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## EFFECTS OF BAIT FORMULATIONS ON TOXICANT LOSSES AND EFFICACY

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**ABSTRACT:** During application by airplane excessive amounts of zinc phosphide were lost from the bait registered to control rat damage in Hawaiian sugarcane. The losses created unnecessary hazards and potentially reduced the efficacy of the control program. In a series of screening tests, alternate adhesives, adhesive concentrations, and bait mixing procedures were evaluated for zinc phosphide retention, acceptance by rats, phosphine residues in sugarcane and operational effectiveness. A formulation was developed that reduced zinc phosphide losses 32% during application, increased acceptance by rats, left residues in sugarcane below the established tolerance and equalled or exceeded the performance of the original formulation.

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### INTRODUCTION

Infield aerial application of zinc phosphide-treated oat groats to control rats in Hawaiian sugarcane was registered in 1970, (EPA Reg. No. 10646-1). This bait was formulated with 2% zinc phosphide (94% A.I.), and 3% corn oil as an adhesive on crimped oat groats. Corn oil was selected as the adhesive because of its low cost, availability, biodegradable properties, and good acceptance by the three pest species -- Polynesian rats, *Rattus exulans*; Norway rats, *R. norvegicus*; and black rats, *R. Rattus* (Hilton, et al., 1972). However, we discovered that up to 50% of the zinc phosphide was lost during the application of this bait (Hilton, Yaeger, and Teshima, 1971). The losses and drift of toxicant potentially posed a hazard and reduced efficacy of the treatment (Yaeger, Teshima and Hilton, 1972).

The objectives of this study were to develop a suitable replacement formulation with all of the positive characteristics of the original (effective against the target species, negligible residues in cane 90 days after application, and effective reduction in cane damage), but alleviating the excessive losses of zinc phosphide during application.

### METHODS

**Screening Trials:** During these preliminary zinc phosphide retention studies, we subjectively evaluated 18 candidate adhesives, 2 to 6 adhesive concentrations, and 3 bait mixing procedures. The test sequence included: (1) hand mixing 200 g batches of each formulation, (2) air drying for 2 days, (3) sifting loose zinc phosphide fines through a 6 mesh/cm sieve onto white paper, and (4) visually ranking the loose fines from each formulation. Formulations with the highest retention rates, compatible with existing mixing and application equipment, were selected for further evaluation in simulated field blower trials.

**Simulated Field Blower Trials:** Batches of 9 kg of six candidate baits selected for advanced screening and two corn oil baits were formulated in a cement mixer, air dried for 2 days, sacked in plastic bags, and stored at room temperature. After 7 days, 8 kg of each bait was passed through a shoulder-mounted powered blower and collected on cheesecloth; the remaining 1 kg was stored. One kg of each formulation passed through the blower was reserved for bioassays; the remaining 7 kg was distributed in covered 1 x 4 m soil flats for weathering trials. Formulations in each flat were then sprinkled with 3-8 cm of water; sprinkling was repeated at 10-day intervals for 30 days. Three randomly selected 10 g samples of each formulation were taken: (1) immediately after mixing, (2-3) after 7 and 87 days storage, (pre-blower sample), (4) immediately after blowing, and (5-7) after 10, 20, and 30 days weathering. Zinc phosphide content was analyzed by the toluene-gas chromatographic procedure developed by Robison and Hilton (1970)

**Bioassays:** The four most promising post-blower formulations from the simulated field trials were each bioassayed on 10 adult, wild trapped, rats of each species. The standard corn oil formulation and untreated oat groats were used as control formulations. Five of the individually caged rats in each test were prebaited with untreated oat groats daily for 3 days, five were not prebaited. Based on average daily consumption, individuals of each species (Polynesian, black, and Norway rats) were offered 5, 10 and 13 g of fresh bait daily for 3 days.

Bait consumption and mortality were recorded daily and rats were observed 7 more days for delayed reactions. Laboratory chow ration and water were offered ad libitum during the test period. ANOVA and multiple range tests were used to compare the data on consumption.

Airplane Trials: In this second series of field-oriented toxicant retention studies, three formulations yielding the most consistent results in previous experiments were again compared with the standard corn oil formulation. Each formulation was prepared using procedures outlined for the Simulated Field Blower Trials. Baits were applied by Stearman biplane equipped with a deflector-type fertilizer spreader (Nass, Hood and Lindsey, 1970) and collected from a 18 x 37 m tarp on the ground. Pre- and post-application bait samples were collected and analyzed chemically to quantify losses of zinc phosphide during operational applications.

