PENETRATION AND TRANSLOCATION OF BAYGON FORMULATIONS IN BALSAM FIR

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by

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INTRODUCTION

The balsam woolly aphid, Adelges piceae (Ratz) is recognized as a serious economic problem in balsam fir stands of the Maritime provinces, Newfoundland and British Columbia (3). Since 1963 the Chemical Control Research Institute has undertaken extensive screening of organic insecticides against this pest (5) under both laboratory (14,19,21) and field conditions (8,9,10,11,12,20,21).

The effectiveness of an insecticide against the balsam woolly aphid presents a difficult toxicological problem due to its feeding behaviour and protective wool mass on its body. Their stylets pass through the epidermis of stem tissue penetrating the parenchyma cells of the cortex where feeding occurs. In younger shoots the phloem is occasionally penetrated (2). Rather than a residual or contact insecticide the most effective means of chemical control may therefore be a systemic insecticide translocated at an effective concentration to the feeding sites.

The insecticide Baygon®, (O-isopropoxyphenyl methylcarbamate), which has been used both as a systemic and residual insecticide, has been found to be potentially useful against several forest insect pests (6,7,15,16,17,18) and for malarial mosquito and blackfly control (27). As a contact insecticide it has been found to be effective against the balsam woolly aphid in laboratory trials (14,19,21) and in controlled field experiments (8,9,10,11,12,20,21). Furthermore, Baygon has been federally approved both in Canada and in the United States for use against household pests (4,25).

We report here some preliminary studies on the foliar application of different formulations of Baygon containing radioactive ^{14}C to assess its potential as a systemic insecticide in the balsam fir. These studies

were carried out on the following aspects:

1. The assimilation of $^{14}CO_2$ by the balsam fir and the movement of ^{14}C metabolites from the leaves in order to assess the rate and direction of compounds moving from the leaf blades during translocation.
2. The volatilization of Baygon from different formulations after application to an exposed surface simulating the plant cuticle.
3. The penetration of ^{14}C-Baygon into the leaf blades of the balsam fir after application in a variety of ways with different formulations and its translocation to other regions of the plant.
4. A bioassay using the balsam fir sawfly feeding on different regions of the plant to determine whether Baygon had been translocated in effective concentrations.

MATERIAL AND METHODS

(i) Plant material

Approximately one foot high balsam fir trees were collected in mid-May from the Ontario Ministry of Natural Resources Nursery at Kemptville. The young trees were transferred to 8" clay pots and maintained outside prior to use. During experimentation the trees were usually maintained in a greenhouse between 60-75^{o}F.

(ii) Baygon formulations

97% technical grade Baygon was supplied by Chemagro Corporation, Kansas City, Mo. A variety of formulations were made from this material that included:

(a) 1% Baygon Emulsion (BE) prepared by dissolving Baygon in a solvent mixture of xylene: AR60: acetone: Tween 80 (4:2:3:1).

(b) 1% Baygon solution (BGD) prepared by dissolving Baygon in 80%

ethylene glycol and 20% Dowanol EPH.

(c) 1% Baygon solution (BOL) prepared by dissolving Baygon in 80% ethanol.

Various other commercial and laboratory prepared formulations of Baygon have been used and are described in the text. ^{14}C-Baygon was added to formulations where appropriate from a sample of radioactive Baygon labelled in the isopropoxy position. This radioactive Baygon, dissolved in benzene, was supplied by the Chemagro Corporation. Where required aliquots of radioactive stock solution were evaporated to dryness and redissolved in the Baygon formulations.

(iii) $^{14}CO_2$ assimilation

This was carried out in a fume hood. The plant part to be labelled was surrounded with a polyethylene bag into which 50µC of $^{14}CO_2$ gas was pumped from a 50 cc hypodermic syringe in which conc. H_2SO_4 had been added to a solution of $Na_2\ ^{14}CO_3$ (98% ^{14}C). The $^{14}CO_2$ was assimilated for 5 minutes under an illumination of 20,000 lux supplied by water filtered incandescent lamps. The remaining part of the plant was covered with Al foil to prevent photoassimilation of any $^{14}CO_2$ which may have leaked from the bay (26). After the assimilation period the bag was removed and the plant taken to an isolated greenhouse and permitted to translocate for periods of 1 or 24 hours before being analysed for ^{14}C distribution.

(iv) Application of ^{14}C-Baygon

Radioactive Baygon in its various formulations was applied to leaves and stems by either brush painting using a very fine camel hair brush, or was layered onto the leaf surfaces by means of a micropipette.

(v) ^{14}C analyses

Following $^{14}CO_2$ assimilation and translocation the ethanol soluble compounds were extracted by rapidly freezing the plant tissue in liquid N_2, grinding to a fine powder, and extracting in 80% ethanol at 70°F for 2 hours (26). The extract was filtered and evaporated down to a small volume. The distribution of radioactivity in the ethanol soluble fraction was determined by 2 dimensional chromatography on Whatmann 3MM paper with butyl acetate: acetic acid: water (3:3:1) and pyridine: 28% ammonia sp g 0.90: isobutyl alcohol (4:2:1). The developed chromatograms were exposed to Kodak No. Screen X-ray films. Labelled compounds with Rf values corresponding to sugars were eluted into 50% alcohol and rerun in n-butanol: pyridine: H_2O (6:4:3) on Whatmann 3MM paper for further identification. Peaks of radioactivity were recorded using a Packard 4M strip scanner and their positions compared to that of known sugars visualized with an alkaline $AgNO_3$ reagent. Following ^{14}C-Baygon application to the plant the lipid soluble material was extracted in either acetone or chloroform after liquid N_2 freezing and grinding of the tissue. Other radioactive analyses were carried out with a Packard Tricarb scintillation counter, Model 3240, using either pure toluene with 0.5% PPO or a methyl hydrate: toluene: PPO (300 ml: 1700 ml: 10g) mixture. Corrections for quenching were made using an external γ source. Leaf autoradiography was accomplished by covering the branches with saran wrap and carefully placing this on Kodak No Screen X-ray plates. The plates were exposed to radioactive plant tissue for one month at -40°C in special light tight covers before development. A single cover of saran wrap was sufficient to prevent chemical artifacts occurring on the plates during processing.

