DIFFUSION OF ^{14}C-FLURPRIMIDOL IN VARIOUS CARRIERS THROUGH EXCISED SILVER MAPLE BARK

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Abstract. Bark banding is one alternative to trunk injection of tree growth regulators (TGRs). The objective of this study was to compare the penetrability of various oils as carriers for a TGR applied to the bark. Five µl of ^{14}C-flurprimidol (225,000 dpm) and unlabeled flurprimidol dissolved in isopropyl alcohol and selected oils (Androc Basal Oil, ArborChem Basal, Arborchem Clean Cut, CWC Hy-Grade I, Exxon Aromatic 200, Exxon Orchex 796, Leffingwell Carrier #9, and N.G. Gilbert Riteway Oil) were applied to bark excised from silver maple (*Acer saccharinum*). Bark discs, 1.35 cm diameter, were sealed in a diffusion chamber and the rates and amounts of ^{14}C-flurprimidol that moved through the bark were measured by liquid scintillation spectrometry. Rate of diffusion, measured as dpm/day for nine days, was similar for each oil. The pattern of penetration was an initial flux the first 24 hours followed by a diminished rate of penetration by the end of day two and a gradual increase during the next seven days.

The high cost of trimming trees growing under power lines has encouraged the electric industry to look at tree growth regulators (TGRs) as a way to extend the time between trimming cycles and to decrease the amount of biomass removed during trimming. The primary method for TGR application is pressurized trunk injection. However, there are a number of problems associated with this technique and that has prompted utilities to seek alternative methods for applying TGRs (6). Problems associated with trunk injection include weeping, non-uniform distribution, branch escape, wood discoloration, bark splitting, and bark blowouts.

Bark banding may be a technique that can be used to overcome some of these problems. The TGR is dissolved in an oil carrier, and the solution is painted on the bark around the tree trunk or a scaffold branch. To reach the site of action, which is the subapical meristem in the shoot, the active ingredient must penetrate the bark and move into the transpiration stream. Bark is a complex tissue system and presents a difficult medium to penetrate because of a layer of cork cells or phellem in the periderm. The phellem consists of dead cells in which the cell walls are impregnated with a water impermeable fatty substance called suberin. Arranged in radial rows that lack intercellular spaces, the suberized phellem in bark presents a barrier to liquid penetration (4,5).

Backhaus et al. (2) used a diffusion chamber to measure the rate and amount of ^{14}C-morphactin movement across excised bark of Monterey pine (*Pinus radiata*) and English walnut (*Juglans regia*). The objective of this study was to use diffusion chambers to compare the effectiveness, i.e., the amount and rate of ^{14}C-flurprimidol diffusion through excised bark, of eight oil-based solvents as carriers for bark applied flurprimidol. Six of these oil carriers have been shown to be effective in moving TGRs across the periderm, cortex, and phloem to the xylem (3), but the amount of active ingredient that reached the xylem was not measured.

Materials and Methods

Chemicals. The ^{14}C-flurprimidol was labeled on the carbonyl group (sp. act. 10.5 µCi/mg). The carriers tested were petroleum-based oils: Androc Basal Oil, ArborChem Basal Oil, ArborChem Clean Cut, CWC Hy-Grade I, Exxon Aromatic 200, Exxon Orchex 796, Leffingwell Carrier #9, and N.G. Gilbert Riteway Oil. Isopropyl alcohol was used as a co-solvent to dissolve the flurprimidol in the carriers.

Plant Materials. Circular bark discs were removed from field-grown silver maple (*Acer saccharinum*) with a 1.35 cm diameter cork borer in late spring and early summer. The discs were

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selected from a smooth, blemish free area of a branch. The branch diameter at the point of disc removal was about 10 cm. The average bark thickness was 2.16 mm.

Diffusion Chamber. Diffusion chambers were built according to the specifications described by Backhaus et al. (2). Each chamber consisted of two cylindrical glass tubes, or cells, that suspended a treated bark disc (Figure 1). Each cell had a 1.3 cm OD opening at one end and was closed at the other. The cell to the bark side or treated side of the bark disc was the donor cell and the receiver cell was on the cambial side of the disc (Figure 1, E, F). A small hole at the top of each cell (Figure 1, C) allowed for air movement on the donor side and the addition and removal of deionized water from the receiver side. Each cell was held in place by wooden blocks (Figure 1, A, B) fastened together with stove bolts (Figure 1, D). The discs were fitted so that the cells formed a pressure seal against the bark requiring no further sealing material.