Residues: The most efficient formulation resulting from previous experiments (highest mortality in bioassays and maximum toxicant retention during application) was aerially applied to 10-month old cane at the registered rate of 5.6 kg/ha. Cane was hand harvested in three randomly located plots (3 m of cane row) one day before, immediately after, and 83 days after application. The leaves were raised and tied to the stems to hold any zinc phosphide particles lodged in the leaf axils. Cane from each plot was ground up in an ensilage chopper, mixed and a 2.3 kg sample was withdrawn. Samples were analyzed for phosphine (Robison and Hilton, 1971) to determine if residues exceeded the established tolerance of 0.01 ppm 90 days after application.

Operational Efficacy: The effectiveness of the selected formulation in reducing rat damage was assessed in three 20 ha cane fields in which the registered formulation had been evaluated during the previous crop cycle. All fields were planted to Variety 59-3775 and bordered by noncrop areas. Each field was divided into an untreated reference block and a treated block. Treated blocks were baited by aircraft at a rate of 5-6 kg of bait per ha when the cane was 10, 13, 16, and 19 months old. Rat damage was assessed at harvest (22-23 months) by the V-cut method (Hood, 1971) Fifty V-cut samples randomly taken in each reference and treated block were examined to determine the number of rat damaged stalks per 100 five-foot lengths of stalk. Paired T-tests were used to determine if damage levels were significantly different between the treated and reference areas for each formulation.

## RESULTS AND DISCUSSION

Preliminary Screening: Zinc phosphide losses from 18 different adhesives, each prepared in 2-6 different concentrations and by 3 different mixing procedures, were compared during the sifting trials (Fig. 1). The formulation producing the fewest zinc phosphide fines was lecithin oil, followed in order, by baits formulated with Rhoplex AC-33<sup>1</sup> (Rohm and Haas Co.), Dow Latex 512R (Dow Chemical Co.), drying oils (i.e., boiled linseed oil), hexadecanol (a fatty alcohol), nondrying oils (i.e., macadamia nut oil) and fatty acids (i.e., lauric acid). The highest concentration of each adhesive usually had the lowest losses; however, many of these remained tacky or soupy and were considered incompatible with the existing bait mixing and application equipment. Of the mixing procedures tested: 1) predusting the zinc phosphide on the oat groats before adding the adhesive, 2) adding an adhesive-zinc phosphide slurry to the groats, or 3) adding the adhesive to the groats and postdusting the zinc phosphide (standard procedure for the registered formulation), the first method had the lowest losses with nearly all adhesives.

Retention rates for the six best formulations are compared with two corn oil formulations in Figure 2. All except the corn oil (3%) formulation were prepared by predusting the zinc phosphide on the oat groats and then adding the adhesive. The lecithin oil consisted of 4 parts lecithin and 1 part white oil. Alcohol was used to dissolve the hexadecanol and then evaporated off after mixing bait. The Rhoplex was added as a 1:9 AC-33 to water dilution. The boiled linseed and coconut and corn oil formulations required no special preparation.

Simulated Field Blower Trials: The replicated analyses of percent zinc phosphide remaining in each formulation during the various sampling periods were consistent (S.E. < 0.10%); therefore, results are expressed as the mean percent reduction in concentration (Figs. 2, 3).

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<sup>1</sup>Use of trade names in this publication does not imply endorsement of commercial products by the federal government.

