USE OF PROPIONIC ACID FOR SHORT DURATION STORAGE OF HIGH MOISTURE PEANUTS

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STORAGE OF HIGH MOISTURE PEANUTS

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CHAPTER I

INTRODUCTION

Background Information

Freshly dug peanuts may be left in the field until adequately cured before harvesting. However, the possibility of inclement weather during this curing period induces most farmers to dry at least a portion of their crops in a commercial dryer. Drying facilities are frequently overloaded, especially when unfavorable weather conditions cause a large number of farmers to harvest high moisture peanuts in a short period of time. Under these circumstances, peanuts may be held as much as three days or more between combining and drying (2, 30). Holding peanuts under conditions of high moisture for a period of time increases susceptibility to aflatoxin contamination (41). Results of holding treatment studies by J. L. Butler (9) showed that traces of aflatoxin were found after holding for as short a time as 24 hours. In a study of 65 truck loads remaining on dryer lots for at least one night, mold was observed in 23 (39).

To appreciate and understand the concern expressed over aflatoxin production, it is imperative to know something about aflatoxin. Aflatoxin is a toxic substance produced by mold (35). It is among the most potent carcinogens and hepatotoxins thus far encountered. Aspergillus flavus, one of the molds found most often in damaged peanuts, is most frequently linked with the production of aflatoxin although other molds

can also be sources of aflatoxin production (2, 6, 7, 41). Some of these alternate aflatoxin-producing molds include Penicillium puberulum, P. citrinum, P. frequentans, P. variable, Aspergillus niger, A. parasiticus, A. ruber, and A. wentii (18, 33).

The potential problem of aflatoxin contamination of food first came to light in Great Britain in 1960 when peanut meals contaminated with A. flavus decimated several poultry flocks (2). Investigation has shown that aflatoxin is toxic to ducklings, turkeys, swine, cattle, sheep, pheasants, chickens, trout, guinea pigs, rats, and monkeys (5, 6, 7, 19, 21, 29, 34, 35, 36). Susceptibility varies among those animals listed with the young being generally more susceptible than the mature of a species (3, 33).

Although aflatoxin toxicity to man has not yet been demonstrated on a large scale, individual cases of hepatitis in man have been traced to consumption of aflatoxin-contaminated food (2, 7, 24, 28, 32, 37). For this reason, growers, processors, and government officials throughout the world are concerned (6). The Food, Drug, and Cosmetic Act permits seizure of adulterated foods to remove them from the marketplace. Because there are as yet no pharmacological data indicating a safe level of aflatoxins in man or in any laboratory test animal (except sheep) and because aflatoxins are carcinogens, no tolerance can be set. The Food and Drug Administration can detect a few ppb of aflatoxins in a finished food (35). So, even though aflatoxin is highly localized, it is possible that a large quantity of peanuts could be condemned even though actually only a very small percentage was contaminated (19).

One channel is open to contaminated peanuts. They may be used for pressing oils, because the refining operation completely removes afla-

toxin. However, the cake cannot be used as a food, only as a fertilizer (6, 33). Although contaminated peanuts may be salvaged in this way, the method is not satisfactory due to the economic losses involved (12).

Up to this point, the entire discussion has been centered around peanuts although many other crops have been shown to produce aflatoxin. Included in this group are rice, barley, Brazil nuts, cassana, cocoa beans, copra, coconut oil cake, locust beans, palm kernels, raisins, sorghum, soya bean meal, wheat, corn, cottonseed, rye, runner beans, soybeans, buckwheat, maize meal, potatoes, and peanut meal-free poultry feed (6, 10, 18, 33). However, peanuts are singled out because: (1) aflatoxins appear more frequently in peanuts; (2) larger quantities of aflatoxins are found in peanuts; and (3) ill-effects of aflatoxin have been traced directly to peanuts (35).

Researchers have tried various methods to control aflatoxin including removal by various chemical reagents, physical separation, chemical inactivation with ammonia, prevention through proper processing procedures, genetic inhibition, various drying techniques and controlled environment storage (2, 6, 12, 13, 18, 23, 31, 41, 42). However, none of these methods has been satisfactory.

A chemical which has not been tested previously with regard to mold control in peanuts is propionic acid. Propionic acid has been shown to act as a good preservative of wet grain (8). When sprayed onto cereals as they were loaded into storage, molds and bacteria were killed within 24 hours. Trials have shown that grain at moisture contents ranging from 17% to 30% can be stored without spoiling after this treatment (11). Experimental work with grain of moisture content greater than 30% suggests that moisture content does not restrict the action

of propionic acid (25).

