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ELEMENTAL MARKING IN DECIDUOUS AND CONIFEROUS TREE SYSTEMS

S. J. Fleischer[1], J. R. Bridges[2], F. W. Ravlin, and W. T. Thoeny[3]

Department of Entomology
VPI & SU
Blacksburg, VA 24061

ABSTRACT

Systemic introduction of Rb into trees as a means of marking insects has been shown to be feasible in two coniferous and two deciduous tree species. Relatively simple application technologies using low rates have proven successful. Injections of solutions, as opposed to implants of dry material, resulted in more rapid uptake and less injury in the tree. Both apoplastic, symplastic and lateral transport occurs within the tree. Distribution within trees varied with tree tissue, height and aspect and was correlated to K levels. Trace element concentration in tree and insect tissue was often directly proportional to application rate. There is a potential of recycling of the Rb within the tree from one year to the next. Insects that have been marked include foliage, fruit, and phloem feeders, and predators and parasitoids.

INTRODUCTION

Elemental enrichment as a technique for marking insects was first demonstrated in the early 1970s (Berry et al. 1972, Stimmann 1974), but the technique was not used in trees until 1984 (Payne and Wood 1984). Subsequently, insects associated with both deciduous and coniferous trees in forest and orchard systems have been labelled using tree injection or implantation. Insect feeding guilds that have been targeted include leaf, phloem, and fruit-feeders. The ability of the mark to be transferred to the F1 generation, and to transfer the mark to predators and parasitoids, has been demonstrated. This review will emphasize adaptations of the elemental enrichment technology for field use in tree systems, and point out significant contributions to the development of the technology that have come from this research.

CHEMOPRINTING

One of the early developments of elemental marks in arthropods associated with trees did not include the idea of enrichment of the elemental concentration at all. Rather, the concept of a "biofingerprint", or "chemoprint", was developed for the western spruce budworm, *Choristoneura occidentalis* Freeman (McLean et al. 1979, 1983). The underlying assumption is that the chemical composition of the insect reflects that of its host plant, and indirectly the soil and parental geologic material. Chemoprints of western spruce budworm developing in two Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco, stands 2.6 km apart were sufficiently different to allow categorization of host location (McLean et al. 1979).

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[1] Current address: Dept. Entomology, The Pennsylvania State University, University Park, PA 16802

[2] USDA - Forest Service, FIDR, Rm 609-RPE, PO Box 96090, Washington, DC 20090-6090

[3] USDA - Forest Service, Southern Forest Experiment Station, 2500 Shreveport Hwy., Pineville, LA 71360

Discriminate analyses also were used to distinguish elemental profiles of western spruce budworms that developed on Douglas-fir, Engelmann spruce, *Picea engelmannii* Parry, and grand fir, *Abies grandis* (Dougl.) Lindl., in the laboratory (McLean et al., 1983).

Problems with the underlying hypothesis and field use of chemoprinting were presented by McLean and Laks (1985). Chemoprinting requires a high heterogeneity of soil types within a study area. Secondly, the assumption of proportional uptake at each trophic level was questioned. Both plants and insects are known to exhibit selective rates of uptake of specific elements, thus changing the relative concentration of elements within their own tissue relative to the nutrients from which they were acquired. Chemoprinting also requires quantitative analysis of several elements, making the logistics relatively difficult.

ELEMENTAL ENRICHMENT: APPLICATION TECHNOLOGY

Selectively increasing the concentration of a single trace element in trees for the purpose of labelling insects was first documented in the middle 1980s in pecans, *Carya illinoensis* (Wangenh.) K. Koch, and Douglas-fir (Payne and Wood 1984, McLean and Laks 1985). Foliar sprays of Rb onto 4-year old Douglas-fir transplants (McLean and Laks 1985) did not preform as well as systemic stem-well introductions (an infusion technique as defined in Stipes and Campana 1986). Sprays of 1000 to 10000 ppm Rb, but not 100 ppm Rb, successfully marked feeding western spruce budworms, but most of the Rb was removed by rinsing the foliage. All other work with trees has used some variation of injection or implantation, as discussed in Stipes and Campana (1986), Dutcher et al. (1985), or Phair and Ellmore (1984). Recent techniques have adapted pressurized syringes (Mauget^R injectors) or implants of the crystalline formulation (Medicap^R implants) similar to those described in Reardon (1984).

