A PERSONAL HISTORY OF THE DEVELOPMENT OF THE RUBIDIUM MARKING TECHNIQUE

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ABSTRACT

Events leading to the development of a rubidium marking technique for the study of insect dispersal are described. Questions of safety in the use of the radioactive isotope P^{32} as a label led to a search for another method. Other typically employed techniques required handling or artificial rearing. Detection by atomic absorption spectrophotometry of enhanced levels of rubidium in insects fed on treated plant material proved to be a relatively inexpensive process compared to other potential techniques such as neutron activation. Rubidium is a safe, relatively rare alkali metal, which mimics potassium in living tissue. Subsequent studies are cited which deal with the advantages and disadvantages of this technique perceived at the time of development.

DEVELOPMENT OF TECHNIQUE

The direct inspiration for the development of the rubidium marking technique stemmed from my introduction to the difficulties and hazards of marking insects with radioactive phosphorous (P^{32}). In May of 1968, I participated in a USDA research project which involved treating an entire corn field with P^{32} in order to mark and follow the dispersal of the corn earworm, *Helicoverpa zea* (Boddie). Although it was someone else's study, I had agreed to help with the labor-intensive field work on the treatment day. At the beginning of the day, we were each given a vial containing a P^{32} solution, a syringe, and basic instructions on how to inject corn plants. At the end of the day, after injecting literally hundreds of corn plants with the P^{32} solution, radioactivity was measured on my hair, hands, body and clothes. Others participating in this field work were similarly contaminated. I went home frightened and distressed. As it turned out, few marked insects were ever recovered, and the experiment came to nothing; clearly, the risks of that field project involving a radioactive marker outweighed the benefits of its use.

When I moved to the USDA's Boyden Laboratory in Riverside, California, I was interested in studying the dispersal of the cabbage looper, *Trichoplusia ni* (Hübner). Early experiments with laboratory-reared loopers proved to be interesting, and somewhat useful, but they could not be expected to give us information about native populations. Our laboratory colony of loopers had been reared on artificial diet for about 200 generations without introduction of new genetic material. Thus, use of our colony moths for experimental studies of dispersal was the equivalent of

using dairy cattle to study wild cattle behavior, *i.e.*, there was little reason to expect that the behaviors we observed in releases of laboratory-reared insects would accurately reflect the behaviors of the wild populations. Furthermore, in our release-recovery experiments, we could not expose the moths to environmental conditions equivalent to those experienced by field-reared natives. I concluded that only by marking native moths in the field could we learn much about movement patterns and dispersal of wild populations of cabbage looper.

In reviewing the available marking techniques for native lepidopterous species (see Akey, in this supplement, for a brief review of various marking techniques), I found that previous workers had either used capture-mark-release-recapture methods or radioactive markers. In the capture-recapture methods, the insects had to be captured by hand from a trap or collected as late instar larvae or pupae and reared to adults. Those captured in a trap would have already responded to the attractant, and we could not know their point of origin. Furthermore, it was possible that their response to pheromone-baited traps a second time would be different. Hand collection is an extremely labor-intensive and time-consuming process to obtain statistically acceptable sample sizes and, again, origins are uncertain. Those collected as late instar larvae or pupae may not have been conditioned to the field environment. Thus, passive, non-disruptive field marking of a native population seemed to be the only effective and efficient way of studying 'normal' responses of moths.

My experience with a radioisotope had frightened me enough that I did not want to work with it under field conditions again. I discussed the problem with Wayne Wolf, an agricultural engineer (USDA-ARS, Tifton, Georgia); we considered the possibility of using some sort of neutron activation marker and analysis (*e.g.*, Monro 1968). We learned that this required an atomic reactor and a great deal of money per analyzed sample. We then mentioned the problem to a plant physiologist, Wade Berry (Dept. of Biological Sciences, University of California, Los Angeles, California), who had worked on potassium metabolism in plants using atomic absorption analysis (*e.g.*, Berry and Smith 1969). When we described the problem to him he replied, "use rubidium".

Rubidium (Rb) is one of the alkali metals and is located between potassium (K) and cesium (Cs) on the periodic table of chemical elements. It is rather evenly distributed through the earth's surface and is rarely found anywhere in high concentrations. As a result, there is a generally uniform low 'background level' of rubidium in the soil and, in turn, the background levels of rubidium in plants and animals are usually very low.

When rubidium is present, it is acquired by plants through the usual channels of uptake, and once in the plant rubidium is a physiological mimic of potassium. This peculiar characteristic of rubidium has made it a favorite of plant physiologists studying potassium. Apparently, an isotope of potassium does not exist which has a convenient half-life for biological studies. Therefore, the plant physiologists have frequently utilized either radioactive rubidium or ordinary rubidium for their studies (*e.g.*, Epstein et al. 1963, Jackson and Adams 1963).

Ordinary rubidium is detectable through the use of a flame emission or flame absorption spectrophotometer (see Akey and Burns, in this supplement, for a review of the current state-of-the-art of instrumentation). Instrumentation at the time of this work (1971) could easily detect concentrations as low as 5 ppb; this represents levels well above the natural background of the element.

As with potassium, rubidium can enter most plants through foliar uptake, through the roots, or as a combination of the two (Murphy et al. 1955). However, most soils will bind rubidium in such a way that it is not readily available, so that foliar applications are most likely to result in increased rubidium concentrations in plants. Rubidium is immediately absorbed into and translocated throughout the plant (Levi 1970). We found rubidium in the bolls, seeds, and various other parts of greenhouse-treated cotton plants.