Objectives

The objective of this study, based on the preceding background information as well as the literature review, is to evaluate the effects of various application rates of propionic acid on: (1) aflatoxin production; (2) germination; and (3) organoleptic properties of peanuts as related to the acceptability of propionic acid for storage of high moisture peanuts.

Engineering Significance

If propionic acid proves successful, the problem of molding in the trucks during peak harvest periods could be eliminated. Acid could be applied in the fields thereby controlling the mold until drying to an acceptable moisture content. This period of time would vary with the amount of acid applied. Low applications could preserve the peanuts in trucks until drying; and higher applications could facilitate storage of the peanuts for months before drying. It is possible that the drying operation could be eliminated altogether depending upon the intended use of the peanuts.

Several British manufacturers have applicators on the market capable of applying propionic acid to moist grains while loading into storage. The acid is applied through nozzles in an auger shaft (1, 16). This type of apparatus could be modified to accommodate peanuts. In the case of truck loads of peanuts at the dryers, however, this method would not be adequate. It would be much more practical to apply the acid in the field during the harvesting operation. This would require

the design and testing of an adequate means of field application.

Propionic acid is corrosive to metal (16). So, prolonged storage of acid-treated peanuts in metal bins may cause corrosion to take place. It may be necessary, therefore, to design storage facilities of non-corrosive materials. Tests on grains have shown that it is only necessary to supply protection from rain in order to maintain the effectiveness of the acid (25). So, most existing storage facilities should be adequate. Non-corrosive wall liners could be developed to line the walls of metal bins as a corrosion deterrent.

CHAPTER II

LITERATURE REVIEW

The use of propionic acid as a moist grain preservative began in 1965 more or less by accident. BP Chemicals (U. K.) Ltd. was seeking more uses for propionic acid. This acid is produced as a co-product from their highly successful "DF" acetic acid from petroleum naptha process, which had made the company Europe's largest producer of acetic acid. Other countries took up licenses to use the "DF" process and it became apparent that more scope for the co-products would make their own plant cheaper to run and make the "DF" process attractive abroad.

A search for other materials suitable for preservation began. From bread, cake and animal feed, it was reasonable that attention should be drawn to grain. Laboratory examination of grain for microbiological development to determine how much acid would be needed as a preservative showed that approximately 0.5 to 1.25 percent by weight of propionic acid would be needed to preserve a given lot of wet grain.

A small field trial in 1965 also showed promise; and in 1966 a small number of controlled experiments were conducted including two at Dayton and High Mowthorpe Experimental Husbandry Farms (EHF). Different rates of acid were applied to small quantities of barley at various moisture contents. These were examined for microflora activity by the British National Agricultural Advisory Service regularly throughout long periods of storage up to a year.

The objective was to study the preserving action of propionic acid and the possibility of treatment becoming feasible for damp grain storage on a large scale. Molds and yeasts were greatly diminished and bacteria considerably reduced at both higher and lower rates of application coupled with higher and lower moisture contents, respectively.

In 1969, a wider range of conditions was covered at 25 locations. Nearly 1500 tons of grain, in quantities varying from as much as 100 tons in bulk to a minimum of 1 ton with a maximum depth of 15 feet, at moisture contents ranging from 16 to 30 percent were treated. The main objective was to determine acid application rates tied to grain moisture content necessary to store grain safely under general farm conditions. Present indications from these trials and from manufacturers are that propionic acid applied at 0.8 percent rate will safely store grain at 20 percent moisture content; and an application rate of 1 percent will safely store grain at 25 percent moisture (8).

In the process of preserving high-moisture grain, the biological properties are utilized to achieve virtual sterilization of the grain. The acid is retained by the grain and prevents subsequent deterioration by microbial activity for periods of at least 12 months without sealed containers or refrigeration. All that is necessary is protection from rain. Suppression of molds and bacteria not only prevents dry matter loss thereby preserving the full feeding value of the grain, but also ensures that the grain remains free-flowing and easily removable from storage (1, 16, 25, 38).

No objections should be raised to the use of propionic acid for health reasons. Following are several arguments to support this statement:

(1) Propionic acid is a product of ruminant digestion;

(2) If it is oxidized like other fatty acids in the body, pyruvic acid, a common metabolite, would be formed;

(3) Propionic acid is found naturally occurring in many foods (e.g. 1% in Swiss cheese);

(4) There is evidence that propionates can be utilized by the body for the synthesis of glycogen;

(5) It has been shown that calcium and sodium propionates (salts of propionic acid) are non-toxic in doses up to 3% of the diet in the case of white rats;

(6) Propionic acid is being used as a preservative to increase shelf life of bread;

(7) Propionic acid is generally recognized as a safe chemical to be used as a preservative without specific restrictions under the Food, Drug, and Cosmetic Act; and

(8) Propionic-acid-treated grain has been fed satisfactorily to beef and dairy cattle, pigs, sheep and poultry. Palatability is not impaired, feed conversions and growth rates are as good or better than with untreated grain, and the livestock show no adverse effects (8, 17, 22, 26, 27).

