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# Evaluation of Davren™, an Amorphous Silica Product, for Protection of Almonds in Storage

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**Objectives:**

Our first objective was to determine the basic susceptibility of seven common stored product pests belonging to the Coleoptera and Lepidoptera that can infest stored almonds to Davren, using small scale laboratory trials/assays. For purposes of comparison, one preharvest insect pest of almonds, the Navel Orangeworm, was also included in this study, bringing our total to eight species tested.

Our second objective, which was not met, was to conduct larger scale trials (storage bin) using Davren.

**Interpretive Summary:**

Tree nut crops in storage can be attacked by several coleopteran (beetle) and lepidopteran (moth) insect pests and currently the almond industry relies on fumigation for the postharvest control of insect pests. The use of fumigants such as phosphine is under intense regulatory scrutiny, on both domestic and international fronts, necessitating alternative methods to protect stored almonds. One alternative is silica/inert dusts, which have a long history of use in agriculture. They function as insecticides by absorbing lipids from the waxy outer layer of insects' exoskeletons, causing them to dehydrate and for some products there is also an abrasive component to their mode of action. However, silica products have significant limitations including a longer insect kill time and higher dosage rates.

This study reports the efficacy of a synthetic amorphous silica (SAS) product called Davren™, against eight insect species in a series of 22 laboratory trials. Seven species are worldwide pests of stored products and one species is a preharvest pest of almonds. Davren primarily consists of SAS, present in a high degree of purity combined with a proprietary mechanical application technology that maximizes insect control. Previous research on the use of SAS has focused on grain and there is little information available about its ability to protect almonds in storage.

The insects assessed were four species of stored product beetles and four species of moths (three species are stored product pests, and one species is a preharvest pest). Davren was directly applied to the top of almonds (7 ounces, 185 nuts per jar) in glass jars and the insects were then added; these trials did not use the proprietary technology to “activate” the product. The beetle assays took 7-21 days while the moth assays were scored after 21 days. With one exception, the moths were much more susceptible to Davren than the beetles. The susceptible beetle, the sawtoothed grain beetle, was killed by very low levels of Davren (100 parts per million) and was as susceptible as any caterpillar to this silica product. Among the four species of caterpillars, with one exception, exposure to Davren decreased almond damage by as much as 58%.

These results are quite promising for controlling stored product moth pests but are problematic for beetles. The low beetle mortality observed may be a function of application, since the proprietary technology was not used, or reflect some innate resistance in the lines of beetles present in the colonies. This last possibility is unlikely and one beetle species, the sawtoothed grain beetle, was quite susceptible at low levels of Davren. Since this is an important stored product pest throughout the world, this result is quite promising.

In conclusion, Davren significantly increased mortality in at least one trial for every species tested, and all caterpillars exposed were vulnerable. Although the beetles tested were vulnerable, with one exception, the mortality produced was not sufficient for control. Further research is needed to determine if the proprietary technology can boost the mortality of these beetles in almonds, as well as to determine if the mortality of caterpillars can be further increased.

### **Materials and Methods:**

Davren: Davren was received as an amorphous powder and stored in the laboratory at room temperature (77-75° F).

Almonds: The almonds used were Extra #1 25/27 count Nonpareil and were stored at room temperature (77-75° F). for at least 7 days before use in the assays

Jars: All assays used glass jars with a metal screened lid. The screen on the inner lid was covered with Whatman Number 5 filter paper to limit dust exiting the jar

Insect species assessed: The four species of Coleoptera assessed were Red flour beetle, Confused flour beetle, Sawtoothed grain beetle and Merchant grain beetle. Only adults were used and were separated from their colony diet using a series of calibrated sieves and divided into groups of 100. Each jar received 100 beetles and the jars were scored at the end of the time interval by pouring the nuts onto large screens and sieving out the adults. The four species of Lepidoptera assessed were Navel Orangeworm, Mediterranean flour moth, Indian mealmoth and Almond moth. One hundred eggs were added to each jar and then incubated. At the end of the interval all nuts were spread

onto trays and the surviving larvae counted. In addition, all nuts in each jar (185 nuts per jar) were scored for feeding damage. The scientific names and number of trials conducted for each species are reported in **Table 1**.