(vi) Bioassay for Baygon

The balsam fir sawfly, Neodiprion abietis (Harris) larvae were collected from a moderately infested balsam fir stand on Highway 27 between Almonte and Arnprior, Ontario. Prior to use the larvae were starved for 24 hours at 45°F. The bioassay was carried out in a standard manner with plant segments containing Baygon or its metabolized derivatives fed to 10 prestarved larvae. The feeding larvae were left in jelly jars at room temperature during the experimental period. Mortality was recorded with time. Controls were fed on untreated branches. All experiments were replicated three times.

RESULTS

(i) Translocation of ^{14}C metabolites

Following a 5 minute period of $^{14}CO_2$ assimilation by a region of new or previous years growth, plants were allowed to metabolize and translocate the assimilated ^{14}C for a further 1 hour or 24 hours. Each plant was then divided into a number of parts and the ethanol soluble fraction of each analysed for radioactive compounds. The distribution of ^{14}C on a percentage basis with time among the plant parts is shown in figures 1-6. Values less than 1% have not been included although traces of activity were measured in regions of the stem a considerable distance from the site of assimilations but their significance has yet to be established. Clearly under these experimental conditions the rate of transport of ^{14}C metabolites in the phloem of the balsam fir is very slow. Chromatography revealed sucrose with some glucose and fructose to be the main ^{14}C labelled compounds translocated.

(ii) Evaporation of ^{14}C-Baygon from a paraffin surface

Formulations BE, BGD, and BOL were prepared containing ^{14}C-Baygon. Aliquots of these formulations were initially radioassayed with the scintillation spectrometer. Similar volumes were applied within circles, ca 2.5 cms diameter, drawn with a wax pencil upon a strip of parafilm. The strip was taped onto a plywood board and maintained under typical light and temperature conditions obtained in the greenhouse. Sampling was carried out at 8, 16, 24 and 48 hours after application. The circles were cut out with a razor blade and each circle extracted in 5.0 ml acetone in a small tube. The tubes were shaken for 2 hours in a gyratory shaker, then allowed to stand overnight. Aliquots of the acetone extracts were radioassayed in the scintillation counter. Each time interval per formulation was triplicated. Figure 7 shows the results where the recovery of radioactivity in the acetone extract is expressed as a percentage of the initial value. This study shows that the evaporational loss of Baygon can be significant. Using the BOL formulation 50% loss was found ca 3 hours after application to the parafilm surface. The BE formulation required ca 48 hours to achieve a similar loss while the BGD formulation by contrast showed a minimal loss (only 16% at 48 hours).

(iii) Penetration of Baygon into excised balsam fir needles

It was considered that an initial appraisal of Baygon penetration may be obtained through a simple study of excised balsam fir needles completely immersed in different formulations of Baygon. Using ^{14}C-Baygon, penetration rates could be readily assayed following acetone extraction of the needles. Studies of cuticular penetration in dicotyledenous plants have indicated that urea, sucrose and dimethyl sulphoxide (DMSO) can stimulate rates of penetration (22).

The following formulations of Baygon were prepared:

1) 0.1% Baygon / 5% sucrose in distilled H_2O
2) 0.1% Baygon / 5% glucose in distilled H_2O
3) 0.1% Baygon / 5% urea in distilled H_2O
4) 0.1% Baygon / 0.1% DMSO in distilled H_2O
5) 5% Baygon in BE
6) BGD
7) BGD containing 5% sucrose
8) 0.1% Baygon containing 10mM urea and 5% sucrose in distilled H_2O

To each of the above formulations ^{14}C-Baygon was added and the solutions radioassayed in the scintillation spectrometer for initial radioactivity. Two and a half ml of each formulation, in triplicate, were placed in test tubes and to each tube 100 mature balsam fir needles were added. The tubes were left at ca 70-75°F in the greenhouse and gently shaken. At 8, 16, 24 and 48 hours after commencement of the experiment 25 needles from each tube were taken for analysis. The needles were surface rinsed with acetone for approximately two minutes. They were then dried, frozen in liquid N_2, ground to a fine powder and extracted with acetone for 24 hours at room temperature. The extract was filtered and 50 µl aliquots taken for radioactive assay in the scintillation spectrometer. The total radioactivity in the extracts was finally expressed as a percentage of the activity initially present in the formulation. The results are plotted in figure 8. It was noted that formulations of 5% Baygon in BE, BGD and BGD containing 5% sucrose caused discolouration and eventual bleaching of the needles. These particular formulations were clearly phytotoxic and also poor media for Baygon penetration.

applied to the leaves of a number of plants as detailed in the previous experiment. At 24, 48 and 72 hours after application samples of the branches including their attached leaves were removed, surface washed in acetone, frozen in liquid N_2 ground to a fine powder and extracted in chloroform. Branch samples were taken both above and below as well as at the site of Baygon application. The chloroform extracts were radioassayed with the scintillation spectrometer and the results expressed as a percentage of the initial application. The results are shown in Table 1.