Treatment Preparation and Application. The treatment solution was applied at a rate of 120 grams flurprimidol per liter. A stock (unlabeled) solution was prepared by dissolving 1 g flurprimidol in 3 ml isopropyl alcohol and 5 ml of each carrier. The ^{14}C-flurprimidol (50.8 μCi) was dissolved in 500 μl isopropyl alcohol making a 0.1016 μCi/μl solution. The treatment solution was made of 4 parts (0.125 mg ai/μl) unlabeled and 1 part (0.01 mg ai/μl) labeled solution.

Before treatment application, the bark discs were pressed between the cells sealing the cambial side of the bark to the receiver cell. The cells were then separated to expose the outer bark and 5 μl of treatment solution were applied with a Hamilton microliter syringe. After the diffusion chamber was resealed, 1.5 ml of deionized water was added to the receiver cell. The cut bark edge was covered with vacuum grease to prevent drying. The treatment application was made within one hour after the bark discs were excised.

The eight oil carrier treatments were randomized in a complete block design with four replications per carrier.

Measurement of Radioactivity. Diffusion of flurprimidol across the bark was determined by measuring ^{14}C accumulation in the receiver cell. The contents of the receiver cell were removed with a Pasteur pipet inserted into the small hole atop the receiver cell. This procedure was repeated every 24 hours for nine days and the solution was counted for radioactivity with a Beckman liquid scintillation counter. After each extraction, fresh deionized water was added to the receiver cell via the small hole.

To recover and measure the amount of ^{14}C-flurprimidol remaining in or on the bark, the discs were dried in an oven at 30°C. The dried bark was oxidized in a Packard 307 Sample Oxidizer and the radioactivity measured by liquid scintillation spectrometry. Total dpm accumulation data were subjected to analysis of variance (ANOVA) procedure.

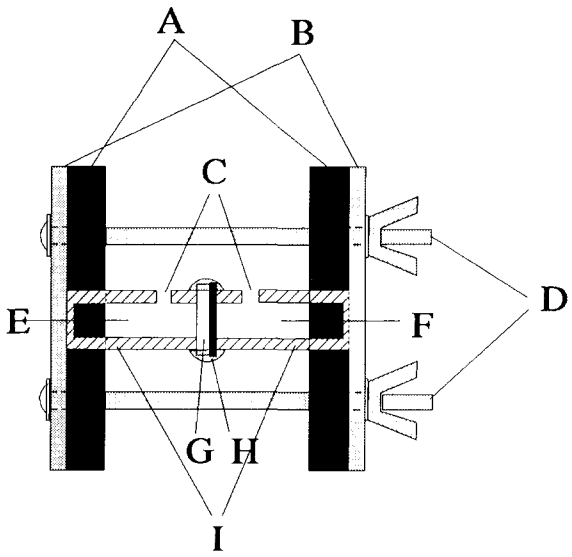

Figure 1. Schematic diagram of a diffusion chamber. A. 7.7 cm x 7.7 cm x 0.6 cm masonite; B. 7.7 cm x 7.7 cm x 0.9 cm plywood; C. air and extraction holes; D. 7.6 cm x 0.54 cm stove bolts, washers and wing nuts; E. receiver cell; F. donor cell; G. bark disc with the receiver to the cambial side and donor cell to the bark side; H. vacuum grease used to prevent drying; I. donor and receiver cells, cylindrical glass tubes, or cells that suspend a treated bark disc. Each cell has a 1.3 cm O.D. opening at one end and is closed at the other (2).

Results

After nine days, the accumulation of ^{14}C-flurprimidol in the receiver cell was similar for each of the oil carriers (Table 1). The greatest accumulation occurred with Leffingwell Carrier #9. Although Leffingwell #9 accumulation was 127 to 490% greater than with the other carriers, high variability between replicated samples resulted in statistically non-significant differences in accumulation between the carriers. Variation in bark properties could account for this variability. Rate of diffusion (dpm/day) of labeled flurprimidol through excised silver maple bark was greatest when dissolved in either Leffingwell Carrier #9 or Arborchem Basal Oil (Figure 2). All carrier/TGR treatments had a similar trend, an initial high rate of penetration through the bark the first 24 hours followed by a drop by the end of day two. With the exception of Androc Basal Oil, there was generally an increase in daily diffusion rates from day two through day eight after which it leveled off.