Incubation temperature: The jars were placed in a walk-in incubator maintained at 80°F with a relative humidity of 60%. The coleopteran trials lasted from 7-21 days and every lepidopteran trial was 21 days, in order to allow time for the eggs to hatch and larvae to infest the nuts.

Addition of Davren to the almonds: All insects were added to jars containing the almonds, typically 185 almonds per jar. Davren was added and the lids were screwed on, before shaking the jars for either 30, 120, 150 and 180 seconds, depending on the trial. The control jars were shaken for a similar time. Initially, the filter paper was retained on the inner lid but in later trials the filter paper was replaced by a metal lid during shaking, in order to maximize breakdown of Davren clumps. The dose of Davren, agitation and incubation time are reported for each trial in **Table 2**.

Statistical Analysis: All trials were paired, treatment and control. For most trials there were 10 jars per treatment, comprising 1,000 adults or eggs. All beetles were scored as living or dead, while for the moths all larvae were counted, and mortality then determined by subtracting the number of larvae recovered per jar from 100. The data were pooled for each trial and analyzed by Chi Square. Relative Risk (RR) a statistic used in epidemiology, was calculated by dividing Davren mortality by Control mortality. A value of 1.0 denotes equal risk and values above 1 indicated an increase in mortality for insects exposed to Davren. For example, a RR of 1.2 indicates a 20% increase in the likelihood that a Davren-exposed individual would die relative to an individual from the control group.

## **Results and Discussion:**

Beetle Mortality: The treatment and control mortality for the four species of beetles is reported in **Table 3**. Mortality for the red flour beetle increased in  $\frac{3}{4}$  of the trials when exposed to Davren, but the highest mortality recorded was 11.6%. The results for the confused flour beetle were similar, except that the maximum mortality was 23.3%. In contrast, the sawtoothed grain beetle was quite susceptible to Davren, with a significant difference in all four trials and a maximum mortality of 95.8%. Interestingly, the closely related Merchant grain beetle was unaffected by Davren, with a maximum mortality of 4.0%. The values for Relative Risk and the statistical significance of the difference is reported in **Table 4**. The RR values for sawtoothed grain beetle are 10X higher than the values for the other three beetle species, once again indicating that this species was the most susceptible. There was no dose-response, but mortality increased somewhat as agitation time increased.

Moth Mortality: The treatment and control mortality for the four species of moths is reported in **Table 5**. Note that the Control mortality was much higher for moths than for the beetles, and most likely resulted from the desiccation of eggs and/or newly emerged

caterpillars eating adjacent eggs, combined with the mortality resulting from feeding on almonds (almonds are protected by chemicals in the outer skin/pellicle such as chlorogenic acid). Nonetheless, caterpillars exposed to Davren experienced significant mortality as high as 87.6%, and had RR as high as 1.9, indicating that they were 90% more likely to die compared to a control larva (**Table 6**). These magnitudes are not as great as those observed for the beetles because the background mortality was much higher. The Mediterranean flour moth was the most susceptible to Davren.

Kernel Infestation by Caterpillars: With the exception of the Almond Moth, Davren exposure reduced kernel damage by as much as 59% in the laboratory trials, **Table 7**. These studies demonstrated that application efficacy is going to determine the success of Davren. There was no straightforward dose response and the factor that was most critical was agitation time. Efficacy will depend on the formation of toxic nanoparticles and any factor that increases clumping and/or results in clumps remaining intact rather than fragmenting will decrease the effect of Davren. The proprietary application technology presumably will increase the number of active/toxic particles per unit of surface or else ensure an even distribution. The importance of application is further underscored by the physical difference between almonds and stored grain. Grain packs tightly and insects moving through grain to feed will come into close contact with any SAS product present, In contrast, almonds do not pack tightly and there are large spaces where beetles can shelter and contact only a small portion of the treated nuts. This is illustrated by the mortality difference between beetles and moths. The overall higher mortality for the lepidoptera tested may simply reflect feeding differences and if the caterpillars spend more time crawling over surfaces this will increase their likelihood of contacting the amount of Davren necessary to kill them. However, the susceptibility of the sawtoothed grain beetle was a surprise. Its size is similar to the other beetles tested, especially Merchant grain beetle, yet it was 15-25 times more likely to die. In contrast, there were no large differences in susceptibility among the four moth species tested.