Insects feeding on the treated plants acquire the rubidium; and, as in the plant, the rubidium mimics potassium. This mimicry is essential to the use of rubidium for marking experiments. Most phytophagous insects have higher levels of potassium than of sodium, and substitution of a small amount of rubidium for some of this potassium does not seem to have any significant effect upon the insects' biology or behavior (*e.g.*, Stimmann et al. 1973). In our tests, we were unable to detect any behavioral changes in insects treated with field dosage levels of rubidium.

The actual field technique, as we conceived it, was to apply a solution of rubidium chloride (RbCl) at a rate of about 1 kg/ha to a crop or other potential host plants. A natural infestation of the insect would develop on the marked host, and when they emerged, their dispersal was to be monitored by placing traps at various distances around the field (see Hopper, in this supplement, for a review of experimental designs and analyses for movement studies). To encourage a larger population size, treatment of the target field with insecticides prior to rubidium application could be used to reduce competitors, predators and parasitoids.

Prior to treating any fields, we determined the background levels of rubidium for native insects arising from that particular host crop. Then, as the experiment progressed, the treated host crop was monitored and the rubidium levels maintained through retreatment at a level high enough to mark the emerging insects. The captured insects were analyzed for their rubidium content and, through statistical analysis of the data, those with significantly elevated levels of rubidium were recognized as being from the treated area.

The analytical technique was simple (see Akey and Burns, in this supplement, for a brief review of sample preparation techniques). The insects were dissolved in a bath of concentrated sulfuric acid and hydrogen peroxide at about 100°C, diluted slightly with water, and part of the sample was injected into the flame emission spectrophotometer (see Akey and Burns, in this supplement, for review of digest techniques).

We followed our laboratory and greenhouse experimentation (Berry et al. 1972 Stimmann et al. 1973) with a series of experiments of field marking (Stimmann 1974). At this time other researchers were also beginning to publish results of their studies involving rubidium labeling; Frazer and Raworth (1974), Shepard and Waddill (1976), Graham et al. (1977, 1978a, 1978b), and Van Steenwyk et al. (1978a, 1978b) all made considerable contributions to the development of the technique. That rubidium labeling was not limited to lepidopterous species was demonstrated in aphids (Frazer and Raworth 1974) and in the Mexican Bean Beetles (Shepard and Waddill 1976). Rapid turnover of rubidium in aphids (Frazer and Raworth 1974) has since been shown to be characteristic of all adult sucking insects studied (*e.g.*, Fleischer et al. 1986). Additional development and application of rubidium labeling in lepidopterous systems were conducted on noctuids (Graham and Wolfenbarger 1977, Graham et al. 1978a, 1978b) and on pink bollworm (Van Steenwyk et al. 1978a, 1978b). To this date, studies by Graham et al. (1978a) and Van Steenwyk et al. (1978b) contribute to a surprisingly small number of actual field applications of the

technology (see Hopper, in this supplement). These authors also reported results which indicated uses of the technique beyond simple dispersal studies, including demonstration of multi-trophic level labeling (Graham et al. 1978b) and the ability to label overwinter insects (Van Steenwyk 1978b).

As with all marking techniques, there are some advantages and disadvantages to consider before undertaking experiments using rubidium as a marker (*e.g.*, Gangwere et al. 1964). Obviously, a number of improvements and advances have been made since we conducted our experiments in the early 1970's. Many of the advantages and disadvantages we perceived at that time (see below) have been addressed and are discussed in the subsequent papers in this supplement (these papers are cited in order of applicability).

Advantages

1. It is simple to apply rubidium to a field (see Hayes for field crops; also Fleischer et al. for tree application).
2. There is no immediate danger from radioactivity. Rubidium has the highest level of naturally occurring radioactive isotope, almost 30%, but its half life is extremely long, so it is not very radioactive. A kilogram of rubidium added to a field results in about the same amount of radioactivity added by fertilizing with potassium which also naturally contains a high proportion of radioactive isotope.
3. There is no phytotoxicity (see Hayes; also, Fleischer et al.).
4. Rb is relatively inexpensive (see Hayes for review of alternative elements; also Hopper).
5. The insects show no behavioral changes (see Hayes; Fleischer et al.; Jackson; and Kimsey et al.).
6. The analysis is simple and fast (one technician was able to analyze up to 500 moths per day in my laboratory) (see Akey and Burns).
7. It works for sucking as well as chewing insects (see Kimsey et al.; Jackson; as well as Hayes; and Fleischer et al.).
8. It apparently can be used to mark insects from any part of the plant (see Hayes; Fleischer et al.).

Disadvantages

1. There is variance in the amount of rubidium per insect; therefore, some statistical analysis of the data may be necessary (see Hopper; Hayes; Fleischer et al.; also Jackson; and Kimsey et al.).
2. The absolute number of marked insects is not known, and must be inferred from samples of the field population (see Hopper, as well as Hayes; and Fleischer et al.).
3. Only one field can be tagged in an area at a time (otherwise, there may be cross-contamination of samples) (see Hayes for review of multiple element markings; also Hopper).
4. A well-equipped laboratory is necessary to analyze for this element (see Akey and Burns).

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