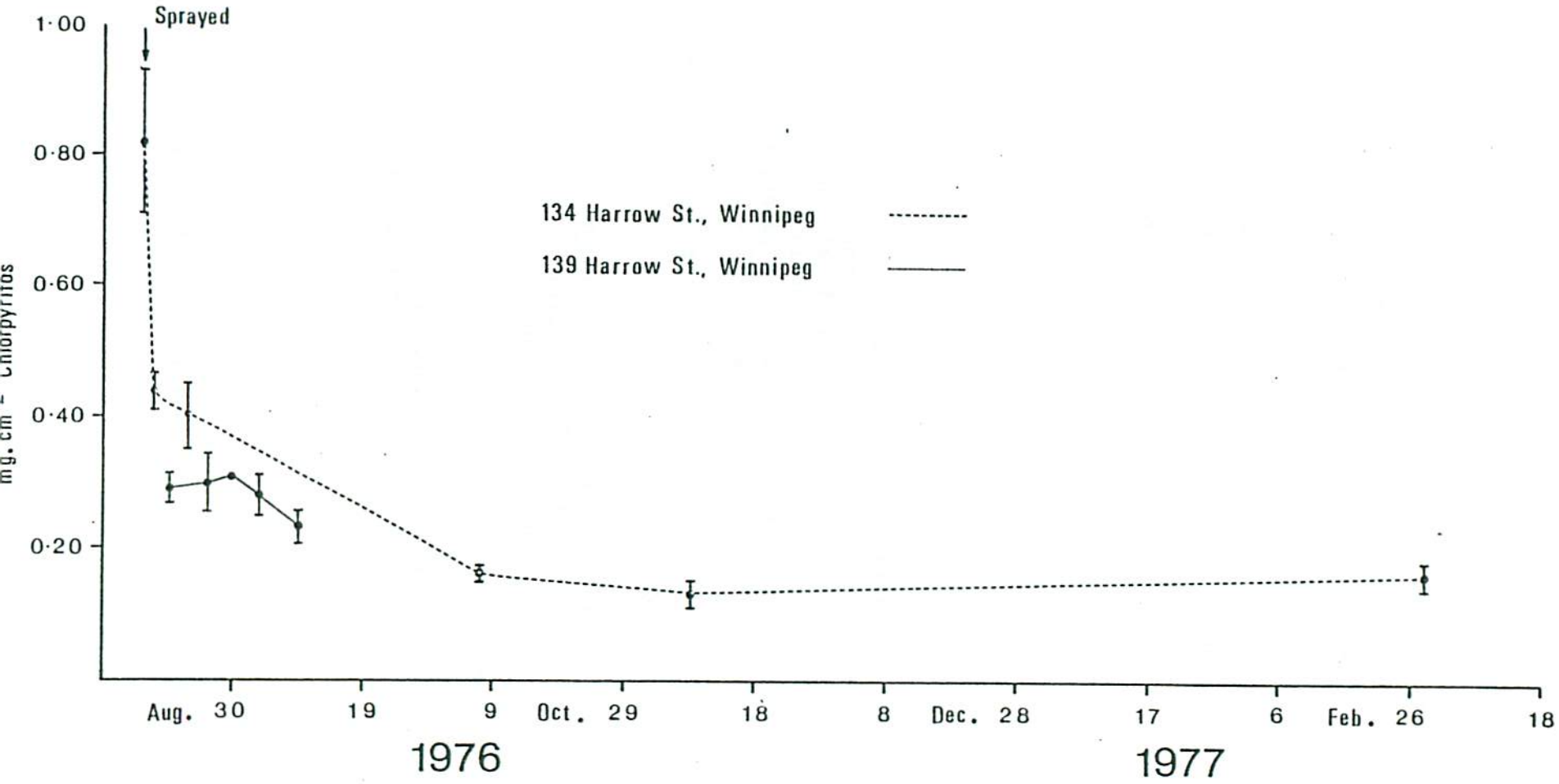


Figure 4. Chlorpyrifos residues on elm bark.

0.005. After dipping, the sticks were allowed to dry, then were sealed in aluminum foil and stored for about a week at -60°C in the dark.

The sticks were then bioassayed for biological activity against the native elm bark beetle, using the technique described previously in this report.

At the time of reading the bioassay, samples of bark, 6 x 6 cm, were removed from the sticks, freeze-dried, pulverized in a Wiley mill and stored in aluminum foil bags in the dark at -60°C. The amount of chlorpyrifos present was later determined for these samples and expressed as mg/cm^2 of bark surface.

