Activity of conventional and reduced-risk insecticides for protection of grapevines against the rose chafer, *Macrodactylus subspinosus* (Coleoptera: Scarabaeidae)

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Abstract: Bioassays were conducted to compare the residual toxicity and leaf protection activity of conventional broad-spectrum and reduced-risk insecticides against the rose chafer, *Macrodactylus subspinosus*. Insecticides were applied to a *Vitis labrusca* (F.) vineyard and residues were aged for 1, 3 or 7 days before leaves were collected and exposed to beetles in no-choice tests. Azinphosmethyl caused rapid knockdown and mortality for up to 1 week after application, with 1-day-old residues providing 95.6% protection against feeding, dropping to 51.6% when residues were a week old. Fenpropathrin caused mortality and knockdown after beetles had been exposed to fresh residues for 72 h. Although these effects diminished as residues aged, this compound provided the best protection of leaves against beetle feeding, with 77.9% reduction in feeding compared with the control after 7 days of aging in the vineyard. Of the reduced-risk insecticides, imidacloprid caused the greatest initial mortality and knockdown of beetles, providing protection against feeding that was equivalent to azinphosmethyl. Exposure to azadirachtin caused a low level of knockdown and mortality when residues were 1- and 3-days old. Protection against feeding was also low, lasting for only 3 days. Beetles were minimally affected by capsaicin and kaolin, with mortality and knockdown seen only when beetles were exposed to 1-day-old residues for 72 h. Foliage protection from these compounds was minimal, with between 10 and 15% reduction in feeding injury. Results are discussed in relation to development of semi-field bioassay methods for evaluating reduced-risk insecticides, and the management of *M. subspinosus* within grape pest management programs.

1 Introduction

Sporadic outbreaks of insect pests in vineyards may require intervention to protect foliage and fruit from economic damage. The rose chafer, *Macrodactylus subspinosus*, is a univoltine scarab beetle that is a pest of many horticultural crops and flowers in eastern North America, particularly in areas with sandy soils (Chittenden, 1916; McLeod and Williams, 1990). Adult beetles emerge in the spring when grapevine clusters are in bloom and when foliage is tender during rapid growth. Because of their relatively thin leaves, vineyards of *Vitis vinifera* L. and hybrid grape varieties are at greater risk than *V. labrusca* L. vineyards, although clusters of all varieties are attacked. Abundance of this beetle varies greatly between years and from vineyard to vineyard, but vines with over 100 rose chafer beetles per plant have been observed (R. Isaacs, unpublished data), and in thin-leafed varieties this degree of pest pressure results in almost total loss of photosynthetically active leaf area. Recent studies on potted *V. labrusca* plants have shown that feeding early in the growing season by rose chafers can negatively

affect subsequent root growth and can increase susceptibility of vines to injury by other foliar herbivores later in the growing season (Mercader and Isaacs, in press). When valuable parts of the crop are attacked by rose chafers, commercial fruit growers require insecticides that will provide protection from defoliation and fruit injury.

Conventional broad-spectrum insecticides such as carbamate and organophosphate compounds can provide good protection of grapevines from feeding by rose chafers and other insects (Williams, 1979; Wise et al., 2002). These neurotoxins have activity through contact and ingestion, and cause rapid knockdown and death in the target insects. Increased restrictions on insecticides in these classes are expected in grapes and other food crops in the United States because of implementation of the Food Quality Protection Act (FQPA) of 1996 by the US Environmental Protection Agency (US EPA). Revocation of some tolerances for use of insecticides in vineyards and extension of re-entry intervals for others are likely to limit the utility of these types of insecticides for this crop, where

hand labour activities are required at multiple stages throughout the growing season. New insecticide chemistries that are achieving registration within the current regulatory environment of the US EPA include compounds that are designated as 'reduced-risk' and that may reasonably be expected to result in one or more of: reduced pesticide risk to human health, reduced pesticide risk to non-target organisms, reduced potential for contamination of valued environmental resources, or broadened adoption of integrated pest management (IPM) (Anonymous, 1996). Reduced-risk insecticides generally have less immediate toxicity to pest insects than conventional insecticides, but they may have sublethal activity such as repellency or antifeedant effects. These types of insecticides are potential new tools for use against rose chafer and other insects in fruit crops because of their suitability for inclusion in IPM programs. Evaluation of these types of compounds is a priority for many pest management research programs in the United States because their use is likely to survive implementation of the FQPA.

