# COMPARATIVE EFFECTIVENESS OF FIVE RADIOISOTOPES AS TRACERS IN STUDYING DISPERSAL OF *CONOTRACHELUS NENUPHAR* (HBST.)

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Investigations on the field behavior of the plum curculio, *Conotrachelus nenuphar* (Hbst.), were begun in 1951 to improve some of the research techniques which are used in studies of insecticidal performance in peach orchards. There is a need not only for improvement in experimental design but also in methods of sampling populations for the evaluation of results of various treatments.

The first phase of field behavior to be studied was the movement of the adult insects after they had emerged from hibernation. In 1951 the author successfully employed radiophosphorus as a tracer to study dispersal of beetles from border rows to the center of the orchard. Nine release points were established along a row of trees bordering a woodland and radioactive insects were liberated at intervals through May and June. An attempt was made to recover the tagged insects at intervals of two to three days. Recovered insects were checked with either a portable El-Tronic Beta-Gamma Survey Meter (Model PR-3) or a Berkeley Decimal Scaler (Model 2000). Although the data from this experiment showed the general trend of dispersal, it was not possible to calculate the exact flight distances because beetles from one release point could not be distinguished from those from another point. In order to get more accurate information on flight range, it appeared desirable to use a variety of marking methods and to employ materials which could be used to designate specific locations. Accordingly, several radioisotopes and various colors of fluorescent lacquer enamels and fluorescent dusts were tested as tracers in the 1952 work.

## METHODS AND MATERIALS

Two general methods were used in an attempt to feed radioactive materials to plum curculios. One method involved the feeding of radioactive solutions while the other involved feeding of fresh, radioactive plant tissue. Radioactive solutions were transferred to shell vials and stoppered with moist cotton plugs. In the late summer and fall of the year when beetles are relatively inactive, they readily ingested radioactive liquid from the moist cotton. In the spring, however, beetles are much more active and thrive better if both food and water are supplied during the activation period. Food was provided in the form of succulent peach terminals placed in small water bottles. When foliage was provided, the insects no longer visited the shell vials and therefore it became necessary to activate both the foliage and the liquid in the shell vials.

The amount of radioactivity in curculios was measured by counting the insects for 10,000 counts in a Model 2000 Berkeley Decimal Scaler having a 10 percent geometry. This number of counts allowed a probable error of only 1 percent. Curculios were placed in metal counting planchets and covered with specially constructed cages. The cages consisted of a circular ring of steel covered on top with sheer nylon. The circular rings were 5 mm. sections cut from steel pipe which measured 9 mm. on the inside diameter and 13.5 mm. on the outside diameter. These cages were designed to confine the insect to a relatively small area for counting purposes yet eliminated the possible injurious effects if anesthetization had been employed.

The thick exoskeleton of the plum curculio absorbed relatively large amounts of radiation. Tests upon radioactive insects before and after dissection and

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maceration indicated that 19 percent of the strontium radiation, 35 percent of the cobalt radiation, and 39 percent of the zinc radiation were lost through self-absorption.

Preliminary activation experiments with $P^{32}$, $S^{35}$, $Cl^{36}$, $Ca^{45}$, $Co^{60}$, $Zn^{65}$, $Sr^{89}$, and $I^{131}$ indicated that highly radioactive plum curculios could be produced with $P^{32}$, $Co^{60}$, and $I^{131}$ and that fair activities could be obtained with $Zn^{65}$ and $Ca^{45}$. Early tests with low activity $Sr^{89}NO_3$ killed curculios but later tests in which high activity $Sr^{89}Cl_2$ was used at low dosages gave very encouraging results. The final choice between $Zn^{65}$ and $Ca^{45}$ as the fifth isotope to be used was based on the fact that the radiation characteristics of $Zn^{65}$ were sufficiently strong and considered to be significantly different from $P^{32}$, $Co^{60}$, $Sr^{89}$, and $I^{131}$. The physical characteristics and chemical forms of the five radioisotopes used in dispersal work appear in table 1.