(vii) Bioassay of Baygon movement

While the ^{14}C data above indicated some movement of radioactive compounds from the sites of Baygon application it does not differentiate between radioactivity in Baygon and its metabolically derived products. It does not therefore indicate how much of the transported material remains toxic. A bioassay using the balsam fir sawfly was therefore set up to complement the ^{14}C studies. Two formulations of Baygon were prepared viz, (1) 5% Baygon in BE and (2) a 5% Baygon solution diluted with H_2O from the Chemagro 17% Baygon commercial emulsifiable concentrate. Suitable young trees were selected and brought into the greenhouse. For each formulation nine trees were used and 10 µl aliquots of the formulation were applied to each needle surface along a 10 mm length of mid stem. Bioassays were commenced at 1 (24 hr), 2 (48 hr) and 3 (72 hr) days after the Baygon application. Three treated branches were cut off per formulation at each assay time and each branch divided into three sections:

1. the site of application plus 10 mm on either side
2. a section of stem above the site of application
3. a section of stem below the site of application.

The sawfly larvae were permitted to feed on these segments and their mortality rate assessed. Mortality counts were recorded at periods of 60, 90 and 120 minutes for these larvae feeding on segments at the site of Baygon application. Mortality counts on segments above and below the site of application were recorded at 24, 48 and 72 hours, after feeding had begun. Controls consisting of three 10 larvae samples feeding on untreated branches were included. The results shown in Fig. 13 are for the residual toxicity at the site of application. Above and below the site of application toxicity was found to be highly erratic and the results not yet sufficiently significant to warrant a clear assessment of Baygon transport.

DISCUSSION

The translocation experiments have clearly demonstrated that during early summer sucrose and possibly hexose sugars, derived from photosynthesis, move through the phloem of the balsam fir both from the most recently matured needles and from needles developed in previous years. However, and as is generally experienced amongst coniferous species, the rate of this sugar movement is slow. From this evidence any insecticide applied to the balsam fir needle that could penetrate the thick cuticle then move to the vein loading sites and gain entry into the phloem with little or no detoxification would act as an effective systemic insecticide especially against phloem feeding insects. However, because of the feeding habit of the balsam woolly aphid the effectiveness of such a systemic insecticide would have to depend on its further transport out of the phloem into the cortical tissue surrounding the vascular channels. Our limited knowledge of this

type of movement in plants suggests that it is facilitated by the cytoplasmic continuity between adjacent cells and is considerably less rapid than phloem transport and furthermore would expose the insecticide to increased metabolic hazards.

One of the major problems of Baygon application to leaf blades appears to be its apparent volatility. However, in these experiments the compound was labelled in the isopropoxy group and the loss may reflect Baygon degradation with the subsequent loss of the isopropoxy group as acetone (24). The rate of evaporational loss can be restrained by less volatile solvent components and varieties of formulations can be made that will retain Baygon on the leaf surface well in excess of 48 hours. While no phytotoxicity was immediately apparent when any of the formulations were applied to the needle surfaces those experiments carried out with excised needles clearly demonstrated that photosynthetic capacity and probably other metabolic systems will be damaged if there is a too liberal application or a too prolonged exposure to certain of those formulations using an 'oil' base to counteract the rapid evaporation of Baygon. From these initial experiments it would appear that a formulation using a water base containing certain 'metabolic' additives such as sucrose or urea will produce a more efficient penetration compared to field formulations using an 'oil' base. That sugars, urea and DMSO were able to enhance penetration does suggest evidence for a metabolic component controlling penetration. This has been reported previously for dicotyledonous plants (22) and is an interesting area that requires further investigation.

Both the autoradiography and the ^{14}C analysis of tissue extracts indicate that Baygon and/or some metabolically derived products can

penetrate the thick cuticles of the fir needles and move away from the application sites. Movement however appears to be very feeble in extent and the movement of 'toxicity' remains to be determined. At present the bioassay remains equivocal on this matter. The ^{14}C analyses may very well be measurements of detoxified metabolic products containing ^{14}C or water soluble conjugates and not Baygon (1, 23). Further studies will have to concentrate on this aspect of the problem. The contrast between ^{14}C movement derived from Baygon application and the movement of ^{14}C metabolites derived from photosynthesis would suggest that Baygon is not readily moved into the phloem system of the fir needles and movement may be one of slow diffusion aided by cytoplasmic streaming between cells, precluding Baygon as an effective systemic agent for the phloem system. In view of the apparent difficulties of phloem penetration it would perhaps be more advisable to attempt Baygon entry either through the root system or into the lower stem where it may move more readily into the water conducting channels of the xylem.

These preliminary studies therefore indicate that the following lines of work should be pursued to prove whether Baygon can be considered as a useful systemic insecticide against the balsam woolly aphid:

1. To improve the formulation for effective penetration and low evaporation or surface degradation.
2. To investigate the metabolic stability of Baygon and any detoxified conjugates.
3. To investigate its systemic potential for distribution in the xylem.
4. To determine the relationship between movement, accumulation and toxicity using the bioassay technique.

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Figures 1 to 6

% Distribution of Radioactivity (values greater than 1%)