This study compared conventional and reduced-risk insecticides registered for use in grapes to determine their immediate and residual activity against adult *M. subspinosus*, using a field based bioassay method recently reported by Liburd et al. (2003), which is similar to the bioassay method of Williams et al. (1990). We tested two conventional insecticides; azinphosmethyl, an organophosphate insecticide with broad activity against insect pests that is used widely in fruit crops and fenpropathrin, a pyrethroid insecticide and miticide recently registered for use on grapes with activity against a broad spectrum of insects as well as certain phytophagous and predaceous mites. Reduced-risk insecticides tested in this study included imidacloprid, capsaicin, azadirachtin, and kaolin clay. Imidacloprid is a neonicotinoid in the chloronicotinyl subclass, registered on US grapes for control of sucking insect pests such as leafhoppers, mealybugs, and aphids. The short duration of residue on the leaf surface results in low activity of this insecticide on parasitoid natural enemies (Wilkinson, 2002) although negative impacts on generalist predators have been reported (Mizell and Sconyers, 1992; Duffie et al., 1998). Additionally, recent research indicates that fecundity of two spotted spider mite, *Tetranychus urticae*, is enhanced in the presence of imidacloprid residues (James and Price, 2002). Thus, some negative side-effects of reduced-risk insecticides can be expected. Of the biological insecticides tested, capsaicin is a pepper extract with primary use as a repellent for protection of plants against mammal and insect herbivores, whereas azadirachtin is a limonoid extracted from the nut of the neem tree, *Azadirachta indica*, with broad activity from antifeedant, reproductive, and developmental effects (Ascher, 1993; Mordue and Blackwell, 1993; Sundaram, 1996). Kaolin clay has been refined into a formulation that is registered for use in wine grapes and other food crops. Application of this product is the basis of the particle film pest management strategy (Glenn et al., 1999) in which a thin layer of kaolin residue is created, to create a barrier to colonization of the crop by pest insects. Use of this strategy has been shown to cause reduced pest damage from whiteflies (Gemei and Liu, 2002) and lepidopteran fruit pests (Knight et al., 2000; Unruh et al., 2000) by disrupting typical host location, selection, and acceptance behaviours.

This study was conducted to compare broad spectrum and reduced-risk insecticides for protection of grapevines from feeding by adult rose chafer beetles. This was achieved by measuring mortality, knockdown, and feeding after 24 and 72 h of exposure of beetles to leaves with differently-aged insecticide residues.

2 Materials and Methods

This research was conducted at the Trevor Nichols Research Complex, Fennville, Michigan in a 2 ha research vineyard of mature *V. labrusca*, cv. Concord vines. Treatments were applied to seven vine rows (one treatment per row) using a FMC Model DP50 air blast sprayer (FMC Corporation, Jonesboro, AK, USA) in 467.7 l/ha of water on June 16, 2001. The following treatments were used; azinphosmethyl (Guthion®, Bayer Crop Science. Kansas City, MO, USA) 50 W at 1.68 kg/ha), fenpropathrin (Danitol®, Valent Walnut Creek, CA, USA) 2.4 EC at 778.9 ml/ha), capsaicin (Capsin®, Kalsec, Kalamazoo, MI, USA) at 4.67 l/ha), azadirachtin (Ecozin®, AMVAC, Los Angeles, CA, USA) 3 EC at 730.7 ml/ha), imidacloprid (Provado®, Bayer Crop Science, Kansas City, MO, USA) 75 WP at 70.05 g/ha), kaolin (Surround®, Engelhard Corporation, Iselin, NJ, USA) WP at 28.04 kg/ha), and an untreated control in which no application was made to the foliage. No other insecticides were applied to these vines, and a standard fungicide program was followed throughout the vineyard. Rainfall occurred twice during the study period; 2 days after treatment (June 18; 14.73 mm) and 5 days after treatment (June 21; 8.64 mm). The mean daily low temperature was 12.3°C and the mean daily high temperature was 23.6°C.