The largest trees into which Rb has been introduced are pecans. Payne and Wood (1984) used low-pressure trunk injection of mature nut-bearing trees (ca. 18 m tall, 75 cm dbh, 60 years old) at the time of pecan shell hardening. Rates were 0, 100 and 200 g RbCl per tree. Levels in pecan foliage, shucks and kernels were significantly elevated. Bracketing these rates, Fleischer et al. (1989) injected pin oak, *Quercus palustris* Muenchh., trees with 0, 100, 200 and 500 g RbCl per tree. To help ensure good distribution they used flare root injection (Stipes and Campana 1986). Also, a relatively large diluent volume (6 liters) was chosen to avoid osmotic injury to phloem tissue at the site of injection. Foliar Rb concentrations were significantly related to injection dosage and time since injection.

McLean and Tuytel (1988) successfully used the Mauget^R injection and Medicap^R implant systems to introduce relatively low Rb rates (up to ca. 50 g RbCl per tree) into 25 year old Douglas-fir trees. Maugets^R injectors, which inject a liquid, generally resulted in higher foliar Rb levels than did implantation of solids using Medicaps^R. Similar adaptations have been successful for the gypsy moth, *Lymantria dispar* (L.), - oak system (Fleischer et al. 1990). Rubidium was introduced into pin oak trees (ca. 16 years old, 14.7 ± 2.5 cm dbh, ca. 12 m tall) using four techniques at two rates (25 or 50 g of RbCl). The four techniques were: (1) flare root injection of a 2 liter solution with a small CO_2-pressurized cylinder, which is an adaptation from the method used by Payne and Wood (1984); (2) flare root injection of a 10 ml solution using a syringe; (3) bole injection of a 10 ml solution using a syringe; and (4) flare root implantation with 3 g of solid RbCl per port. Syringe injections were drawn from a stock solution of 5 g RbCl per 10 ml. The number of ports into the tree was varied in methods 2 through 4 to achieve the desired rate (25 or 50 g RbCl). Foliar Rb concentrations were generally higher from the 50-g compared to the 25-g treatment in all but the flare root injection treatments, where the rate of Rb application appeared to have little effect. Introduction of solutions showed a more rapid increase in foliar Rb concentration than implantation of a solid, which supports McLean and Tuytel's (1988) conclusions. Syringe injections resulted in higher foliar Rb concentrations than the CO_2-pressurized cylinder or the implants.

Techniques have been developed for injecting both Rb and Sr into loblolly pine, *Pinus taeda* L., for marking southern pine beetle, *Dendroctonus frontalis* Zimm. Bridges et al. (1989) used a low-pressure trunk injection technique similar to one developed by Phair and Ellmore (1984) to apply Rb and Sr to 25-cm dbh loblolly pines. The injectors delivered solutions of RbCl and $SrCl_2$ into the xylem through holes drilled into the first 3-4 growth rings. Injection ports were spaced about 5 cm apart around the bole at ca. 60 cm above the ground. To determine the amount of Rb or Sr required to mark southern pine beetles, they injected loblolly pines (25 cm dbh) with 0, 5, 10, 20, 30, or 50 g of RbCl and $SrCl_2$ in 150 ml of water. The amount of Rb and Sr in tree tissues was proportional to the amount injected per tree. Southern pine beetles have been successfully marked in loblolly pines by applying a solution of RbCl to shallow cuts in the bole made with an axe (Thoeny et al. unpublished data).

Rb UPTAKE AND DISTRIBUTION WITHIN TREES

Work with elemental enrichment in trees has required information on within-tree uptake and distribution. Uptake of the trace element over time has been traced in pin oaks, loblolly pines and Douglas-fir. McLean (1988) concluded that there was a steady increase over time in new and old Douglas-fir foliage. Fleischer et al. (1989) fit linear regression models to oak foliar Rb levels as a function of application rate (0 to 500 g RbCl per tree) for different times. Uptake rate was most rapid within a few days of injection, and stabilized thereafter. In loblolly pine trees, weekly sampling indicated elevated levels of Rb in xylem and inner bark tissues one week after injection (Bridges et al. 1989). Concentrations of Rb in xylem remained fairly constant through the 15 week sampling period. In inner bark tissue, there was a general trend of increasing concentrations for the first 5 weeks followed by a gradual decline. Concentrations of Rb in outer xylem and inner bark were linearly proportional to the rate of Rb application in loblolly pines (Bridges et al. 1988) and in pin oaks five months after Rb introduction (Fleischer et al. 1988). McLean and Tuytel (1988) also demonstrated appreciable Rb concentrations in both phloem (i.e., inner bark) and outer sapwood of Douglas-fir trees. Transport in plants can occur in the transpiration stream (apoplastic transport, movement primarily in dead tissue) or in the phloem (symplastic transport, transport through the living cells). Rubidium is highly mobile in phloem tissue, and lateral movement of minerals between xylem and phloem has been documented (Kramer and Kozlowski 1979). The rapid uptake of the Rb into leaves soon after introduction coupled with documentation of Rb in inner bark (phloem), especially long after introduction, illustrates that Rb is moved by both transport mechanisms.