Results and Discussion

The results of bioassay of dipped sticks are given in Table 9. The three highest concentrations of chlorpyrifos prevented any penetration of the bark and killed all beetles in the 6-day period. Concentrations of 0.01% and 0.005% permitted some serious penetration of bark, although control of penetration at these levels was excellent and no beetles survived. The two lowest concentrations did not provide acceptable control of insect attack.

Table 9. Fate of beetles 6 days after introduction on elm sticks dipped in diminishing concentrations of chlorpyrifos

Treatment	No. of beetles				Control (%)[a]
	N	Into phloem	Scored xylem	Alive	
0.5%	30	0	0	0	100
0.1%	30	0	0	0	100
0.05%	30	0	0	0	100
0.01%	30	0	1	0	93
0.005%	30	2	0	0	100
0.001%	30	21	9	14	40
0.0005%	30	23	18	23	0
Untreated	30	25	15	27	---

[a]Measure of prevention of xylem scoring.

The data in Table 9 suggest, therefore, that reliable prevention of serious beetle feeding may be expected when the bark on elm branches contains the amount of chlorpyrifos equivalent to that deposited by dipping in an aqueous mixture of Dursban 2E containing 0.01% chlorpyrifos.

In Figure 5, which shows the curve generated by the chemical determinations of chlorpyrifos on the dipped sticks, it may be seen that this amount is approximately 0.001 mg/cm^2 of bark surface, since this is equal to a dip concentration value of 0.01%.

Thus, the minimum amount of chlorpyrifos on a branch that may be expected to prevent serious feeding is 0.001 mg/cm^2 of bark surface. It must be emphasized, however, that this is the *minimum* and that it cannot be expected to afford any lasting protection in the field. In the 1978 crown spraying experiment in Sault Ste. Marie the chemical residue at the end of the season amounted to 10 times this minimum and afforded good protection. This had disappeared by the following spring, however.

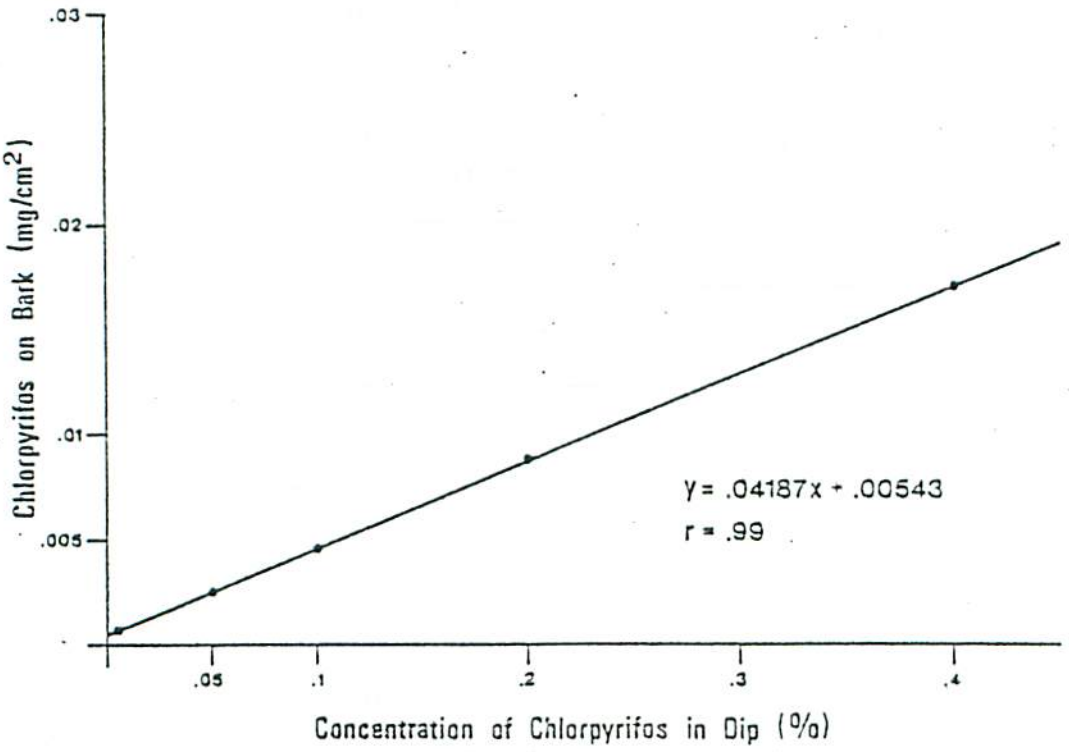


Figure 5. Curve generated by the chemical determinations of chlorpyrifos on dipped sticks.

LITERATURE CITED

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