Individual leaves were removed from the vines by cutting the petiole and immediately placing the petiole in a floral water pick (Dakota Plastics, Watertown, SD, USA) to maintain leaf turgor throughout the study. Baited rose chafer traps (Great Lakes IPM, Vestaburg, MI, USA) were placed in a grass field in Oceana County, Michigan, emptied 12 h prior to collections, and then beetles were collected less than 12 h before the evaluation of each residue age to provide fresh insects for the assays. Captured beetles were transported to the laboratory in large plastic containers on untreated grape foliage. Insecticides were applied to the vineyard on the same day as the first collection of rose chafers, and 24 h later leaves were collected from the treated rows and transported to the laboratory in test tube racks inside coolers for use in the bioassay.

Bioassay chambers consisted of a 32 oz circular plastic container with holes in the lid to improve ventilation. A grape leaf was placed inside with the bottom of the water pick inserted in a hole in one end of the box. For each combination of residue age and treatment, 10 replicate containers were used. Ten active beetles collected within the previous 24 h were added to each container, and the lid of the container was closed. Chambers were arranged on a shelf under incandescent lighting on a 16 : 8 L : D cycle.

Beetles were assessed after 24 and 72 h exposure to determine the lethal and sublethal effects of the insecticides. At each assessment, the number of dead beetles and number of beetles displaying knockdown symptoms (immobilized but

trembling) were recorded. To assess leaf protection, the area of feeding damage was recorded on each leaf at the end of the 72 h exposure period using methods similar to those described by O'NEAL et al. (2002). An image (at 150 dots per inch resolution) of each leaf was gathered using a ScanJet 6200C scanner (Hewlett Packard, Cupertino, CA, USA) and HP Precision Scan Pro (Version 1.1) software. The saved images were processed in the Scion Image 4.0.2 program (National Institutes of Health, Bethesda, MD, USA) to determine the total area of the leaf and the area of the leaf surface that had been fed upon by the beetles. When beetles did not fully remove leaf area of these *V. labrusca* leaves, damaged tissues could be distinguished easily from undamaged tissues because of the different colour of damaged and undamaged tissues.

For each combination of residue age and duration of exposure, analysis of variance was conducted to determine whether the treatments affected the proportion of beetles that were dead or exhibiting knockdown symptoms. All analyses of mortality and knockdown were conducted on arcsin transformed proportion values to normalize variances, and significant differences among treatments were determined using Fishers Protected Least Squares method. The same method was used to compare the area of leaf removed by beetle feeding, except that these data were not transformed prior to analysis.

3 Results

The mortality of rose chafer beetles was significantly different among treatments when beetles were exposed to 1-day-old residues for either 24 h ($F_{6,63} = 47.3$, $P < 0.0001$) or 72 h ($F_{6,63} = 34.7$, $P < 0.0001$). After 24 h, azinphosmethyl caused the greatest mortality and this increased to almost 100% after 72 h of exposure (table 1). Imidacloprid did not cause mortality different from the untreated leaves after 24 h of exposure, but after 72 h there was a moderate level of mortality evident in this treatment. Beetles were not killed very quickly by fenpropathrin, with low levels of mortality after 24 h, rising to over 50% after 72 h exposure. The four reduced-risk insecticides did not cause significant mortality after 24 h, but by 72 h, imidacloprid, azadirachtin, kaolin, and capsaicin all caused significantly greater mortality than the untreated leaves. At the 72 h exposure readings, mortality from azadirachtin residues was equivalent to that from fenpropathrin.

The level of mortality caused by 24 h of exposure to 3-day-old residues was lower than for 1-day-old residues, although there was still a significant effect of treatment ($F_{6,63} = 2.41$, $P = 0.037$). Only the broad-spectrum insecticides caused significant mortality (table 1). Mortality on leaves treated with all other insecticides was not significantly different from the untreated leaves. After 72 h exposure to the residues azinphosmethyl, fenpropathrin, and azadirachtin caused numerically more mortality than the untreated, but no treatments were significantly different from the untreated leaves because of variability among replicates and high control mortality.

After 1 week of leaf aging under field conditions, no differences in the number of dead beetles were found among the different insecticides after beetles were exposed for 24 h to residues ($F_{6,63} = 1.83$, $P > 0.05$). After beetles were held with leaves for 72 h, there was a significant effect of treatment ($F_{6,63} = 1.83$, $P = 0.015$), with azinphosmethyl the only treatment causing significantly greater mortality than the untreated. The high degree of control variability in the untreated leaves meant that no other treatments caused a statistically-different mortality from the untreated.