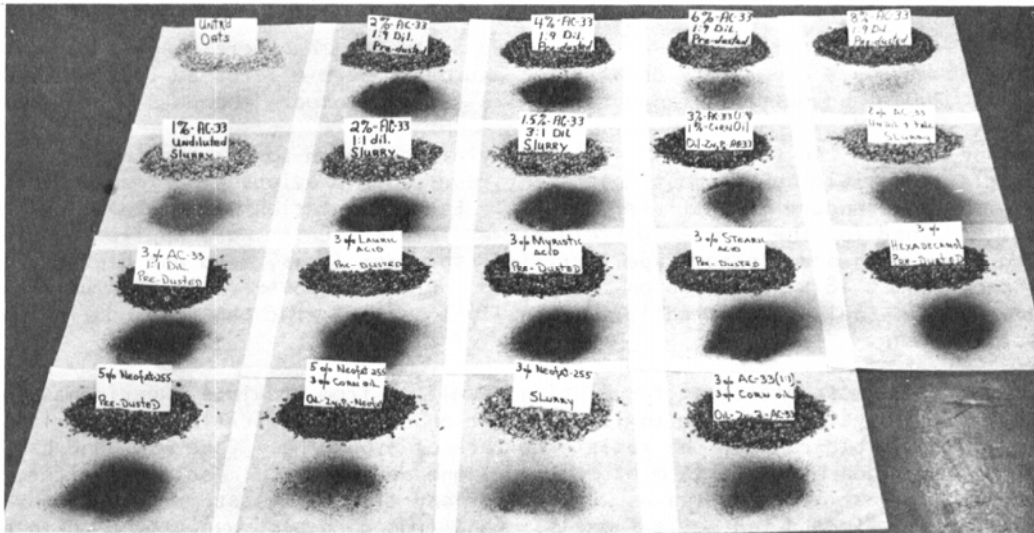


Figure 1. One of a series of preliminary screening trials ranking the adhesive properties of various formulations by the amount of zinc phosphide sifted from the bait onto white paper.

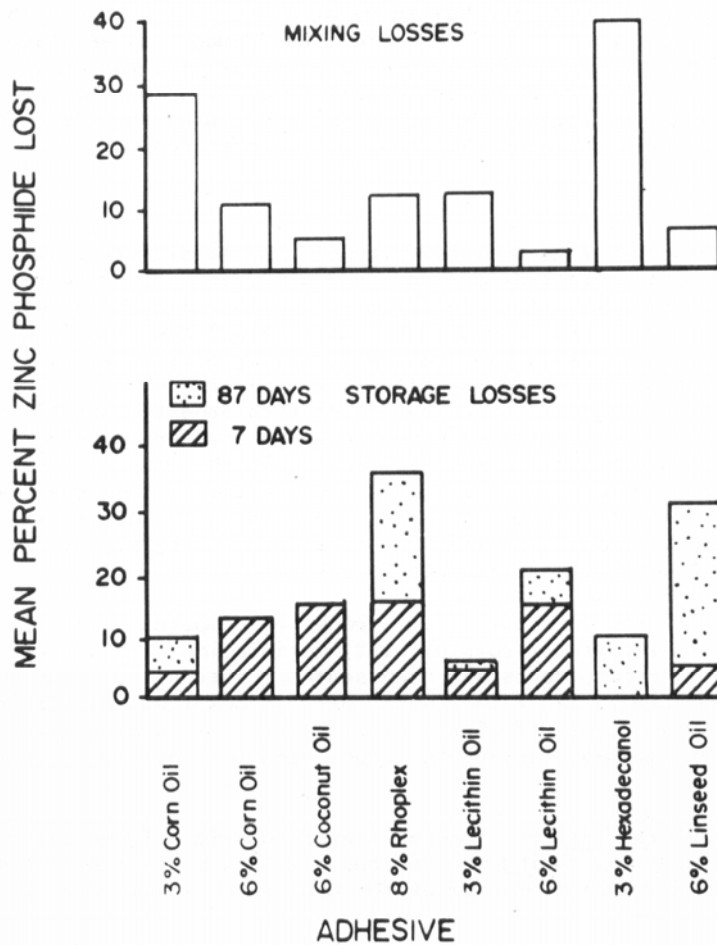


Figure 2. Zinc phosphide losses from 8 adhesive formulations during mixing and storage.