As of 1970, merchants in Great Britain were beginning to accept propionic-acid-treated feed grain (1). This would indicate they are satisfied that the acid is acceptable for feed grains.

Propionic acid does have some disadvantages. For instance, concentrated propionic acid is irritative in smell, highly corrosive and splashes of this chemical could cause serious damage to skin and eyes (8). However, if routine precautions are taken, the acid is safe

enough to handle. Equipment corrosion through using the acid is negligible since only a minute dressing is applied (1).

Acids should not be used on grain for malting or seed since germination is killed (11, 25). This should present no particular problem. It merely implies that the peanut seed producer must forego this system of mold prevention. In Great Britain, propionic acid cannot be used on grains destined for flour milling since the amount of acid needed is greater than that legally permitted (11, 15, 25). However, as stated earlier, no tolerance is set by the Food, Drug and Cosmetic Act.

An important advantage of propionic acid treatment over other methods of storage is that its effectiveness persists even after removal from storage. So, moist grain can be handled and transported without subsequent deterioration. Treated grain can even be dried, ground or rolled without losing its preservative action (1, 25, 38).

Although unaware of trials being conducted on high moisture peanuts, Taylor (40) stated that tests have been conducted on wheat, oats, barley, corn, field beans, soybeans, peas, ground coconut shells and bagasse. He stated that similar rates have proven effective in all these tests. These rates form a straight line relationship varying from 1/2% for 15% grain to 2% for 35% grain.

CHAPTER III

EXPERIMENTAL DESIGN

The design of the experiment consisted essentially of setting treatment levels to effectively accomplish the objectives.

Two field treatments used were: (1) harvest immediately after digging and (2) field cure 3 days. It was anticipated that these field treatments would produce moisture contents of 40 and 30% (wet basis), respectively. These field treatments provide two possibilities which could conceivably occur during actual harvesting conditions. Those harvested immediately after digging give an extreme case with an excessive moisture content. The second treatment with a field curing of 3 days is more realistic as an event that is likely to occur under actual harvesting conditions.

Ten application rates of propionic acid were to be tested for each field treatment. These were 0.00, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00, 2.50, and 3.00% by weight. Based on recommended rates from the literature it was reasonable to assume that similar rates would be effective on peanuts and that the rates chosen would span the range satisfactorily. Figure 1 shows graphically the rates recommended by Bee (8) and Taylor (40). In the case of Bee the recommended rates were based on tests with barley. Those rates recommended by Taylor have been proven effective in tests conducted on wheat, oats, barley, corn, etc. as stated in the literature review.