The percentage of adults displaying knockdown symptoms after 24 h was significantly different among treatments ($F_{6,63} = 4.38$, $P = 0.009$) (table 2). Fenpropathrin and imidacloprid caused the greatest amount of knockdown of beetles, while azinphosmethyl caused significantly less knockdown than these insecticides, with activity that was not significantly different from the rest of the reduced-risk compounds. As the residues aged in the vineyard, there was an overall reduction in the proportion of beetles exhibiting knockdown symptoms, although a significant treatment effect remained after 3 days of residue aging ($F_{6,63} = 4.38$, $P = 0.009$) and 1 week of residue aging ($F_{6,63} = 6.23$, $P < 0.0001$). Three days after application, residues of every treatment except kaolin caused some knockdown, with the greatest activity from imidacloprid and azinphosmethyl. By 1 week after application, the knockdown activity of azinphosmethyl and azadirachtin had expired, but a significant degree of knockdown was retained by imidacloprid and fenpropathrin (table 2).

Table 1. *Percent mortality of adult rose chafer beetles after 24 or 72 h exposure to leaves with residues of different insecticides aged under vineyard conditions for 1, 3, or 7 days*

| | Age of residue (days) | | | | | |
| | 1 | | 3 | | 7 | |
Treatment	24 h	72 h	24 h	72 h	24 h	72 h
Azinphosmethyl	69.0 ± 6.0 a	99.0 ± 1.0 a	9.0 ± 2.8 a	48.0 ± 9.8 a	13.0 ± 4.2 a	29.5 ± 4.3 a
Fenpropathrin	14.0 ± 3.4 b	53.0 ± 3.9 b	6.0 ± 3.4 a	21.0 ± 7.4 ab	5.0 ± 2.2 a	16.7 ± 4.1 bc
Imidacloprid	8.0 ± 2.5 bc	38.0 ± 5.7 c	5.0 ± 1.7 ab	17.0 ± 3.7 ab	6.0 ± 3.1 a	21.0 ± 4.2 ab
Azadirachtin	11.0 ± 3.5 bc	57.0 ± 6.2 b	5.0 ± 2.7 ab	33.0 ± 8.9 ab	3.0 ± 1.5 a	10.4 ± 1.7 c
Capsaicin	3.0 ± 2.1 bc	30.0 ± 3.7 c	1.0 ± 1.0 ab	12.0 ± 2.9 b	3.0 ± 1.5 a	11.1 ± 2.6 bc
Kaolin	5.0 ± 3.0 bc	42.0 ± 5.9 c	1.0 ± 1.0 ab	4.0 ± 1.6 b	3.0 ± 2.1 a	16.4 ± 2.1 bc
Untreated	2.0 ± 1.3 c	14.0 ± 3.0 d	0.0 ± 0.0 b	18.0 ± 4.2 ab	6.0 ± 2.7 a	15.8 ± 2.2 bc

Values are mean ± SE, and values within a column followed by the same letter are not significantly different ($P < 0.05$).

Table 2. *Per cent of rose chafer beetles displaying knockdown symptoms after 24 h exposure to leaves of* Vitis labrusca *cv. Concord treated with different insecticides. Insecticide residues were aged for 1, 3, or 7 days under vineyard conditions before exposure to beetles*

Treatment	Age of residue (days)		
	1	3	7
Azinphosmethyl	11.0 ± 2.3 b	12.0 ± 2.9 ab	1.0 ± 1.0 b
Fenpropathrin	34.0 ± 4.8 a	10.0 ± 2.9 b	8.0 ± 2.5 a
Imidacloprid	34.0 ± 7.5 a	18.0 ± 3.3 a	10.0 ± 2.9 a
Azadirachtin	6.0 ± 2.2 b	11.0 ± 2.3 b	0.0 ± 0.0 b
Capsaicin	3.0 ± 2.1 b	8.0 ± 2.9 b	0.0 ± 0.0 b
Kaolin	2.0 ± 2.0 b	5.0 ± 3.0 c	1.0 ± 1.0 b
Untreated	0.0 ± 0.0 c	0.0 ± 0.0 c	2.0 ± 1.3 b

Values are mean ± SE, and values within a column followed by the same letter are not significantly different (P < 0.05).