In conclusion, moths were more susceptible to Davren than beetles, with the exception of the sawtoothed grain beetle, in this assay system. Susceptibility was species dependent, and closely related beetles varied dramatically in their vulnerability to this toxicant. Sawtoothed grain beetle mortality was as much as 40-fold higher than Merchant grain beetle, and Confused flour beetle mortality was as much as 5-fold higher than Red flour beetle mortality. These results demonstrate a basic susceptibility to this product, but further research is needed utilizing the proprietary application technology to determine if the beetle toxicity can be boosted. Since the sawtoothed grain beetle is a very important and difficult to control beetle pest, this technology may be promising to develop a novel strategy for its control. Unfortunately, we were unable to conduct the mid-scale and large-scale trials originally envisioned in our timeframe, and those studies will require a separate project.

**Table 1.** The insect species tested and number of trials.

<b>Order</b>	<b>Scientific Name</b>	<b>Common Name</b>	<b>Trials</b>
Coleoptera	<i>Tribolium castaneum</i>	Red flour beetle	4
	<i>Tribolium confusum</i>	Confused flour beetle	4
	<i>Oryzaephilus surinamensis</i>	Sawtoothed grain beetle	4
	<i>Oryzaephilus mercator</i>	Merchant grain beetle	2
Lepidoptera	<i>Amyelois transitella</i>	Navel Orangeworm	2
	<i>Ephestia kuehniella</i>	Mediterranean flour moth	3
	<i>Plodia interpunctella</i>	Indian mealmoth	2
	<i>Cadra cautella</i>	Almond moth	1

**Table 2.** Trial information in chronological order for each species assayed

<b>Species</b>	<b>Dose in parts per million (ppm)</b>	<b>Agitation time</b>	<b>Days exposed</b>
Red flour beetle	100	30 sec	7
Red flour beetle	100	120 sec	14
Red flour beetle	250	180 sec	21
Red flour beetle	500	180 sec	21
Confused flour beetle	100	30 sec	14
Confused flour beetle	100	120 sec	14
Confused flour beetle	250	180 sec	21
Confused flour beetle	500	180 sec	21
Sawtoothed grain beetle	100	150 sec	7
Sawtoothed grain beetle	100	150 sec	7
Sawtoothed grain beetle	100	120 sec	21
Sawtoothed grain beetle	100	180 sec	14
Merchant grain beetle	100	180 sec	14
Merchant grain beetle	250	180 sec	21
Navel Orangeworm	100	180 sec	21
Navel Orangeworm	250	150 sec	21
Mediterranean flour moth	100	150 sec	21
Mediterranean flour moth	200	150 sec	21
Mediterranean flour moth	250	150 sec	21
Indian mealmoth	100	120 sec	21
Indian mealmoth	250	150 sec	21
Almond moth	100	150 sec	21

**Table 3.** Mortality following exposure to Davren for beetles. There were 1,000 beetles per treatment unless otherwise specified

<b>Species</b>	<b>Dose*</b>	<b>Days</b>	<b>Davren Mortality%</b>	<b>Control Mortality %</b>
Red flour beetle	100	7	4.2	2.2
Red flour beetle	100	14	11.6	7.2
Red flour beetle	250	21	5.8	6.6
Red flour beetle	500	21	4.8	0.8
			(29/600)	(5/600)
Confused flour beetle	100	14	3.1	2.5
Confused flour beetle	100	14	5.0	3.0
Confused flour beetle	250	21	9.9	1.6
Confused flour beetle	500	21	23.3	9.0
			(140/600)	(54/600)
Sawtoothed grain beetle	100	7	86.3	3.0
Sawtoothed grain beetle	100	7	69.7	5.4
Sawtoothed grain beetle	100	21	95.8	5.5
			(1,532/1,600)	
Sawtoothed grain beetle	100	14	62.8	5.1
Merchant grain beetle	100	14	2.2	1.5
Merchant grain beetle	250	21	4.0	0.8