Bridges et al. (1989) also concluded that lateral movement of Rb from xylem to phloem occurred in loblolly pine trees, as illustrated by the simultaneous occurrence of elevated levels of Rb in phloem and xylem. Concentrations of Rb were higher in phloem than xylem (McLean and Tuytel 1988, Bridges et al. 1989). Bridges et al. (1989) found that ratios of Rb/K in phloem and corresponding xylem tissues were highly correlated. They concluded that the higher levels of Rb in the inner bark compared to the xylem were due to a greater concentration of K in inner bark.

Recent studies have shown that Rb is not uniformly distributed even within the same tree tissue. Phloem concentrations were generally greater in the upper portions of Douglas-fir trees. Significant differences due to height in foliar Rb concentrations also were present and varied with the technique of Rb introduction. Aspect (compass direction) affected concentrations at low Rb application rates for foliar Rb levels and at all rates for phloem Rb levels (McLean and Tuytel 1988). In pin oaks, there was a higher rate of foliar Rb accumulation in the top and middle strata, as compared with the bottom strata. Variation among leaves not accounted for by Rb application rate was higher in the lower strata (i.e., the unexplained variation in a regression model where application rate was the independent variable was higher from leaf samples at lower strata) (Fleischer et al. 1989). Bridges et al. (1989) found discernable channels of high inner bark Rb and Sr concentrations above the injection ports of loblolly pines. These channels had a slight spiral ascent as might be expected for apoplastic transport in pines (Rudinsky and Vite 1959). However, Rb concentrations became more evenly distributed within a few meters of the injection ports. In the pecan fruit, exocarp tissue held higher Rb concentrations than endocarp tissue (Payne and Wood 1984).

TRANSFER AMONG YEARS IN THE FIRST TROPHIC LEVEL

Trees are relatively long-lived compared to herbaceous species, and nutrients may be cycled in long-lived systems. Rubidium has been used in a "reverse tracer" technique to estimate the retention of potassium in forest systems (Stone 1981). Fleischer et al. (unpublished data) found significantly elevated Rb levels in pin oak foliage injected with 100 to 500 g RbCl in the year following injection. Whether this would hold true at lower injection rates, and the implications of this for using the elemental enrichment technique for tracing insect movement, remains to be determined.

TRANSFER TO INSECTS

Many of the studies that involved Rb introductions into trees reported Rb concentrations in insects collected or reared from the enriched tree tissue. In pecans, larval and adult hickory shuckworms, *Cydia caryana* (Fitch), developing from treated trees carried significantly elevated levels of Rb (Payne and Wood 1984). Larvae whose guts were voided prior to chemical analysis were marked at the 200 g/tree rate, but ca. 20% of those larvae were not marked at the 100 g/tree rate.

Gypsy moths were reared on foliage from trees injected with 100 to 500 g RbCl/tree (Fleischer et al. 1989). Rubidium concentrations in all life stages, and in the frass and pupal exuviae, were directly proportional to the amount injected per tree. Greater than 82% of the moths reared on foliage at the 100 g rate, and greater than 95% of those reared at the higher rates, carried the Rb mark. Rubidium concentrations in gypsy moth larvae, expressed as a linear function of application rate, did not show a change with head capsule width. This suggests that Rb accumulation rate was fairly consistent among larval instars, at least when measured in larvae without voiding the gut. Sex influenced Rb concentrations in the latter life stages, with gypsy moth females retaining more of the pupal Rb than males. Even in the absence of enrichment, Rb concentrations in female adults and pupal exuviae were higher than males. Western spruce budworm females also took up a higher proportion of the Rb available in artificial diet (17 to 32 %) than did males (10 %) (McLean and Laks 1985). This was also true for the tufted apple budmoth, *Platynota idaeusalis* (Walker), where females incorporated 15% and males 10% of Rb in diet (Knight et al. 1989). This sexual dimorphism may be associated with retention or transport of the Rb to the developing eggs.