Fig. 1. 1 hour after ^{14}C application

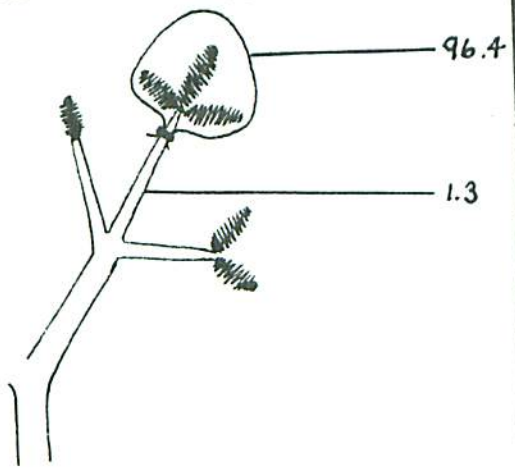


Fig. 2. 24 hours after ^{14}C application

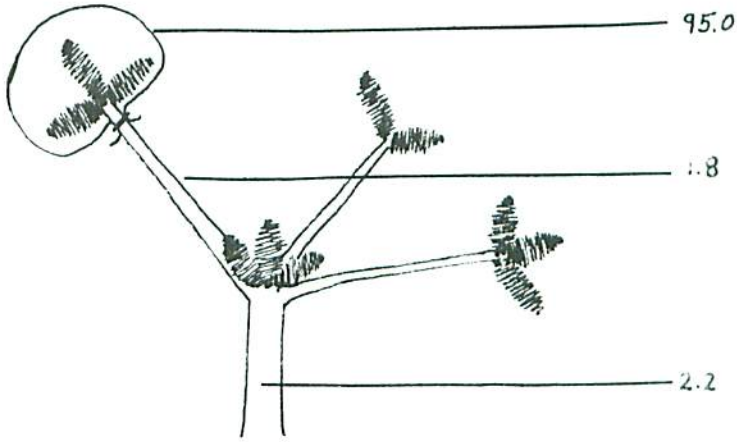


Fig. 3. 1 hour after ^{14}C application

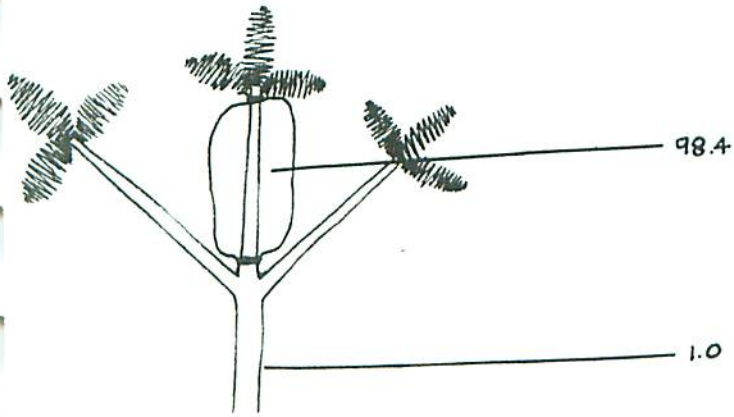


Fig. 4. 24 hours after ^{14}C application

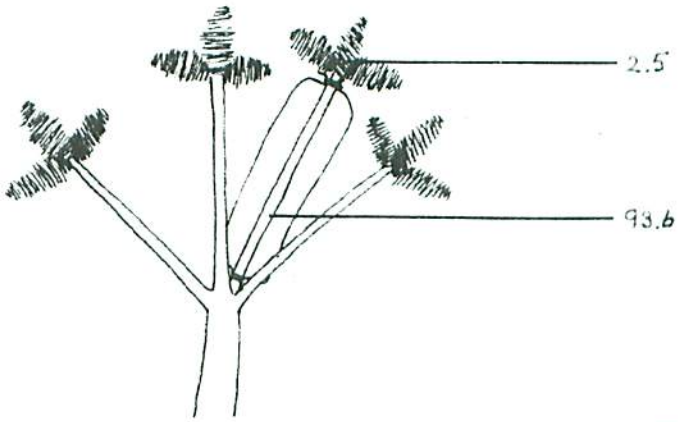


Fig. 5. 24 hours after ^{14}C application

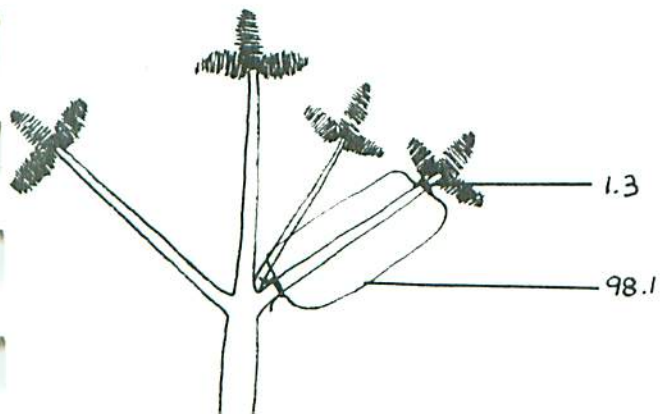


Fig. 6. 24 hours after ^{14}C application

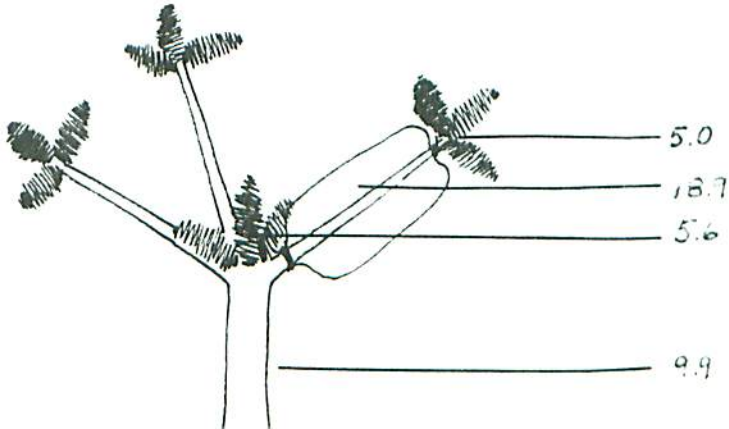


FIG. 7 EVAPORATION OF BAYGON C^{14} FROM A PARAFILM SURFACE

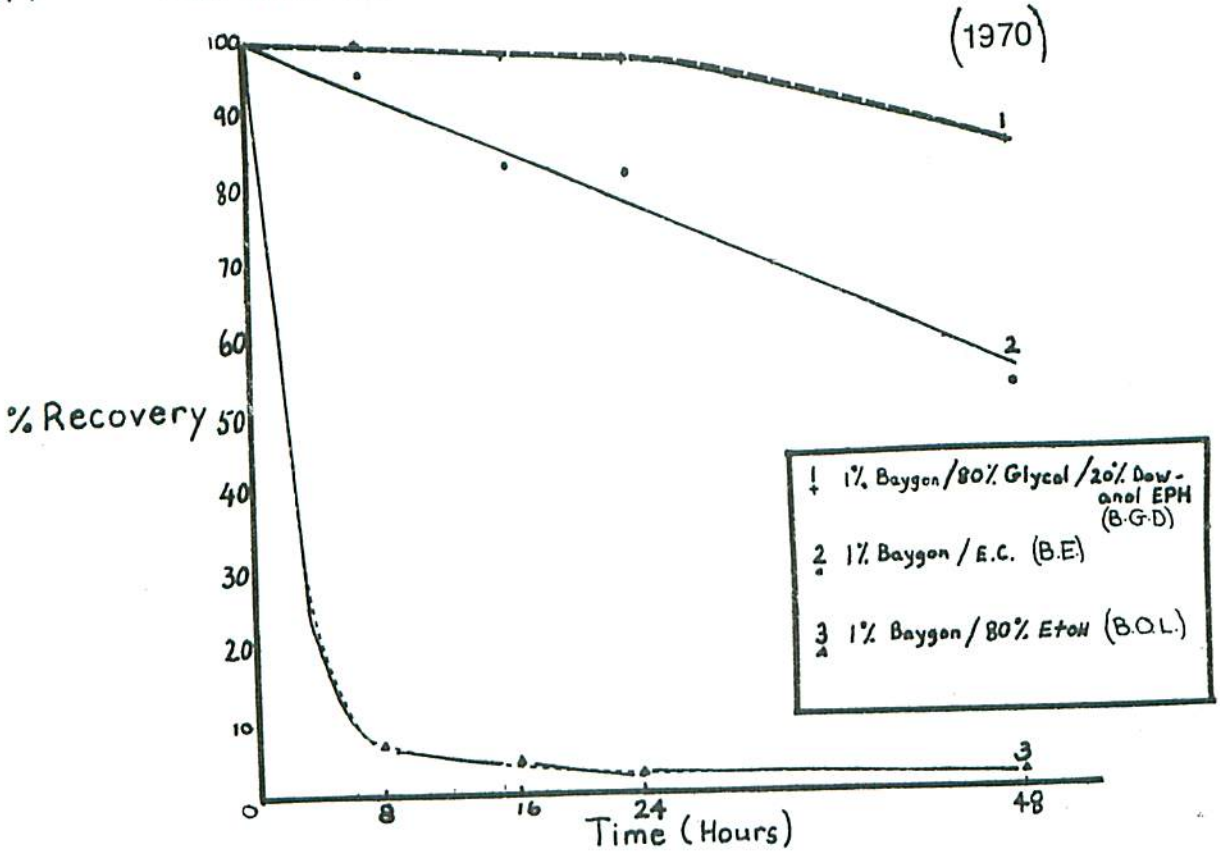


FIG. 8 IN VITRO PENETRATION OF BAYGON C^{14} IN VARIOUS FORMULATIONS IN EXCISED BALSAM FIR NEEDLES (1970)