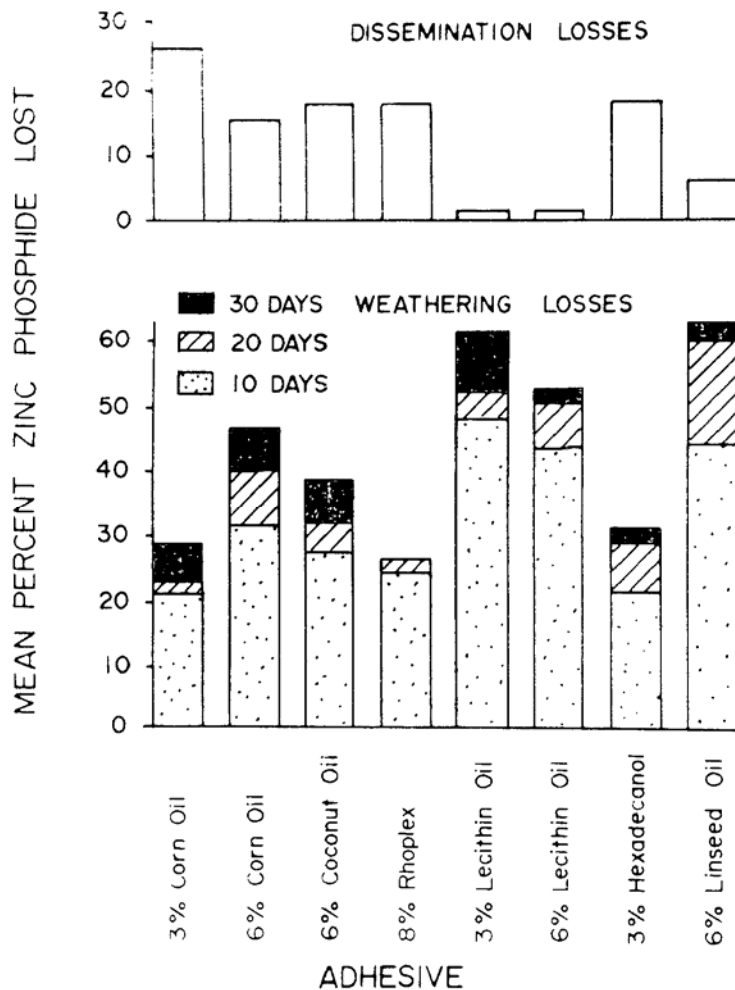


Figure 3. Zinc phosphide losses from 8 adhesive formulations during simulated application and weathering trials.

Zinc phosphide losses during the mixing process were excessive with 3% hexadecanol and the 3% corn oil formulations (Fig. 2). Losses were related, respectively, to the evaporation of alcohol for the hexadecanol formulation on contact with the mixer wall and the postdusting of zinc phosphide on mixer walls already coated with corn oil. Short-term storage losses were lowest with the hexadecanol formulation. The lecithin (3%) formulation had the lowest losses during long-term storage (Fig. 2). All formulations retained greater amounts of zinc phosphide than the registered corn oil formulation during the blower trials (Fig. 3). The lecithin oil formulations lost only 2.1 percent of the zinc phosphide compared to losses of 27-1% with the corn oil formulation. The lecithin oil formulations improved retention during application by 92 percent. Linseed oil also substantially improved retention.

Major losses of zinc phosphide from all formulations occurred during the first 10 days of artificial weathering (Fig. 3). Cumulative losses over 30 days were highest with the 3% lecithin oil and 6% linseed oil formulations. In contrast, concentrations of zinc phosphide on the Rhoplex formulation declined very little after the first 10 days of weathering. Zinc phosphide breaks down rapidly when in contact with moist Hawaiian soils (Hilton and Robison, 1972); therefore, nontarget hazards and residue levels associated with food-crop applications are primarily related to the persistence of zinc phosphide on baits and decomposition of the oat groats. Under these conditions, a bait that withstood weathering for 5-10 days and then rapidly broke down was preferred, however, none weathered in this manner. In terms of residues and nontarget hazards, the 3% lecithin oil and 6% linseed oil formulations were the safest and the 8% Rhoplex formulation potentially the most hazardous.

Bioassay: The 3% lecithin oil, 6% linseed oil and 6% coconut oil formulations were selected for bioassays. Due to the superiority of the lecithin oil formulation, three additional lecithin products (Alcolec S, SLV-42 and 211, American Lecithin Co.) were compared using procedures outlined in the preliminary screening trials. Of these, a 2% Alcolec S formulation had less sifting losses than the 3% lecithin oil formulation and was included in the series.

