Project Number 78-D4

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PROJECT NO.: 78-D - Navel Orangeworm Research
(Continuation of Project No. 77-D)
Part A - Controlled Atmospheres
Part B - Varietal Resistance

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Part 1 - Controlled Atmospheres

I. Objectives: To determine (1) effectiveness of a low oxygen controlled atmosphere for disinfesting stored almonds; (2) time for complete navel orangeworm mortality; (3) the most effective method of controlled atmosphere application to stored almonds.

II. Interpretive Summary: A 3,000 cubic foot bin of inshell Nonpareil almonds was successfully treated with controlled atmosphere. Within 18 hours, the oxygen content was reduced to 0.5% with a purge rate of 500 cu ft/hr. Merchant grain beetle died 28 hrs after 0.5% oxygen was attained and red flour beetle and Indian meal moth in 40 hours. Seventy-five percent of the navel orangeworm pupae were killed in 5.5 days. Low temperatures occurring during the test probably contributed to the navel orangeworm survival.

Laboratory studies of twelve gas mixtures with oxygen concentration at 0, 0.3 or 1%; carbon dioxide concentration at 0, 2, 10 or 20%; and the balance nitrogen were tested against the navel orangeworm to determine the most effective combination for killing this pest. The results indicate that the best mixture consists of 0.3% oxygen with the balance nitrogen. This gas reduced the time-to-kill by about 20% as compared to the mixture 0.3% oxygen and 10% carbon dioxide, which approximates the product gas of a controlled atmosphere generator. Generally the results indicate that it is desirable to have some oxygen in the atmosphere (0.3%), and the presence of some carbon dioxide tends to inhibit the mortality of this pest.

Other laboratory studies of the navel orangeworm are continuing. Temperatures of 60, 70 or 80°F; relative humidities of 40 or 60%; with oxygen concentrations of 0.5, 1, 2, or 5% are under evaluation. Preliminary evaluation indicates that reductions in treatment times by 35-45% can be made if the relative humidity is 40% as compared to 60%. Treatment at 80°F will reduce treatment times by 15-38% compared to 70°F.

III. Experimental Procedure:

Field Study:--A field test utilizing a bolted steel bin 12' diameter by 23' high (ca. 3000 cu ft) was conducted. The bin was filled to within 5' of the top with inshell Nonpareil almonds. A plastic pipe was placed down the center of the bin so that the atmosphere was released at the bottom center of the bin. Gas sample lines and temperature probes were attached to the plastic pipe. Gas sample ports were located at 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26 and 28 feet from the bottom and temperature sensors at 0, 14, and 28 feet from the bottom. Sites above 24 feet were in the headspace.

Insect samples were suspended in the headspace about 1' above the almond surface. Time of sampling was at 12 hour intervals; from 26-106 hours for the merchant grain beetle and red flour beetle, 58-112 hours for Indian meal moth and 70-154 hours for navel orangeworm.

An experimental 500 cu ft/hr generator operating at 50 cu ft/hr natural gas and 500 cu ft/hr air was utilized as the atmosphere source. The operational parameters of the machine were determined daily by sampling the output gas and determining its oxygen, carbon dioxide, nitrogen, argon, carbon monoxide and methane content with a Fischer gas chromatograph. Oxygen content within the bin was determined with a paramagnetic oxygen analyzer. Oxygen sample times were 0, 1, 2, 3, 4, 6, 12, 18, 24 hrs and every 12 hrs thereafter until the end of the test. Insect mortality was determined 1½ weeks after exposure.

Laboratory Study A:--Laboratory studies of twelve gas mixtures with oxygen concentration at 0, 0.3 or 1%; carbon dioxide at 0, 2, 10 or 20%; and the balance nitrogen were tested against the navel orangeworm (NOW) to determine the most effective mixture for killing this pest. Quart jars containing thirty 25-day old NOW larvae were treated with the atmosphere at 80°F and 50% relative humdity. At time intervals of 12 hrs jars were removed from treatment, aired and held for adult emergence. Treatment times ranged from 12 hrs to 84 hrs. All treatments were replicated a minimum of 3 times.

Laboratory Study B:--Laboratory studies to evaluate the parameters of temperature, relative humdity, and oxygen concentration on the navel orangeworm are continuing. Temperatures of 60, 70 or 80°F; relative humdities of 40 or 60%; with oxygen concentrations of 0.5, 1, 2 or 5% are under evaluation. Quart jars containing thirty 25-day old NOW larvae are being treated under the above conditions with treatment times from 24 hrs to 192 hrs in 12 hr increments. All treatments were replicated a minimum of three times. At this time pending statistical evaluation of the data by computer all treatments at 70 and 80°F have been completed.

IV. Results:

Field Study:--Oxygen content within the bin was reduced to 0.5% within 18 hours in all parts of the bin. Merchant grain beetle adults died 28 hrs after 0.5% oxygen was attained, red flour beetle adults and Indian meal moth pupae in 40 hrs, and 75% of the navel orangeworm pupae in 5.5 days. The emerged navel orangeworm did not produce viable eggs. Temperature varied from 93°F to 33°F in the overhead space. Nut temperatures averaged 66°F at the start and 48°F at the end of the test.