The area of leaf feeding by rose chafer beetles was reduced by insecticide treatments for each of the residue ages tested ($F_{6,63} > 12.21$, P < 0.0001), with some treatments providing long-term protection against defoliation (table 3). Feeding damage by rose chafers was reduced by over 90% in the presence of 1-day-old residues of azinphosmethyl, fenpropathrin, and imidacloprid, compared with the untreated leaves. When residues were 1-day old, leaves with kaolin or capsaicin residues had significantly less feeding damage than the untreated leaves but allowed greater feeding than leaves treated with azinphosmethyl, fenpropathrin, or imidacloprid. Thereafter, kaolin and capsaicin afforded no protection of leaves from feeding under these bioassay conditions. Azadirachtin residues provided some protection for the first 3 days, but their activity was gone by 1 week after application. The high degree of feeding protection afforded by 1-day-old residues of imidacloprid was much reduced by 3 days after application, and by 1 week after application, imidacloprid reduced feeding by 40.2% compared with the untreated leaves. Residues of azinphosmethyl maintained leaf protection for 7 days, as shown by a 51.6% reduction of feeding compared with the untreated. Fenpropathrin was the

Table 3. *Average area (cm^2) of grape leaves eaten (± SE) by adult rose chafers when leaves were treated with different insecticides that were aged under vineyard conditions for varying lengths of time*

Treatment	Age of residue (days)		
	1	3	7
Azinphosmethyl	1.36 ± 1.78 d	16.56 ± 4.37 b	17.32 ± 2.89 b
Fenpropathrin	0.94 ± 0.97 d	9.57 ± 2.60 b	7.97 ± 1.69 c
Imidacloprid	3.81 ± 3.38 d	25.72 ± 4.15 b	21.41 ± 2.28 b
Azadirachtin	16.90 ± 2.67 c	27.58 ± 4.36 b	34.65 ± 4.77 a
Capsaicin	28.45 ± 4.52 b	40.37 ± 2.64 a	39.76 ± 2.19 a
Kaolin	26.82 ± 3.79 b	40.91 ± 3.36 a	33.08 ± 2.77 a
Untreated	39.15 ± 2.45 a	44.44 ± 4.50 a	35.80 ± 2.63 a

Average area of grape leaves: 1 day: 154.45 ± 2.97 cm^2; 3 day: 125.29 ± 1.87 cm^2; 7 day: 158.13 ± 2.58 cm^2.
Values within a column followed by the same letter are not significantly different (P < 0.05).

most consistently active residue for protecting leaves against feeding by rose chafer, providing 78% reduction of feeding damage after 7 days of aging under vineyard conditions.

4 Discussion

The relationships between mortality, knockdown and leaf feeding reported above provide insights into differences in activity characteristics of the insecticides tested, and provide information on potential options for control of rose chafer beetles in vineyards. Both the pyrethroid and the organophosphate insecticides provided a high degree of foliage protection, but they differed in how this effect was reached. Azinphosmethyl primarily caused mortality of beetles, which increased with the duration of exposure. Beetles in this treatment were observed to be exhibiting sublethal effects soon after their placement in the chamber. The volatility of this insecticide may have contributed to its high activity in the confines of the bioassay chamber. The combined effect of the mortality and knockdown resulted in little feeding on fresh residues, but as the residues aged and the immediate mortality declined, feeding levels increased. In contrast, the initial activity of fenpropathrin was primarily through strong knockdown activity, as reported for other pyrethroids against beetle pests (PREE et al., 1996). Beetles in this treatment were quickly affected by the fresh residues, and although the level of knockdown declined after 3 days of aging, beetles were still highly unlikely to feed on leaves that were treated 7–10 days earlier.