Mean day 1 consumption by all species was significantly less with formulated vs. untreated oat groats (Table 1). Prebaiting increased consumption of nearly all formulations by all species. The Alcolec S formulation was consumed in significantly ( $P < 0.05$ ) greater amounts by non-prebaited and prebaited Norway rats and, excluding the corn oil formulation, by prebaited black rats. Mean consumption of all formulations over 3 days was proportionately similar to the first day for all species. The Alcolec S, corn oil and lecithin formulations had the lowest percentage of noneaters and linseed oil had the highest. Although the Alcolec S formulation offers some improvement in bait acceptance over the corn oil formulation, differences were of minor importance. Of greater importance, the principal differences between adhesive formulations were related to zinc phosphide concentrations on the post-blower samples and are reflected in the amount of zinc phosphide consumed per rat (Table 1). The  $LD_{50}$ 's (95% C.L.) in mg/kg of zinc phosphide for Polynesian, black and Norway rats were 23.1 (6.3-33.4), 21.3 (19.3-23.9) and 27.0 (12.0-61.0), respectively. The number of  $LD_{50}$ 's consumed by all species was significantly ( $P < 0.05$ ) higher with the Alcolec S formulation than all others in four of six comparisons. It was the only formulation producing 100 percent mortality among both non-prebaited and prebaited rats of all species (Table 1). It was exceeded only by lecithin oil when fed to prebaited Polynesian rats.

Airplane Trials: The Alcolec S formulation reduced zinc phosphide losses 92 percent during aerial application when compared to the corn oil formulation (Table 2). Retention of zinc phosphide with the Alcolec S formulation was also better than both the lecithin and linseed oil formulations. The mixing time required to obtain a uniform bait with the Alcolec S formulation was approximately double that required for the corn oil formulation, and with repetitive mixing, residues tended to harden and accumulate in the mixer. These disadvantages were considered acceptable when compared to the benefits of improved retention, acceptance and mortality. Although storage losses were not determined for the Alcolec S formulation, they are expected to be similar to the lecithin oil formulation. Operationally, bait is usually applied within 1 to 2 days of mixing thus minimizing the importance of storage losses.

Residues: No phosphine residues in sugarcane were detected ( $< 0.001$  ppm) one day before application. Residues averaged 0.087 ppm (range = 0.072-0.104) immediately after the application of Alcolec S bait but dropped to 0.001 ppm 83 days later. Residues immediately after application were attributed to bait lodged in the leaf axils. Residues after 83 days were well below the established tolerance of 0.01 ppm phosphine 90 days after application.

Efficacy: Rat damage to sugarcane at harvest in the three Alcolec S-zinc phosphide-treated blocks was 68, 49 and 63 percent less than in the paired untreated blocks (Table 3). The corn oil formulation applied on the same treated blocks during the preceding crop cycle reduced damage 36, 43 and 69 percent, respectively. A paired t-test showed that differences in damage between the treated and untreated blocks were significant ( $P < 0.05$ ) for both formulations. Damage was reduced an average of 60 percent with the Alcolec S formulation and 49 percent with the corn oil formulation; however, we cannot show a significant difference between the two formulations (Table 3).

## CONCLUSION

The zinc phosphide - Alcolec S formulation is an effective substitute for the original corn oil formulation--losses of zinc phosphide during operational application were reduced 92%. Acceptance and mortality increased during laboratory bioassays with all species of rats, reductions in damage equalled or exceeded levels achieved with the corn oil formulation, and phosphine residues remained below the established tolerance. Following this series of tests, the original corn oil registration was amended to substitute Alcolec S for operational use.

Table 1. The effects of adhesives on bait consumption and toxicity.