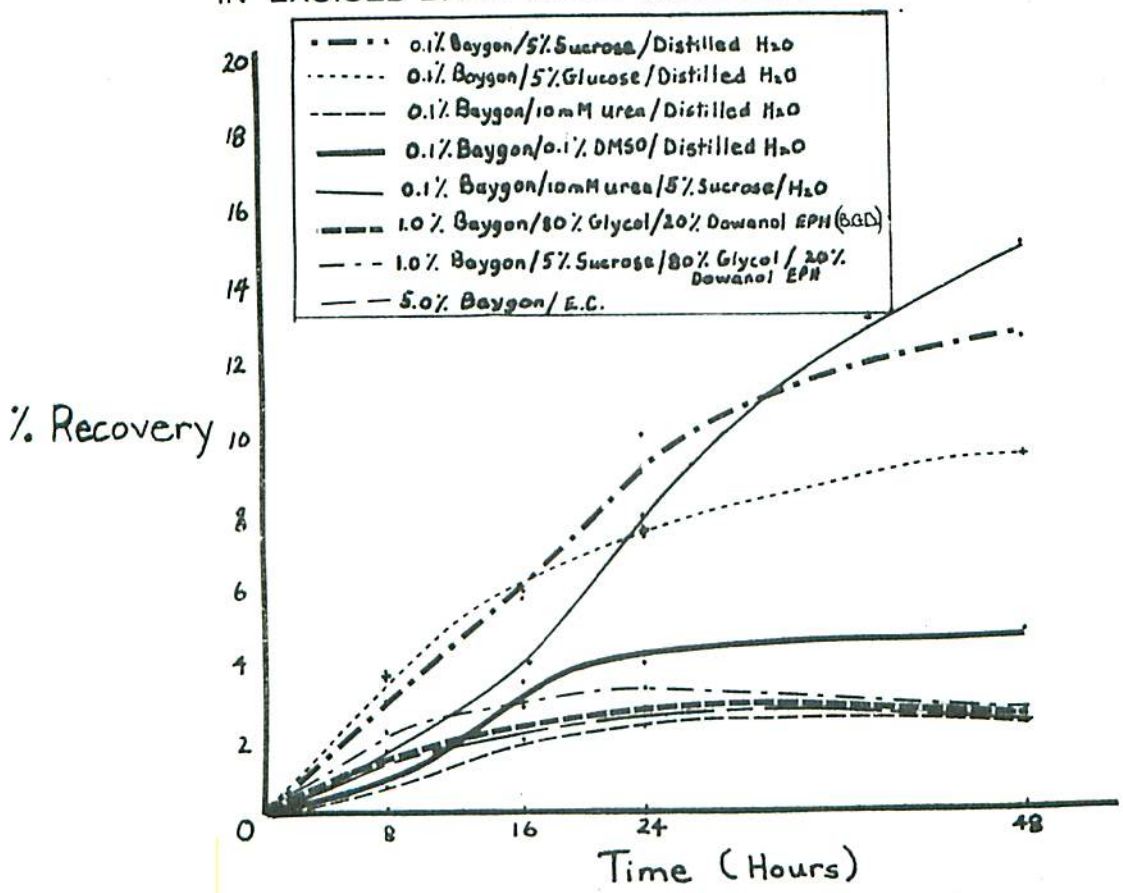
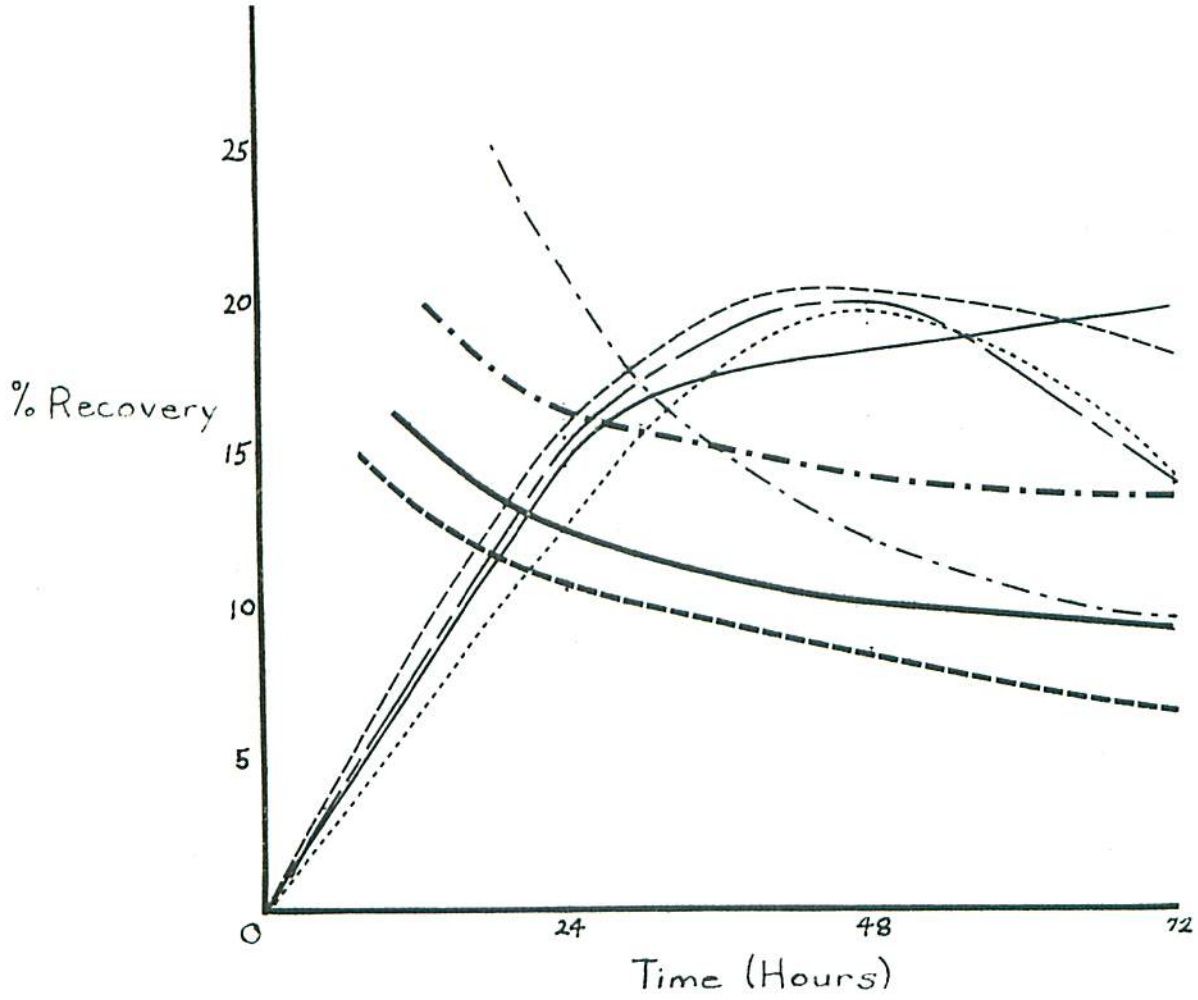