Elemental concentrations in frass may be useful for understanding utilization. Concentrations in western spruce budworm frass exceeded that in artificial diet (McLean and Laks 1985), and concentrations in gypsy moth frass from high treatment rates were well above concentrations in larvae (Fleischer et al. 1989). These data suggest that excess salts are removed in the frass.

In studies with loblolly pine, the Rb mark was detected in over 86% of the beetles developing in trees injected with 50 g of RbCl, but Sr was not detected in individual beetles (Bridges et al. 1989). Because Sr was not detected in individual beetles, was not as uniformly distributed in the tree, and was more slowly transported through the tree, it was considered less suitable for marking southern pine beetles (Bridges et al. 1989).

TRANSFER TO THE F_1

Strontium has been incorporated as an elemental marker into the larval diet of gypsy moth parentals, with the aim of detecting the larvae and adults of the F_1 used in sterile egg release programs (Tanner et al. 1984, 1985). The element was transferred to the Fı, even using non-toxic concentrations (lower than 0.1% $SrCl_2$). However, rapid elimination in the F_1 larvae when feeding on nonenriched diet prevented the ability to distinguish the mark in latter life stages.

Rubidium has been transferred to F_1 gypsy moths also. Gypsy moths reared on foliage from pin oaks injected at 0 and 500 g RbCl per tree were mated with individuals reared from the same treatment. Eggs from the 500 g treatment held Rb concentrations three orders of magnitude higher than controls (Fleischer et al. 1989). A Rb mark was also easily detected in all individual first instars collected prior to feeding (Fleischer, unpublished data).

In apple, *Malus sylvestris* (L.) Mill., orchards an understanding of movement is useful for developing resistance management programs and mating disruption strategies. Data on tufted apple bud moth movement, however, have been limited to male captures in pheromone traps, while information on female movement has been inferred from infestation data. Sampling for females has not been feasible, although the egg stage can be sampled, usually after eclosion. A Rb mark has been successfully transferred from parentals reared on diet to hatched eggs (Knight et al. 1989) for the purpose of studying female movement. Also, males reared on Rb-enriched diet mated to females reared on control diet resulted in marked hatched egg masses. Field trials have resulted in a Rb mark in 39 to 67% of the sampled egg masses within an orchard (Knight et al. 1990).

TRANSFER TO HIGHER TROPHIC LEVELS

Payne and Wood (1984) demonstrated the ability of the Rb mark to be transferred to parasitoids. Significantly elevated levels of Rb were found in *Calliephialtes grapholithae* (Cresson), an ichneumonid parasitoid of the hickory shuckworm. Payne and Wood (1984) suggested this to be the first demonstration of transfer of the Rb mark to parasitoids. Thoeny et al. (unpublished data) found that the Rb mark could be transferred from the southern pine beetle to three predator and four parasitoid species. A review of transfer of trace elements to parasites and predators is presented by C. G. Jackson in this supplement.

TOXICITY

Phytotoxicity due to excess accumulation of Rb salts can occur at the site of introduction or at more distal sites. Fleischer et al. (unpublished data) found cupping, yellowing and premature drop of foliage in pin oak trees injected with 200 or 500 g RbCl/tree (ca. 16 cm dbh and 12 m tall trees). Symptoms took several weeks to become apparent, but they occurred in only one replication which was higher on a slope, and may have had a drier soil profile. No toxic symptoms were observed in these Rb-stressed trees the following year. Toxic symptoms were not detected in pin oaks or loblolly pines treated with lower rates (Fleischer et al., up to 100 g RbCl, unpublished data, Bridges et al., up to 50 g, unpublished data, respectively).

McLean and Tuytel (1985) cut disks from injected Douglas-fir trees, photographed the Rb introduction site , and digitized the area of current springwood that was not properly formed. They concluded that implants were more damaging than injections of solutions. Not all of this damage was caused by the Rb per se, as some damage was observed in controls (injections or implants without Rb). Reardon (1984) also noted discolored areas associated with holes drilled for systemic insecticide introductions into trees. Neely (1988) reviewed wound closure in trees.