| Species                               | Adhesives        | Zn <sub>3</sub> P <sub>2</sub> | Percent concentration |                         | Mean (S.E.) consumption per rat (N=5)** |         |       |         |       |         | Mortality % |
|---------------------------------------|------------------|--------------------------------|-----------------------|-------------------------|---|---------|-------|---------|-------|---------|-------------|
|                                       |                  |                                | Bait (g)              | No. LD <sub>50</sub> 's | Days                                    |         | Days  |         | Days  |         |             |
|                                       |                  |                                |                       |                         | Day 1                                   | Day 1-3 | Day 1 | Day 1-3 | Day 1 | Day 1-3 |             |
| <u>R. rattus</u><br>(w/o prebait)     | Corn oil 3.0     | 0.76                           | 1.3(0.9)a*            | 1.5                     | 3.0(2.1)a                               | 3.3     | 0     | 0       | 20    |         |             |
|                                       | Lecithin oil 3.0 | 1.53                           | 0.8(0.3)a             | 1.0                     | 3.8(1.4)a                               | 4.7     | 0     | 0       | 60    |         |             |
|                                       | Linseed oil 6.0  | 1.53                           | 0.8(0.2)a             | 0.9                     | 3.7(1.1)a                               | 4.0     | 20    | 0       | 40    |         |             |
|                                       | Coconut oil 6.0  | 1.14                           | 0.5(0.2)a             | 0.7                     | 1.8(0.5)a                               | 2.4     | 20    | 0       | 20    |         |             |
|                                       | Alcolec S 2.0    | 1.83                           | 0.8(0.3)a             | 0.9                     | 4.6(1.4)a                               | 5.2     | 0     | 0       | 100   |         |             |
| Untreated oats                        | ----             | ----                           | 8.9(0.5)b             | 27.7                    | -----                                   | -----   | 0     | 0       | 0     |         |             |
| (w/prebait)                           | Corn oil 3.0     | 0.76                           | 2.0(0.4)a             | 2.1                     | 4.5(0.9)a                               | 4.8     | 0     | 0       | 60    |         |             |
|                                       | Lecithin oil 3.0 | 1.53                           | 0.8(0.2)b             | 1.3                     | 3.8(0.8)a                               | 6.1     | 0     | 0       | 60    |         |             |
|                                       | Linseed oil 6.0  | 1.53                           | 1.0(0.3)b             | 1.3                     | 4.7(1.2)a                               | 6.0     | 0     | 0       | 60    |         |             |
|                                       | Coconut oil 6.0  | 1.14                           | 1.0(0.1)b             | 1.0                     | 3.3(0.4)a                               | 3.6     | 0     | 0       | 80    |         |             |
|                                       | Alcolec S 2.0    | 1.83                           | 2.4(0.5)a             | 2.4                     | 13.4(2.7)b                              | 13.4    | 0     | 0       | 100   |         |             |
| Untreated oats                        | ----             | ----                           | 9.6(0.2)c             | 28.2                    | -----                                   | -----   | 0     | 0       | 0     |         |             |
| <u>R. norvegicus</u><br>(w/o prebait) | Corn oil 3.0     | 0.76                           | 0.6(0.2)a             | 1.2                     | 1.0(0.4)a                               | 2.0     | 0     | 0       | 0     |         |             |
|                                       | Lecithin oil 3.0 | 1.53                           | 0.7(0.2)a             | 0.8                     | 2.4(0.5)a                               | 2.7     | 0     | 0       | 0     |         |             |
|                                       | Linseed oil 6.0  | 1.53                           | 0.5(0.1)a             | 0.9                     | 1.8(0.5)a                               | 2.9     | 0     | 0       | 0     |         |             |
|                                       | Coconut oil 6.0  | 1.14                           | 0.6(0.1)a             | 1.1                     | 1.5(0.4)a                               | 2.2     | 0     | 0       | 0     |         |             |
|                                       | Alcolec S 2.0    | 1.83                           | 3.1(0.6)b             | 3.1                     | 12.3(2.3)b                              | 12.3    | 0     | 0       | 100   |         |             |
| Untreated oats                        | ----             | ----                           | 4.0(1.8)c             | 21.2                    | -----                                   | -----   | 0     | 0       | 0     |         |             |
| (w/prebait)                           | Corn oil 3.0     | 0.76                           | 1.1(0.4)a             | 1.3                     | 1.8(0.6)a                               | 2.1     | 20    | 0       | 40    |         |             |
|                                       | Lecithin oil 3.0 | 1.53                           | 1.3(0.4)a             | 1.5                     | 4.4(1.4)b                               | 5.1     | 20    | 0       | 40    |         |             |
|                                       | Linseed oil 6.0  | 1.53                           | 0.7(0.3)a             | 1.0                     | 2.4(0.9)a                               | 3.4     | 20    | 0       | 0     |         |             |
|                                       | Coconut oil 6.0  | 1.14                           | 1.0(0.2)a             | 1.1                     | 2.6(0.4)a                               | 2.8     | 0     | 0       | 20    |         |             |
|                                       | Alcolec S 2.0    | 1.83                           | 2.7(0.5)b             | 2.7                     | 10.8(1.8)c                              | 10.8    | 0     | 0       | 100   |         |             |
| Untreated oats                        | ----             | ----                           | 9.2(0.3)c             | 27.8                    | -----                                   | -----   | 0     | 0       | 0     |         |             |
| <u>R. exulans</u><br>(w/o prebait)    | Corn oil 3.0     | 0.76                           | 0.2(0.0)a             | 0.5                     | 1.0(1.6)a                               | 2.3     | 0     | 0       | 60    |         |             |
|                                       | Lecithin oil 3.0 | 1.53                           | 0.3(0.1)a             | 0.5                     | 2.6(0.8)a                               | 5.1     | 0     | 0       | 60    |         |             |
|                                       | Linseed oil 6.0  | 1.53                           | 0.1(0.1)a             | 0.3                     | 1.0(0.5)a                               | 3.2     | 40    | 20      | 0     |         |             |
|                                       | Coconut oil 6.0  | 1.14                           | 0.2(0.1)a             | 0.6                     | 1.2(0.5)a                               | 4.1     | 20    | 0       | 40    |         |             |
|                                       | Alcolec S 2.0    | 1.83                           | 0.8(0.3)a             | 1.0                     | 9.2(3.2)b                               | 11.6    | 20    | 0       | 100   |         |             |
| Untreated oats                        | ----             | ----                           | 3.4(1.4)b             | 13.9                    | -----                                   | -----   | 0     | 0       | 0     |         |             |
| (w/prebait)                           | Corn oil 3.0     | 0.76                           | 1.7(0.5)a             | 1.7                     | 8.3(2.5)a                               | 8.4     | 0     | 0       | 80    |         |             |
|                                       | Lecithin oil 3.0 | 1.53                           | 1.4(0.3)a             | 1.4                     | 13.4(2.9)a                              | 13.4    | 0     | 0       | 100   |         |             |
|                                       | Linseed oil 6.0  | 1.53                           | 1.0(0.2)a             | 1.0                     | 9.9(1.8)a                               | 9.9     | 0     | 0       | 100   |         |             |
|                                       | Coconut oil 6.0  | 1.14                           | 1.1(0.5)a             | 1.1                     | 8.2(3.7)a                               | 8.3     | 0     | 0       | 80    |         |             |
|                                       | Alcolec S 2.0    | 1.83                           | 0.9(0.3)a             | 0.9                     | 10.6(3.9)a                              | 10.6    | 0     | 0       | 100   |         |             |
| Untreated oats                        | ----             | ----                           | 8.0(1.0)b             | 23.7                    | -----                                   | -----   | 0     | 0       | 0     |         |             |