While radioactivity accumulated in the receiver cell solutions, the greatest amounts of radioactivity were recovered from the bark discs at the end of the treatment period (Table 1). Bark treated with Leffingwell Carrier #9 and Androc Basal Oil displayed the greatest and least accumulation of radioactivity in the bark, respectively. There was variability among the oil carriers in the amount of total radioactivity recovered. Recovery ranged from 52% for Androc Basal Oil to 129% for N.G. Gilbert Riteway Oil, respectively.

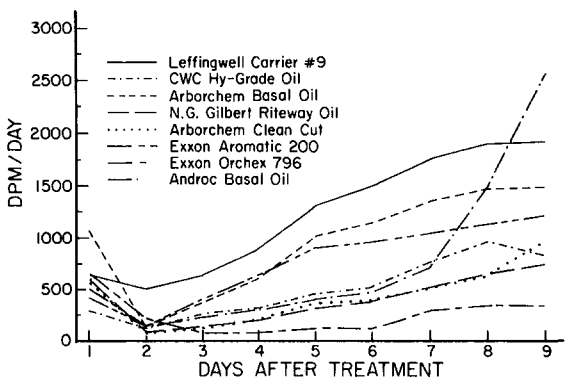

Figure 2. The diffusion rates (disintegrations/minute/day) of ^{14}C-flurprimidol through excised silver maple bark over a 9 day period. Each point represents the mean of four replications.

Table 1. Comparison of total ^{14}C-flurprimidol diffusion, radioactivity in the bark, and percent of total applied radioactivity recovered from excised silver maple bark discs nine days after treatment with ^{14}C-flurprimidol dissolved in each of the eight carriers. Values are the mean of four replications.

Carrier	Total recovered from diffusion chambers	Recovered from bark	Total recovered compared to applied
	(DPM)[1]	(DPM)	(%)
Leffingwell Carrier #9	11,011	269,445	125
Arborchem Basal Oil	8,621	215,971	100
Exxon Aromatic 200	6,826	166,873	74
Androc Basal Oil	6,806	110,331	52
CWC Hy-Grade I	4,526	208,797	95
Arborchem Clean Cut	3,809	204,179	92
N.G. Gilbert Riteway Oil	3,586	287,184	129
Exxon Orchex 796	2,243	267,106	120

[1]Column means are not significantly different at the 95% level.

Discussion

The expected movement of a bark-applied TGR is diffusion through the bark to the xylem, and then translocation to growing points throughout the tree crown. However, there are possible fates that could influence the amount that reaches the site of action. The active ingredient may 1) volatilize and be lost into the atmosphere, 2) remain on the surface of the bark in a viscous liquid or crystalline form, 3) penetrate the suberized phellem cell walls and remain there in solution in the lipoidal layer, 4) penetrate the periderm, cortex, and phloem and be absorbed by the symplast (be phloem-mobile), or 5) penetrate the suberized phellem layer of the periderm, cortex, and phloem, and move in the apoplast with the transportation stream to the growing points in the apex (be xylem-mobile) (1).

Leffingwell Carrier #9 caused the greatest penetration of ^{14}C-flurprimidol over the 9 day period, but the difference from the other carriers was not significant. Leffingwell Carrier #9 was the only carrier tested that was developed specifically for use with a TGR, Maintain CF 125. The others are used in other aspects of vegetation manage-

ment, such as basal and penetrating oils for bark applied herbicide treatments.

Diffusion rate patterns were consistent for each of the eight carriers. The diffusion curve is S-shaped (Figure 2). The influx of radioactivity during the first 24 hours may be due to rapid infusion of flurprimidol through the suberized phellem cells and the larger pore spaces of the cortex and phloem. Typically, after the drop in diffusion rates the second day (48 hours after application), the rate of flurprimidol diffusion increased with time until day eight. At that time the rate levels off, possibly due to breakdown of bark tissue on the receiver side or the tissue drying on the donor side of the bark. Androc Basal Oil was the exception in that the rate of diffusion continued to increase.