TABLE 1

*Characteristics of various radioisotopes used in activation of plum curculio.*

| ISOTOPE | HALF-LIFE[1] | ENERGY OF RADIATION[1] (MEV)[2] | | CHEMICAL Form |
|---|---|---|---|---|
| | | Beta | Gamma | |
| Phosphorus 32 | 14.3 days | 1.71 | None | $KH_2P^{32}O_4$<br>$H_3P^{32}O_4$ |
| Cobalt 60 | 5.3 yr. | 0.31 | 1.10<br>1.30 | $Co^{60}Cl_2$ |
| Zinc 65 | 250 days | 0.4 | 1.14<br>0.44 | $Zn^{65}Cl_2$ |
| Strontium 89 | 55 days | 1.5 | None | $Sr^{89}Cl_2$<br>$Sr^{89}(NO_3)_2$ |
| Iodine 131 | 8 days | 0.6 | 0.367<br>0.80 | Elemental $I^{131}$ in weak $Na_2SO_3$ solution |

[1] Lapp and Andrews, *op. cit.*

[2] Million electron volts.

### *Radiophosphorus*

Phosphorus 32 in the form of potassium acid phosphate was received as a 31 gram unit having a total activity of 350 millicuries (mc.). Since this type of $P^{32}$ is not processed, there was considerable variation in the activity of different shipments. The salt was dissolved in distilled water and diluted to concentrations of 10 to 363$\mu$c./ml. for activation experiments.

Processed phosphorus was used only in preliminary work because of its relatively high cost. This material was received as orthophosphoric acid having a concentration of 3.87 mc./ml. on October 23, 1950. In exploratory studies this material was diluted to concentrations of 1, 2, 5, and 10 $\mu$c./ml.

### *Radiocobalt*

Cobalt 60 was received as cobalt chloride in acid solution having an original concentration of 34.2 mc./ml. ±3 percent. This material had a specific activity of 4,816 mc./gm. In preliminary work $Co^{60}$ solutions were diluted to concentrations of 5.5, 11, 22 and 44 $\mu$c./ml. Later concentrations of 50 to 100 $\mu$c./ml. were used successfully in producing high activities in adult insects.

### *Radiozinc*

Zinc 65 was obtained as zinc chloride in acid solution having an original concentration of 3.14 mc./ml. ±10 percent on April 11, 1952. The specific activity of this material was quite low (28 mc./gm.) as compared with other radioisotopes used in these experiments. $Zn^{65}$ was used at concentrations of 2, 4, 8, 16, 22, 44 and 290 μc./ml.

### *Radiostrontium*

Strontium 89 was received in two distinct forms and these varied widely in specific activity. One unit of $Sr^{89}$ in the form of strontium nitrate with a total activity of approximately 1.5 mc. was received on August 16, 1951. The other shipment of strontium consisted of 31.3 ml. of carrier free $Sr^{89}Cl_2$ in acid solution having a concentration of 1.60 mc./ml. ± 10 percent. This material was employed at concentrations of 0.375 to 20 μc./ml.

### *Radioiodine*

Iodine 131 was received as a 50.435 mc. carrier free unit having a concentration of 9.17 mc./ml. ± 10 percent. A concentration range of from 8 to 75 μc./ml. was used in preliminary activation tests.

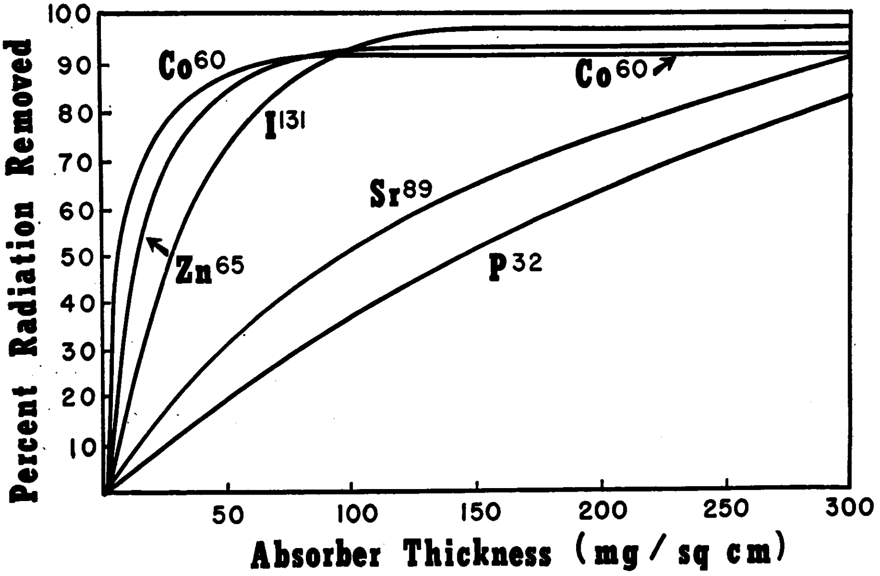

FIGURE 1. Absorption curves for various radioisotopes used to activate plum curculio.