\* Figures not followed by the same letter are significantly different (P 0.05)  
 \*\*Noneaters are included in the means.

Table 2. Zinc phosphide losses from four adhesive formulations during application by airplane.

| Adhesive     | Concentration (%) | Mixing order | Percent zinc phosphide |           | Percent loss |
|--------------|-------------------|--------------|------------------------|-----------|--------------|
|              |                   |              | Pre-drop               | Post-drop |              |
| Alcolec S    | 2                 | Post-dust    | 1.90                   | 1.83      | 4            |
| Lecithin oil | 3                 | " "          | 1.90                   | 1.54      | 19           |
| Linseed oil  | 5                 | " "          | 1.88                   | 1.25      | 34           |
| Corn oil     | 3                 | Pre-dust     | 1.83                   | 0.95      | 48           |

Table 3. Comparative operational efficacy of the corn oil and Alcolec S formulations of zinc phosphide treated oat groats.

| Field                         | Zn <sub>3</sub> P <sub>2</sub> | n  | Mean percent (S.E.) damaged stalks per V-cut sample |           |
|-------------------------------|--------------------------------|----|---|-----------|
|                               |                                |    | Corn oil  | Alcolec S |
| 43B                           | Treated                        | 75 | 3.88(0.31)  | 50        |
|                               | Untreated                      | 75 | 6.94(0.39)  | 50        |
|                               | % reduction                    | 75 | 35.8  | 67.6      |
| 51A                           | Treated                        | 75 | 6.41(0.40)  | 50        |
|                               | Untreated                      | 75 | 11.20(0.61)   | 50        |
|                               | % reduction                    | 75 | 42.7  | 49.3      |
| 61B                           | Treated                        | 75 | 2.72(0.27)  | 50        |
|                               | Untreated                      | 75 | 8.87(0.82)  | 50        |
|                               | % reduction                    |    | 69.3  | 62.7      |
| Mean percent damage reduction |                                |    | 49.3  | 59.9      |

#### ACKNOWLEDGMENTS

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