Symptoms of Rb toxicity to tree herbivores have also been observed at high application rates. Dry weights of F_1 gypsy moth neonates were significantly reduced when parentals were reared on trees injected with 500 g RbCl (Fleischer et al. unpublished data), although pupal weights of the parentals were not affected (Fleischer et al. 1989). Using Rb-enriched diet, Knight et al. (1989) found significant decreases in tufted apple bud moth larval developmental rate and in adult female longevity and fecundity at 6000 mg Rb/liter of diet (33,234 ppm Rb on a dry weight basis). No effects on these fitness parameters were observed at lower rates, and no effects were observed on male or female pupal developmental rate at any Rb concentration tested. Tanner et al. (1984) found concentrations of $SrCl_2$ above 0.1% in artificial diet were detrimental to gypsy moth F_1 neonates.

ANALYTICAL TECHNIQUES

The analytical techniques used for sample digestion and Rb determinations in tree systems have varied. Most workers have investigated elemental concentrations in individual insects, although samples have been pooled in some of the work with southern pine beetle due to its small size.

When atomic absorption spectrophotometry has been used for quantification, samples were generally collected and held frozen. Samples have been dried with heat (Fleischer et al.

1989), or lyophilized (Payne and Wood 1984). Dry samples have been dry-ashed [560 to 600^0C for 6 hrs to overnight (Fleischer et al. 1989)], or wet ashed [6 N HCl for insect tissue and 0.1 N HCl for plant tissue (Payne and Wood 1984); 70% HNO_3 for insect tissue and 70% $HClO_4$ for plant tissue (Bridges et al. 1989)]. In some studies the potential suppression of Rb signal due to ionization interferences has been reduced by incorporation of ion suppressants such as Li (Bridges et al. 1988) or Cs (Fleischer et al. 1989). Some researchers aspirated directly from the solubilized ash (Payne and Wood 1984), whereas others diluted to ensure reading with a narrower, and presumably more linear, range.

Quantification of Rb has been with atomic absorption spectrophotometry using the emission mode (Bridges et al. 1989) or with a hollow cathode lamp (Payne and Wood 1984). Working with Douglas-fir trees, McLean and Tuytel (1988) used X-ray dispersive spectrophotometry . Samples were ground, formulated into pellets, and subjected to the X-ray energy.

SUMMARY AND FUTURE DIRECTIONS

Application of Rb into trees for use in studies of insect dispersal and host-plant relations appears to be feasible using relatively low application rates. Rates in future field tests need to be extrapolated with a consideration of the amount of tree tissue into which the element needs to be distributed. For example, Bridges et al. (unpublished data) have experimented with using bole surface area to estimate the required dosage into loblolly pine to mark southern pine beetle. Baseline data are now available from both hardwoods and softwoods, from young transplants to mature 60 year old pecans, and from leaf, phloem, and fruit feeding arthropods on these trees. Trace elements can be injected as solutions or implanted as powders, but injections as solutions appear to be more efficient and less damaging to the tree. Rubidium is transported in both the symplastic and apoplastic transport systems. The distribution of Rb within trees appears to be strongly correlated to K distribution and is influenced by tree tissue, height and aspect. The Rb is relatively long-lived in the tree and may be recycled within tree tissues.

Development of the elemental enrichment technique in tree systems is occurring rapidly, and significant contributions to the elemental enrichment technology are coming from work in these systems. Original application technologies have been developed, and information on Rb transfer within the first trophic level, mechanisms of transport, and the potential of Rb recycling within the first trophic level are being determined. Work in tree systems has added to information on transfer of the mark to the F_1 generation. In addition to transfer to the eggs, transfer to F_1 larvae has been documented in gypsy moths. Payne and Wood (1984) have suggested that the first documentation of transfer of the mark to a parasitoid came from work with pecans. Few studies in tree systems, however, have addressed bioelimination. Studies in herbaceous systems have shown that rapid bioelimination in some species can significantly limit the time that the mark is detectable (Mitchell et al. 1982, Fleischer et al. 1986).

To date, elemental marking experiments in tree systems primarily have been developmental or feasibility studies. Field trials of this technique have occurred in loblolly pine (Bridges and Thoeny, unpublished data) and in apples orchards (Knight et al., 1990). In loblolly pine, marked southern pine beetles were captured 0.5 km distant from the site of Rb introduction, which was as far as traps had been placed. In apple orchards, the spatial pattern of Rb-labelled egg masses was primarily within 65 m of release sites, although distances ranged up to 250 m, and the spatial pattern was influenced by temperature. Hopefully, we will soon see empirical information related to insect movement and ecology in tree systems coming from use of this technology in the field.

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