### *Release and Recovery*

Adult plum curculios which had been collected from unsprayed peach and plum trees were fed $KH_2P^{32}O_4$, $Co^{60}Cl_2$, $Zn^{65}Cl_2$, $Sr^{89}Cl_2$, and elemental $I^{131}$ in solution or in peach foliage. Each of these materials was used to designate one of the five different release points.

Releases were made simultaneously at each release point at weekly intervals from May 12 to June 15. Activated insects were released at the base of the release trees in early morning or after sundown. Recoveries were made by jarring all of the peach trees in the orchard at three to four day intervals from May 22 to June 25.

Beetles which were collected in this manner from release trees were checked for radioactivity with an El-Tronic portable beta-gamma survey meter. Individuals showing activity were recorded and released with the assumption that the identity of the radioisotope in the insect corresponded with that of the release point.

Plum curculios which were recovered after they had flown at least 25 ft. from the point of liberation were brought into the laboratory and measured for radioactivity in a Berkeley Decimal Scaler. Radioactive insects were then killed and cemented to the bottom of a metal counting planchet. Next an attempt was made to determine the point of release by identifying the radioisotope present in the insect on the basis of its radiation characteristics. First, the relative energy of the radiation was determined by means of a graduated series of aluminum foil absorber screens. Absorption curves were then prepared and in this manner the pure beta emitting materials could be separated from the beta-gamma emitting materials. Absorption curves for five radioactive materials used in these experiments are shown in figure 1. The curves for pure beta emitters such as $Sr^{89}$ and $P^{32}$ are shown to be only slightly curved while the curves for the materials emitting both beta and gamma radiation are true exponential curves. Counts were made at 14-day intervals for half-life determinations.

TABLE 2

*Degree and persistence of radioactivity of ingested radiophosphorus, radiocobalt, radiozinc and radiostrontium in adult plum curculios. Figures in body of table refer to counts per minute.*

| ISOTOPE | CONCENTRATION ($\mu$c/ml) | DAYS AFTER INITIAL EXPOSURE | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Activation Period | | | | RETENTION PERIOD | | | | | | | |
| | | 1 | 2 | 3 | 4 | 15 | 30 | 45 | 60 | 75 | 90 | 105 | 120 |
| $P^{32}$ | 10 | 5,200 | 17,900 | 20,200 | 20,700 | 12,000 | 3,900 | 1,040 | 350 | 150 | 74 | 41 | 0 |
| | 25 | 5,300 | 17,900 | 20,300 | 22,300 | 13,400 | 5,000 | 1,500 | 480 | 194 | 104 | 65 | 46 |
| | 50 | 15,500 | 35,400 | 40,500 | 48,700 | 30,000 | 8,600 | 2,080 | 665 | 290 | 154 | 92 | 56 |
| | 100 | 26,700 | 50,900 | 58,500 | 64,000 | 44,000 | 13,000 | 3,000 | 1,100 | 440 | 200 | 106 | 66 |
| $Co^{60}$ | 5.5 | 30 | 50 | 100 | 1,900 | 1,400 | 760 | 580 | 310 | 215 | 155 | 128 | 118 |
| | 11 | 135 | 1,600 | 2,300 | 2,500 | 1,700 | 900 | 465 | 300 | 200 | 140 | 110 | 100 |
| | 22 | 1,000 | 3,000 | 3,900 | 5,500 | 5,000 | 4,200 | 3,650 | 2,750 | 2,370 | 2,100 | 1,950 | 1,900 |
| | 44 | 1,400 | 4,200 | 6,400 | 6,600 | 6,000 | 3,500 | 2,000 | 1,350 | 1,000 | 940 | 900 | 880 |
| $Zn^{65}$ | 2 | 22 | 37 | 24 | 131 | 78 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 4 | 25 | 97 | 115 | 293 | 130 | 62 | 41 | 0 | 0 | 0 | 0 | 0 |
| | 8 | 36 | 89 | 229 | 337 | 220 | 135 | 86 | 58 | 45 | 38 | 35 | 0 |
| | 16 | 75 | 172 | 264 | 415 | 240 | 130 | 80 | 62 | 54 | 52 | 50 | 48 |
| $Sr^{89}$ | 0.375 | 24 | 67 | 1,156 | Beetles dead | | | | | | | | |
| | 0.75 | 24 | 901 | 998 | Beetles dead | | | | | | | | |
| | 1.5 | 366 | 523 | 714 | Beetles dead | | | | | | | | |
| | 3.0 | 289 | 502 | 561 | Beetles dead | | | | | | | | |