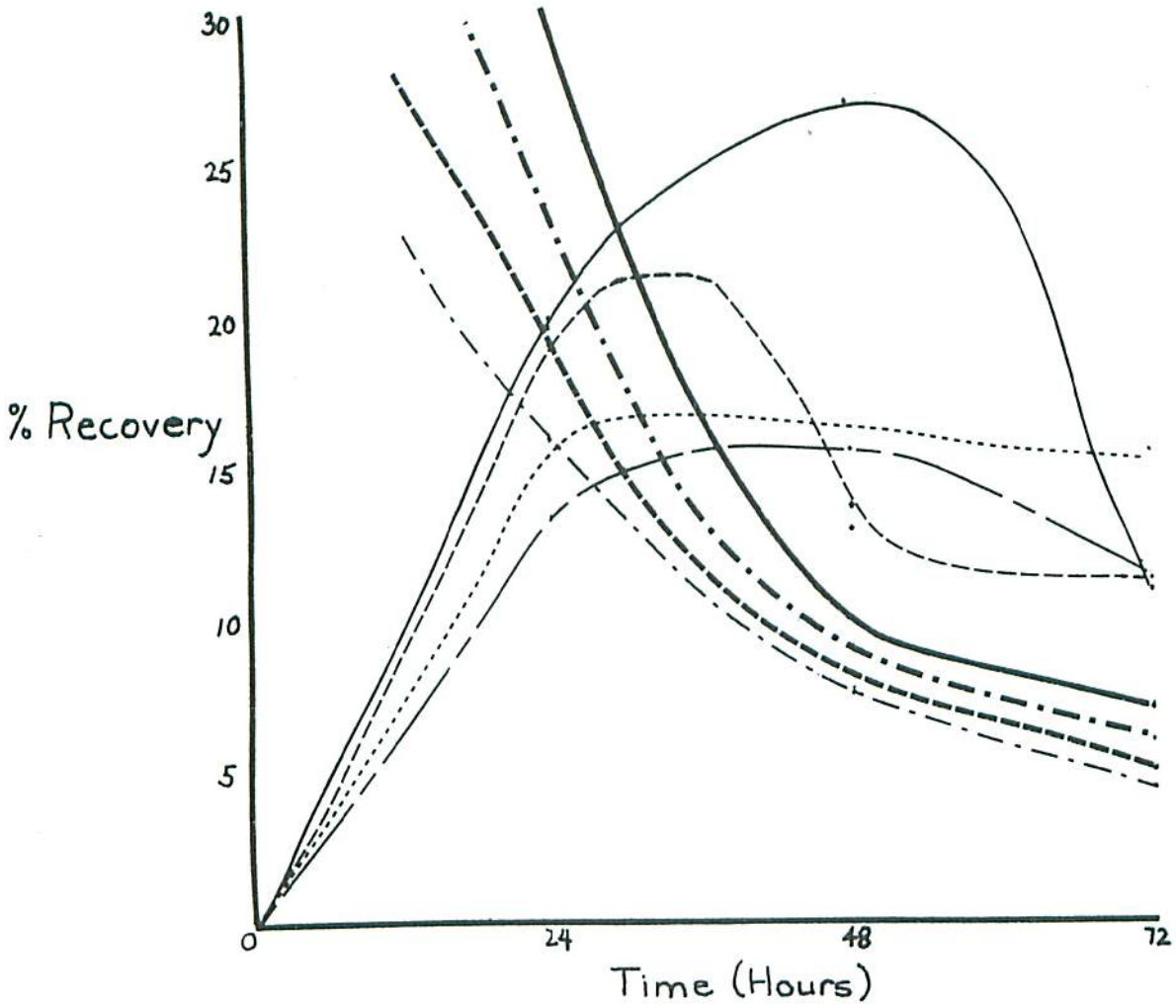