Laboratory Study A:--To statistically determine the time required to kill 95% (LT_{95}) of the insects, the percent killed at each time interval was transformed to logits and plotted against time for each treatment gas mixture. The LT_{95} values (in hours) as determined by computer analysis for the mixtures are:

% oxygen	% carbon dioxide 0	2	10	20
		LT_{95} (hr)		
0	65.2	71.5*	76*	77*
0.3	52.8	59.0	67.9	68.0
1	79.7	85.0	84.3	70.8

*These values were determined by an eye fit of the logit line.

Laboratory Study B:--The percent killed at each time interval was transformed to logits and plotted against time for each treatment. A line fitted by eye on each graph was used to estimate the LT_{95} value. The estimated LT_{95} values (in hours) at the two completed temperatures are:

	Temperature 70°F RH		80°F RH	
% oxygen	40%	60%	40%	60%
		LT_{95} (hrs)		
0.5	66	103	45	69
1	69	124	43	77
2	68	123	48	86
5	79	154	68	not determined

V. Discussion:

Field Study:--This test showed that a 3,000 cu ft bin of almonds could be successfully treated with a 500 cu ft/hr volume of low oxygen atmosphere. Insect mortality occurred as expected for the low temperatures encountered.

Laboratory Study A:--Several trends appear from these values. The most effective oxygen concentration is 0.3%, apparently a small amount of oxygen somehow contributes to a faster mortality rate. Increasing the carbon dioxide inhibits mortality of the insects at 0% and 0.3% oxygen concentrations, but at 1% oxygen the higher carbon dioxide level was most effective. At this concentration the effects of carbon dioxide toxicity are probably being expressed. Higher concentrations of carbon dioxide (40-60%) in air have been shown to be insecticidal.

The fastest killing mixture (0.3% O_2, balance N_2) gave a 20% reduction in time to kill as compared to the mixture approximating the product gas of a controlled atmosphere generator (0.3% O_2, 10%, CO_2, balance N_2) This study indicates that it is desirable to have a small amount of oxygen present (0.3%) and preferably no carbon dioxide. The carbon dioxide may be scrubbed from the generator gas but the cost of doing so would exceed the benefits derived.

Laboratory Study B:--A thorough evaluation will have to await completion of treatments at the third temperature and computer analysis of the data. Preliminary evaluations indicated that a reduction in treatment times by 35-45% can be achieved when treated at 40% RH as compared to 60% RH. If the relative humidity is about 40% the oxygen content can be as high as 2% without appreciably lengthening the treatment time. The relative humidity of almonds in storage is determined by commodity moisture content and cannot be controlled by the treatment gas. Treatment at 80°F can reduce the time from 15-38% as compared to treatments at 70°F.

VI. Publications:

D. G. Guadagni, E. L. Soderstrom, and C. L. Storey 1978. Effect of controlled atmosphere on flavor stability of almonds. Journal of Food Science, Vol. 43: 1077-1080.

Part 2 - Varietal Resistance

I. Objectives: To investigate the nonvolatile chemical components of almond hulls that are responsible for navel orangeworm delayed development.

II. Interpretive Summary: Previous studies determined that there is a factor in Mission cultivar hulls which inhibits navel orangeworm development. Studies this past year comparing two quantities of almond hulls incorporated in an agar diet have demonstrated that a large dose (4X) of hulls even further inhibits development. A water extraction of hulls does not have this effect, indicating that there is a water insoluble factor or factors in Mission hulls that inhibits NOW development. Mission hulls from several locations have been collected from the 1978 crop and sent to Dr. Ron Buttery at USDA, Western Regional Laboratory for chemical analysis. Extracts will be incorporated into navel orangeworm diet for analysis and identification of the factor(s).

III. Experimental Procedure: Ground almond hulls were incorporated into an agar diet (Bio Serv Cabbage Looper Rearing Diet 773-A) used to rear navel orangeworm. Seventy grams of Mission variety hulls were soaked in 500 ml H_2O and 50 or 200 ml aliquots were incorporated in the agar diet. A 275 ml aliquot plus an additional 100 ml water was centrifuged for 5 min at 1500 rpm the supernatant then recentrifuged at 2500 rpm for 30 min. A 155 ml aliquot of the supernatant was substituted for water in the diet. The diets were poured into 35 x 10 mm petri dishes. One navel orangeworm egg was placed in each dish. The cultures were reared at $80^{o}F$ and 60% RH. Daily observations were made for adult emergence. Twenty individuals were observed for each of three replicates. Elapsed times for emergence were averaged and analysis of variance determined.

IV. Results: The 50 ml aliquot produced a 3 day delay in navel orangeworm development at the 50% emergence; while the 200 ml aliquot produced an additional 6 day delay. The water extract did not produce a delay.

V. Discussion: The hull resistance factor is shown to be nonnutritional, insoluble in water, and quantitative. This is encouraging for finding a possible chemical that may be exploited in breeding programs and as a possible spray to retard navel orangeworm development thus preventing recycling of navel orangeworm on almonds between hull split and harvest.