## RESULTS

In radiophosphorus activation experiments with solutions, a concentration range of from 1 to 363 $\mu$c./ml. was tested over a two year period. The most practical dosage when using shell vials appeared to be around 100 $\mu$c./ml. Plum curculio adults exposed to this concentration of $P^{32}$ for 2 days averaged approximately 50,000 counts per minute (cpm.). The amount of radioactivity produced by ingesting various levels of radiophosphorus and the retention of this radioactivity is shown in table 2. It is evident that curculios reach relatively high levels of activity in two or three days. The figures in table 2 indicate that about 30 percent of the maximum activity is reached in the first day, 80 percent the second day, 90 percent the third day, and approximately 100 percent the fourth day. A saturation level of activity was reached on the fourth day. These data are based upon tests conducted from August 21 to December 17, 1951. In the spring of the year when physiological processes in the beetles are more rapid, the retention period of radiophosphorus is probably greatly reduced. When the concentration of $P^{32}$ is increased, a given activity is reached in a shorter time but concentrations in the range of 350 $\mu$c./ml. proved to be toxic to adults.

When peach foliage was employed to activate beetles, concentrations of from 10 to 122 $\mu$c./ml. were tested. The lower ranges resulted in activities of from 1,900 to 10,000 cpm. per beetle in 5 days as compared with an activity of 14,000 cpm. in the upper ranges. The optium concentration in solution was considered to be approximatley 100 $\mu$c./ml. At this strength beetles reached high activities in 4 to 5 days.

Radiocobalt was the most persistent of the five radioisotopes tested and therefore lends itself particularly well to long range studies. The degree and persistence of ingested radiocobalt at low concentrations is summarized in table 2. In comparison with radiophosphorus the activation of insects with radiocobalt was much slower since by the end of the second day an average of less than 50 percent of the maximum activity was reached. Later and more comprehensive experiments carried on in the spring of 1952 showed that a radiocobalt chloride solution at a concentration of 100 $\mu$c./ml. gave high and more rapid activation. Beetles feeding upon these solutions averaged about 12,000 cpm. in 5 days. Attempts to activate peach foliage with $Co^{60}$ solutions were unsuccessful.

In the fall of 1951 $Co^{60}$ solutions with concentrations of 5.5, 11, 22, and 44 $\mu$c./ml. were compared. Four days after exposure the respective activities of the insects were 1,900 2,500, 5,500, and 6,600 cpm.

Preliminary tests with $Zn^{65}$ at concentrations of 2, 4, 8, and 16 $\mu$c./ml. indicated that much higher ranges would be needed for field recoveries. Accordingly, the concentration was raised to 290 $\mu$c./ml. but this concentration proved toxic to beetles. When the activity of the radioactive solution was increased to 44 $\mu$c./ml. succulent peach foliage was killed very rapidly. This was undoubtedly due to the toxic effects of the non-radioactive carrier zinc in solution. When this solution was reduced to half strength, fair activities were obtained in feeding beetles upon activated foliage. A group of 21 insects which had fed upon activated foliage at the above concentration averaged 758 cpm. This material was the least effective of the five radioactive materials employed in feeding tests.

Initial experiments with radiostrontium were carried on with $Sr^{89}(NO_3)_2$ at concentrations of 0.375, 0.75, 1.5, and 3 $\mu$c./ml. Table 2 shows that after curculios had reached an activation of several hundred cpm. they exhibited symptoms of poisoning and died within a few days. Later tests with the non-radioactive salt $Sr^{89}(NO_3)_2$ proved that typical symptoms of poisoning were produced by chemical, rather than radiation, effects.