FIG. 9 IN VIVO PENETRATION OF BAYGON C^{14} USING A 13.9% BAYGON COMMERCIAL FORMULATED E.C.



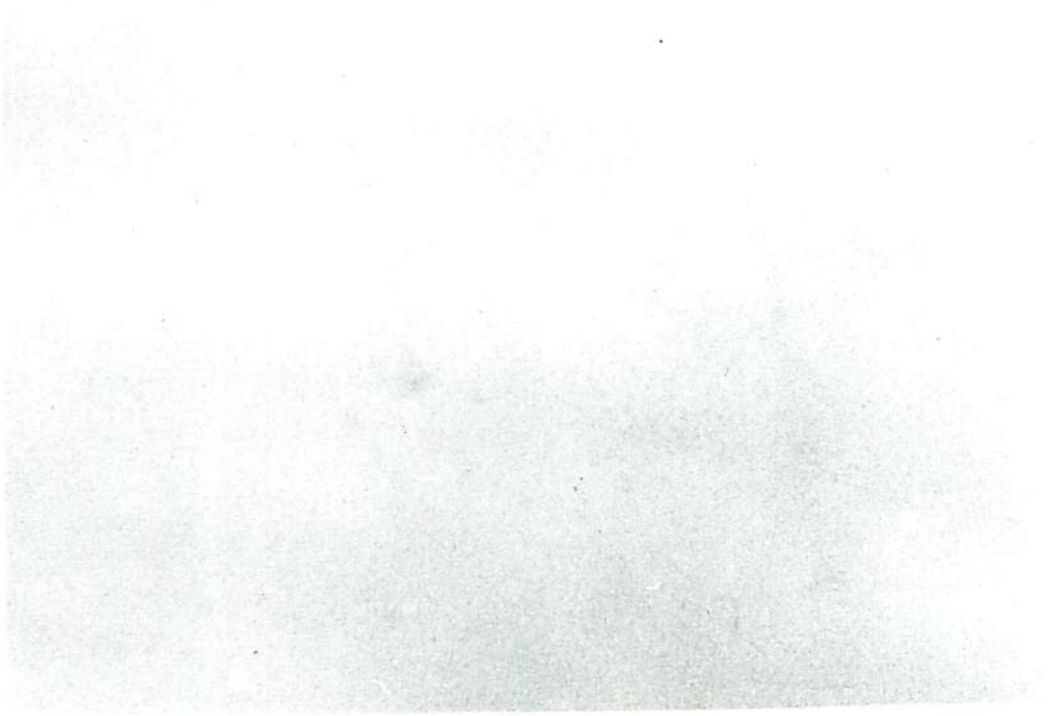
Acetone Rinse	Acetone Extract
1% Baygon	1% Baygon
1% Baygon /5% Sucrose	1% Baygon /5% Sucrose
1% Baygon /2.5% Urea 2.5% Sucrose	1% Baygon /2.5% Urea 2.5% Sucrose
1% Baygon /5% Urea	1% Baygon /5% Urea

FIGURE 10. IN VIVO PENETRATION OF BAYGON C^{14} USING A 5% LABORATORY FORMULATION (1970).