Imidacloprid was the most active reduced-risk insecticide tested, providing immediate and sustained mortality and knockdown, and protection of leaves from feeding. This product is currently used in grapes primarily for leafhopper control. However, because the perceived spectrum of activity for this insecticide is restricted to homopteran insects, it has been considered of limited use to grape growers seeking coincident protection against insects from different families. The activity against Coleoptera demonstrated here may provide for greater use of this reduced-risk insecticide rather than broad-spectrum alternatives in eastern US vineyards, particularly if antifeedant sublethal effects can achieve pest management goals (e.g. NAUEN et al., 1998). The primary need may be around the time of bloom, when rose chafer and leafhoppers may require control at the same time. Additionally, vines in many areas of eastern North America are fed upon by adult Japanese beetles, *Popillia japonica* Newman, during periods when leafhoppers require control. Given the results presented here on a related member of the Melolonthinae, activity against Japanese beetles in vineyards would be expected. Recent bioassay and field evaluations in highbush blueberry, *Vaccinium corymbosum*, support this expectation, with foliar application of imidacloprid providing rapid knockdown and mortality followed by residual antifeedant activity (J. Wise and R. Isaacs, unpublished data). Imidacloprid and other neonicotinoids, such as acetamiprid that has recently received registration in US

grape production, are expected to play an expanded role in future viticultural crop protection, and may help minimize the ecological impact of pest management programs.

Azadirachtin's activity against rose chafer for 1–3 days provides evidence that this biological insecticide can be incorporated into pest management programs for short-term activity against this pest. This was the most promising treatment that can be used in organic production, and although there was little evidence of knockdown activity, 1-day-old residues caused a moderate level of mortality and reduction in feeding damage. Antifeedant activity of this compound has been reported previously for other insects (MORDUE and BLACKWELL, 1993; MORDUE et al., 1998; ENRIZ et al., 2000). The very low level of activity against the beetles exhibited by capsaicin and its minimal protection against feeding by adult beetles indicates that this is not an effective option for control of this pest. Capsaicin was also ineffective as an olfactory repellent against the cabbage maggot, *Delia radicum*, in trials where it was added to a physical barrier placed around the base of cabbage plants (HOFFMANN et al., 2001).

Kaolin particle films have has been shown to be an effective alternative approach for some pest management needs (GLENN et al., 1999; KNIGHT et al., 2000; UNRUH et al., 2000), but results from our assays provided little evidence of activity against rose chafers. However, more repeated applications may be required to achieve sufficient coverage of the leaves to deter feeding by this pest, and the compound's activity may be greater under field conditions where beetles can disperse from treated areas. Application of a coating to the plant may be expected to have detrimental horticultural impacts. Although benefits in terms of reduced sun scald and less overheating in fruit have been reported (GLENN et al., 2002), reduced carbon assimilation and yields have been found in apples treated with kaolin (GLENN et al., 2003). The short growing season and relatively low light intensity in many eastern US grape growing regions indicates that the horticultural effects of kaolin should be determined before widespread use of this product on grapes.

The vineyard-based bioassay approach described here, similar to that of WILLIAMS (1979), provided an effective method for comparison of lethal and sublethal activities of different insecticides against this pest, and should find utility for other crop–pest combinations. Conducting bioassays in this way, with controlled laboratory evaluation of mortality, knockdown and feeding on residues aged under field conditions provided insight into the activity characteristics of different compounds under evaluation. Azinphosmethyl provided the overall highest and consistent levels of lethal activity, but as residues aged in the field, longer exposure time and ingestion were increasingly important for causing beetle mortality. The lethal activity of fenpropathrin was comparatively short-lived, but its knockdown effect was significant across all of the aged residue tests. This finding, along with the consistently best protection from defoliation, suggests that the knockdown activity is caused by beetle contact with surface residues, rather than ingestion. The imidaclo-

prid data trends suggest that freshly applied surface residues will kill the beetles before defoliation can occur, but later as residues are absorbed into the plant, ingestion is important to attaining lethal or sublethal responses. This also indicates that the beetle knockdown from imidacloprid is sublethal poisoning rather than true repellency. Capsaicin and kaolin provided limited lethal and sublethal activity and only after the maximum exposure periods. A similar trend in defoliation and knockdown data suggests that these compounds do not act as repellents to the rose chafer, but that ingestion may play a role in mortality. Although residues of azadirachtin were lethal only when 1-day old, knockdown effects persisted for up to 3 days. The high initial mortality required extended exposure and was accompanied by relatively high defoliation (compared with imidacloprid), suggesting that ingestion may be important for lethal activity.

If further restrictions on broad-spectrum insecticides are implemented for this sector of the US fruit industry, some of the reduced-risk insecticides tested here can be integrated into management programs to protect vines against defoliation by this pest when populations reach damaging levels.

Acknowledgements

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