Despite a consistent pattern of radiolabel movement through the bark, the greatest amounts of radioactivity were associated with the bark tissue. There did not appear to be an association between the movement of radioactivity into the bark and transit through the bark into the receiver solution. For example, Exxon Aromatic 200 facilitated moderate diffusion through the bark but accumulation in the bark was only 74% of the applied activity. The bark is a complex tissue consisting of several layers, each having different physical and chemical characteristics. It may be that each carrier/TGR combination has a different interaction with the various components of the bark tissue. A carrier (like Leffingwell Carrier #9) may show good movement into and through the bark because it is well balanced in respect to all barriers to movement into and through the bark, while another carrier has limited movement into and/or through the bark because its physical or chemical properties are not well suited to both penetration and movement.

In these experiments we expected to recover 95 to 105% of the total radioactivity that was applied. Judged by the percent of total applied radioactivity recovered, there was both a loss and 20 to 30% over recovery. Swipe tests of the drying oven walls confirmed the loss of radioactivity from the bark surface by volatilization. The apparent loss of radioactivity by volatilization was particularly pronounced for Androc Basal Oil and Exxon Aromatic 200. A review of the physical data for each carrier failed to indicate a correlation between recovery of applied radioactivty and physical properties, e.g., vapor pressure, of the carrier. While a 1 µl application error could account for the 20 to 30% over recovery, it seems unlikely that either an over application or deposition of radioactivity volatilized from bark treated with other carriers would occur so uniformly in replicated samples of three out of the eight carriers. We do not know why more radioactivity than applied was recovered in bark treated with these three carriers.

This research has suggested the complexity of tree bark tissue and the related uncertainty of choosing a carrier to optimize both penetration and movement of flurprimidol. Progress in bark application will come from understanding the interactions between the physical and chemical properties of bark and carriers, and the effect of environmental conditions on the stability of carrier/TGR combinations. In view of the lack of significant differences found in this study, there are a number of oil carriers which are effective for carrying flurprimidol through the bark of silver maple. Some of these oil carriers are already present in the right-of-way management arena as carriers for herbicides applied as basal bark treatments.

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Résumé. Les bandages sur l'écorce sont une alternative à l'injection des troncs d'arbres de régulateurs de croissance. L'objectif de cette étude était de comparer la pénétrabilité de diverses huiles comme véhicule de transport de régulateurs de croissance appliquées sur le tronc. Cinq l de ^{14}C-flurprimidol (225 000 ppm), de flurprimidol non catalogué dissous dans de l'alcool isopropylique et des sélections d'huiles étaient appliqués sur des morceaux d'écorce excisés d'érable argenté (*Acer saccharinum*). Les disques d'écorce, de 13.5 mm de diamètre, étaient scellés dans une chambre de diffusion; les taux et les quantités de ^{14}C-flurprimidol qui traversaient l'écorce étaient mesurés par un spectromètre de scintillement liquide. Le taux de diffusion, mesuré en ppm/jour durant neuf jours, était similaire pour chaque huile. Le patron de pénétration était un flux initial durant les 24 premières heures suivi d'une diminution du taux de pénétration à la fin du second jour avant de reprendre sur un accroissement graduel au cours des sept jours suivants.

Zusammenfassung. Eine Alternative zur Stamminjektion von Baumwachstursregulatoren (TGRs) ist das Binden der Rinde. Die Zielsetzung dieser Studie war, die Durchdringbarkeit verschiedener Öle als Träger von einem TGR zu vergleichen, die auf die Rinde aufgetragen wurden. Fünf ul von ^{14}C-Flurprimidol (225,000 dpm) und nicht näher gekennzeichnetes Flurprimidol, gelöst in Isopropylalkohol, und ausgewählte Öle wurden auf entnommene Rinde vom Silberahorn (*Acer saccharinum*) aufgetragen. Es wurden Rindenscheiben mit 13.5 mm Durchmesser in einer Diffusionskammer versiegelt, und die Rate und Menge von ^{14}C-Flurprimidol, die durch die Rinde drangen mit Leuchtflüssigkeitsspektometrie gemessen. Die Diffusionsrate, gemessen in dpm/Tag über neun Tage, war für jedes Öl gleich. Die Form der Penetration war anfänglich ein gleichmäßiger Fluß in den ersten 24 Studen, gefolgt von einer verminderten Penetrationsrate am Ende des zweiten Tages und einem graduellen Anstieg während der nächsten sieben Tage.