Acetone Rinse	Acetone extract
1% Baygon	1% Baygon
1% Baygon/5% Sucrose	1% Baygon/5% Sucrose
1% Baygon/2.5% Sucrose 2.5% Urea	1% Baygon/2.5% Sucrose 2.5% Urea
1% Baygon/5% Urea	1% Baygon/5% Urea

FIGURE 11.



AUTORADIOGRAPH OF BRANCHES AFTER ACETONE RINSING;
APPLICATION OF 5% BAYGON PREPARED FROM 17% BAYGON E.C.

FIGURE 12.

AUTORADIOGRAPH OF BRANCHES AFTER ACETONE RINSING;
APPLICATION OF 5% BAYGON PREPARED IN UNDILUTED LAB

Figure 13.

BIOASSAY OF RESIDUAL TOXICITY

A

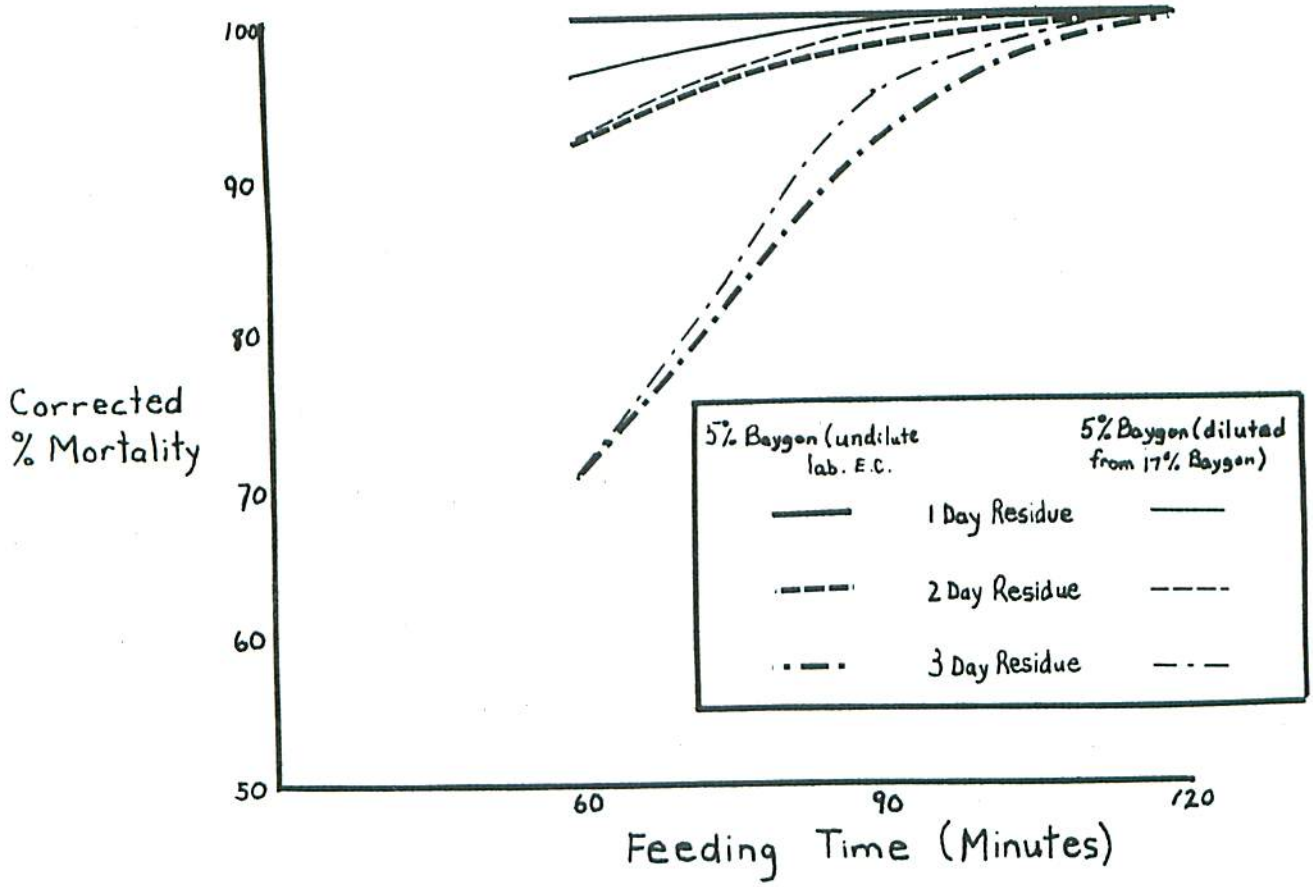


TABLE 1

QUANTITATIVE MEASUREMENT
OF
MOVEMENT OF BAYGON C_{14}

Formulation	Days After Application	% Recovery			
		Acetone Rinse	Chloroform extract		
			Above Site of Application	At Site of Application	Below Site of Application
5% Baygon (undilute lab prepared E.C.)	1	36.6	0.4	6.5	0.4
	2	23.4	0.2	7.8	0.7
	3	18.5	0.2	8.0	0.4
5% Baygon (diluted from commercial 17% Baygon)	1	84.6	0.3	0.2	1.0
	2	83.9	0.6	0.1	0.4
	3	75.0	0.8	0.6	0.2

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