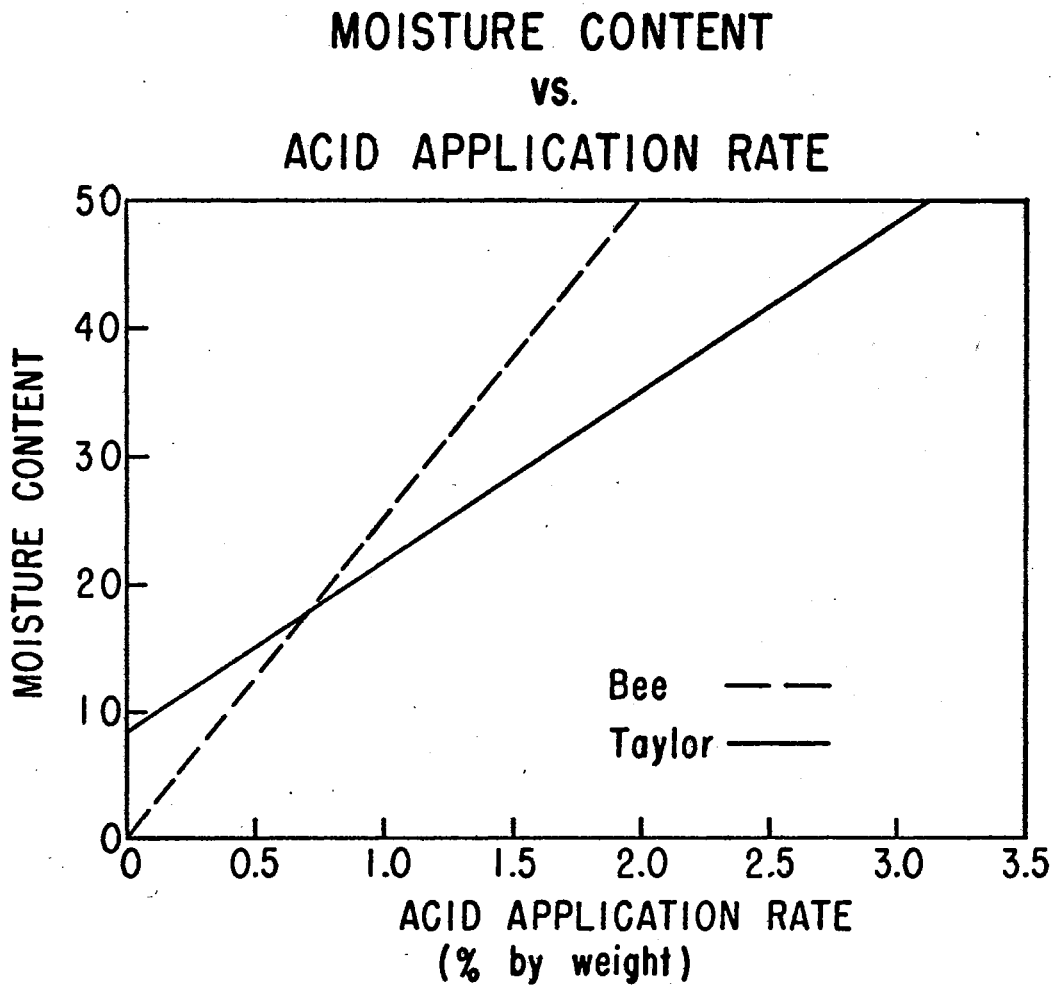

Figure 1. Acid Application Rates Recommended by Bee (8) and Taylor (40)

One temperature level, the ambient temperature of the laboratory, was decided upon for this study. This temperature (70-75°F) would be conducive to mold production and, therefore, should give a good measure of the efficacy of propionic acid for aflatoxin prevention.

It was decided that four replications of each application rate would be necessary. Three replications were to be inoculated with Aspergillus flavus spores to be used for aflatoxin assays and germination tests. The fourth replication was not to be inoculated and was to be used for organoleptic evaluations.

A summary of the experimental design based on the preceding discussion is shown in Table I. In all subsequent discussions, the following notation will be used: the first number denotes the initial moisture content (wet basis); the second number denotes the propionic acid application rate (% by weight); the third number designates the replication; and the fourth number designates the number of days following the acid application until the sample was taken. Example: 40 - 1.00 - 2 - 2.

TABLE I

THE EXPERIMENTAL DESIGN

Test No.	Temperature °F	Anticipated Moisture Content, % w.b.	Acid Application Rate, % by wt.	Replications	Sampling Days*
1	70 - 75	40	0.00	1, 2, 3, 4	1, 2, 3, 5, 8, 14
2			0.50		
3			0.75		
4			1.00		
5			1.25		
6			1.50		
7			1.75		
8			2.00		
9			2.50		
10			3.00		
11	70 - 75	30	0.00	1, 2, 3, 4	1, 2, 3, 5, 8, 14
12			0.50		
13			0.75		
14			1.00		
15			1.25		
16			1.50		
17			1.75		
18			2.00		
19			2.50		
20			3.00		

*Sampling Days refers to the number of days of storage between the acid application and sampling.

CHAPTER IV

EXPERIMENTAL PROCEDURES AND EQUIPMENT

Prior to bringing the peanuts into the laboratory the individual quantities of propionic acid to be applied were weighed into bottles and stored. The acid was a reagent grade purchased from Curtin Scientific Co. Also, a dense suspension of A. flavus spores supplied by the Botany and Plant Pathology Department was produced by washing cultures of the spores into a water suspension. These spores were then stored under refrigeration the night prior to actual inoculation of the peanuts.

For each field treatment, 1000 pounds of Starr variety Spanish peanuts was brought into the laboratory and sorted to remove much of the foreign material and many immature peanuts. The first load of peanuts was sorted by hand. However, this was a formidable task. For the succeeding field treatments a mechanical peanut cleaner was borrowed from the Agronomy Department. Visual examination of the peanuts sorted mechanically suggested they were as free of foreign material as those sorted by hand.