The following year solutions of $Sr^{89}Cl_2$ of high specific activity and comparatively low chemical concentrations were used successfully in both foliage and vials. Relatively low levels of activity of $Sr^{89}Cl_2$ were needed to produce high activity in the plum curculio. The optium concentration was approximately 2 $\mu$c./ml. in vials and 20 $\mu$c./ml. in foliage.

Radioiodine was employed in concentrations of 8, 32, 40, and 75 $\mu$c./ml. in activation work. At the higher concentrations beetles after five days exposure averaged 26,864 cpm. while at lower levels they averaged 2,680 cpm. $I^{131}$, like $Co^{60}$, was not readily taken up by peach foliage and therefore aqueous solutions in vials were used in all activation work.

## DISCUSSION

A total of 473 beetles were activated and released and 193 recoveries were made. Release points were determined for 93 percent of the beetles which were recovered at various points in the orchard. Table 3 shows the number of beetles released, the number of beetles recovered, and the average flight distance.

Thirty-one beetles were recovered on trees exclusive of release trees. No recoveries were made of insects activated with radioiodine on other than the release trees. This was believed to have been due to the short half-life of $1^{131}$.

There was considerable variation in the average and maximum flight distance of curculios activated with different isotopes. Although the numbers recovered were comparatively small, there was some indication that materials with half-lives comparable to $P^{32}$ *or shorter may be too short lived for this particular type of field* investigation. Since soil contamination is not a problem with the small amounts of radioactive material employed in such studies it would seem desirable to employ those isotopes, such as $Sr^{89}$ and $Co^{60}$ which have relatively long half-lives for field behavior investigations extending over a period of 6 to 8 weeks. However, the hazard involved in fruit contamination must also be taken into consideration.

TABLE 3

*Release, recovery and flight distance of plum curculios marked with various radioisotopes.*

| Radioisotopes | No. Beetles Released | No. Beetles Recovered | Average Flight Distance in ft. | Maximum Flight Distance in ft. |
|---|---|---|---|---|
| $P^{32}$ | 62 | 49 | 61.2 | 90 |
| $Co^{60}$ | 127 | 50 | 82.7 | 870 |
| $Zn^{65}$ | 23 | 7 | 82.5 | 135 |
| $Sr^{89}$ | 175 | 70 | 135.8 | 403 |
| $I^{131}$ | 86 | 17 | 0 | 0 |
| Totals | 473 | 193 | | |

## CONCLUSIONS

It may be concluded from these experiments that it is possible to activate plum curculios with any of the five radiosotopes. There is, however, considerable variation in the rate of uptake and in the length of time the materials are retained by adults. $Sr^{89}$ appeared to be the most useful radioisotope for these studies since it can be presented to insects in either solutions or in plant tissue, it has a relatively long half-life, and the radiation is comparatively strong. It is a pure beta emitter and hence is less hazardous than gamma emitting substances. The chief danger in the use of strontium is the possible accumulation of this element in bone tissue of those handling it through accidental inhalation or ingestion.

$Co^{60}$ was also a very effective and persistent tagging agent. The only disadvantages are that it is a gamma emitter and it is not well distributed in plant tissues. Cobalt was retained longer than any of the other isotopes which were employed in field work.

$P^{32}$ has all the advantages of $Sr^{89}$ and in addition is much less hazardous to the investigator. Its relatively short half-life may limit its usefulness in the field except in certain instances where insects can be fed for relatively long periods so that a large quantity of the material is present in the insect tissue.

$Zn^{65}$ was not very satisfactory because of the low specific activity of the sample employed. Radiozinc may prove to be most useful when used as a high specific activity material either in solution or in topical application with adhesive preparations. $I^{131}$ was quite unsatisfactory because of its very short half-life.

These experiments have shown that it is possible to mark insects with several different radioisotopes, release them simultaneously in the field, and later identify the isotope with which recoveries were activated. However, for this particular problem the use of these particular radioisotopes was impractical because it required such a long time for half-life determinations. In certain types of experiments where half-life determinations can be made at the convenience of the investigator, the use of $Sr^{89}$, $P^{32}$, and $Co^{60}$ should prove to be quite practical and useful in designating different release points or release dates.

## LITERATURE CITED

**Lapp, R. E.,** and **H. L. Andrews.** 1948. Nuclear radiation physics. Prentice-Hall, Inc. New York. 487 pp.