**Table 4.** Results for Davren exposure to beetles expressed as Relative Risk

Species	Dose*	Days	Relative Risk	Chi Square
Red flour beetle	100	7	1.8	4.6; 0.05 > P > 0.025
Red flour beetle	100	14	1.6	10.9; P < 0.001
Red flour beetle	250	21	1.0	<b>ND**</b>
Red flour beetle	500	21	5.8	16.0; P < 0.001
Confused flour beetle	100	14	1.0	<b>ND**</b>
Confused flour beetle	100	14	1.7	4.7; 0.05 > P > 0.025
Confused flour beetle	250	21	6.2	62.0; P < 0.001
Confused flour beetle	500	21	2.6	44.4; P < 0.001
Sawtoothed grain beetle	100	7	28.8	203.7; P < 0.001
Sawtoothed grain beetle	100	7	12.9	878.8; P < 0.001
Sawtoothed grain beetle	100	21	17.7	2,107.8; P < 0.001
Sawtoothed grain beetle	100	14	12.3	739.8; P < 0.001
Merchant grain beetle	100	14	1.0	<b>ND**</b>
Merchant grain beetle	250	21	5.0	20.5; P < 0.001

\* Parts per million

\*\* No Difference



**Table 5.** Mortality following exposure to Davren for moths.

<b>Species</b>	<b>Dose*</b>	<b>Days</b>	<b>Davren Mortality%</b>	<b>Control Mortality %</b>
Navel Orangeworm	100	21	74.7	49.8
Navel Orangeworm	250	21	75.4	50.1
Mediterranean flour moth	100	21	80.2	58.4
Mediterranean flour moth	200	21	80.4	45.4
Mediterranean flour moth	250	21	82.3	51.7
Indian mealmoth	100	21	58.6	45.0
Indian mealmoth	250	21	60.2	32.3
Almond moth	100	21	87.6	80.7

**Table 6.** Results for Davren exposure to lepidoptera expressed as Relative Risk

<b>Species</b>	<b>Dose*</b>	<b>Days</b>	<b>Relative Risk</b>	<b>Chi Square</b>
Navel Orangeworm	100	21	1.4	89.8; <i>P</i> < 0.001
Navel Orangeworm	250	21	1.5	151.5; <i>P</i> < 0.001
Mediterranean flour moth	100	21	1.4	110.7; <i>P</i> < 0.001
Mediterranean flour moth	200	21	1.8	261.0; <i>P</i> < 0.001
Mediterranean flour moth	250	21	1.6	126.6; <i>P</i> < 0.001
Indian mealmoth	100	21	1.3	36.5; <i>P</i> < 0.001
Indian mealmoth	250	21	1.9	92.4; <i>P</i> < 0.001
Almond moth	100	21	1.1	17.3; <i>P</i> < 0.001

\*Parts per million

**Table 7.** Infestation of almond kernels following exposure to Davren for moths. All differences are significant ( $P \leq 0.05$ ) except for Almond moth

<b>Species</b>	<b>Dose*</b>	<b>Days</b>	<b>Davren Infestation%</b>	<b>Control Infestation%</b>
Navel Orangeworm	100	21	23.2	33.4
Navel Orangeworm	250	21	19.5	40.7
Mediterranean flour moth	100	21	39.7	82.3
Mediterranean flour moth	200	21	31.2	75.3
Mediterranean flour moth	250	21	22.3	47.8
Indian mealmoth	250	21	42.9	70.6
Almond moth	100	21	24.0	24.3