Following the sorting process, the peanuts were weighed into 25-pound lots and placed in the storage bins. As stated previously there were four replications for each acid application rate. Three were inoculated with A. flavus spores at this stage. The inoculation process consisted of spreading each lot of peanuts on a tarp. One person would

continuously stir them while another sprayed them with the dense suspension of A. flavus spores. The fourth replication was not inoculated, but was stored as it was. All were then left overnight at which time the acid was applied. It was felt that this would give the spores some time to take hold before the acid could affect them.

Application of the propionic acid was done in a manner very similar to the A. flavus inoculation. The major difference was in the spray apparatus. A pressure regulator and a fan tip spray nozzle were incorporated so the acid could be metered out at a relatively slow rate. Admittedly, some of the acid was lost, both as a mist and on the tarp. However, it was felt that this was the most satisfactory method available. It is also felt that satisfactory coverage was attained.

The acid was applied in an enclosed area of the laboratory to minimize drift of the acid mist. Due to the pungency of the odor it was necessary to wear gas masks when in the area until application was complete. As an added safety precaution rubber gloves were worn when handling the acid.

Figure 2 shows the layout of the bins as well as covers which were constructed. These bins were made of stainless steel and measured approximately 15 inches in diameter and 23 inches tall. The decision to use these bins was based mainly on the fact that they were readily available as well as adequate for the intended use. The covers were constructed of 1/4" plywood. Their function was to help keep moisture in the peanuts and to keep mice out.

Sampling was done on each of 1, 2, 3, 5, 8, and 14 days after applying the acid. Samples were of approximately one pound each. This was sufficient in the case of the inoculated peanuts to run germination

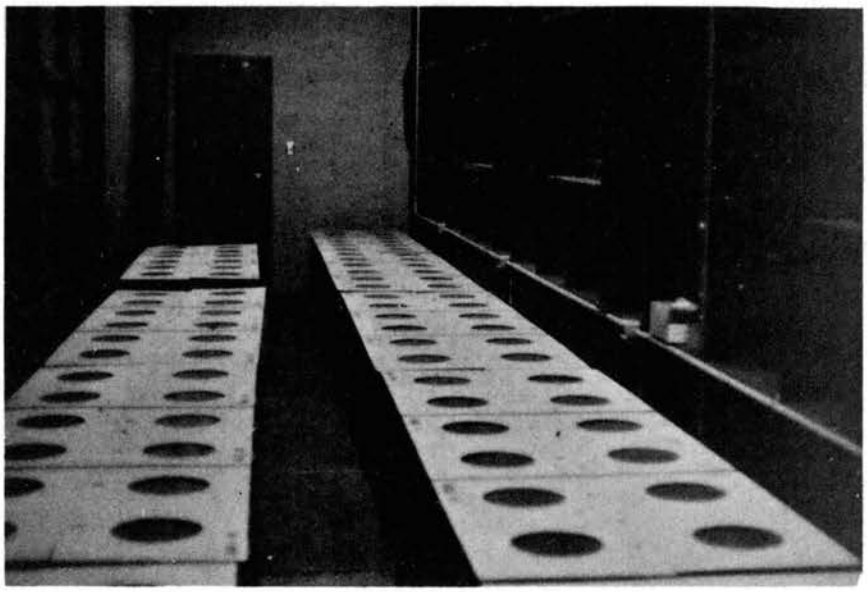

Figure 2. Bin Layout

tests and to perform an aflatoxin analysis. In the case of the uninoculated peanuts, one pound was sufficient for organoleptic (taste) evaluation. Following sampling the peanuts were dried to a safe storage moisture content (less than 10%).

A peanut dryer was needed to dry these samples. Since no dryer was available in the laboratory, it was necessary to design and construct one which would fit the needs of the project.

Based on recommendations by Allen (4), the maximum temperature allowable was 95°F and the maximum air flow was 20 cfm/ft^3 of peanuts. This led to a minimum heat requirement of 720 watts and a fan capacity of 120 cfm. The dryer was constructed of 1/2" exterior grade plywood.

Figure 3 shows the dryer as constructed. It was divided into 12 shafts of 10 drying compartments each. This gave the dryer the capacity to dry up to 120 samples simultaneously while, at the same time, being able to dry as few as 10 samples at any given time. Each shaft inlet was fitted with a sliding door with which the flow to each shaft was to be balanced. In addition each drying compartment was fitted with an equalization plate whereby the flow through each could be equalized.

Heat was supplied to the dryer by conical electric heaters. The temperature was controlled with a Brown Electronic Control Millivoltmeter Temperature Indicator and Controller.

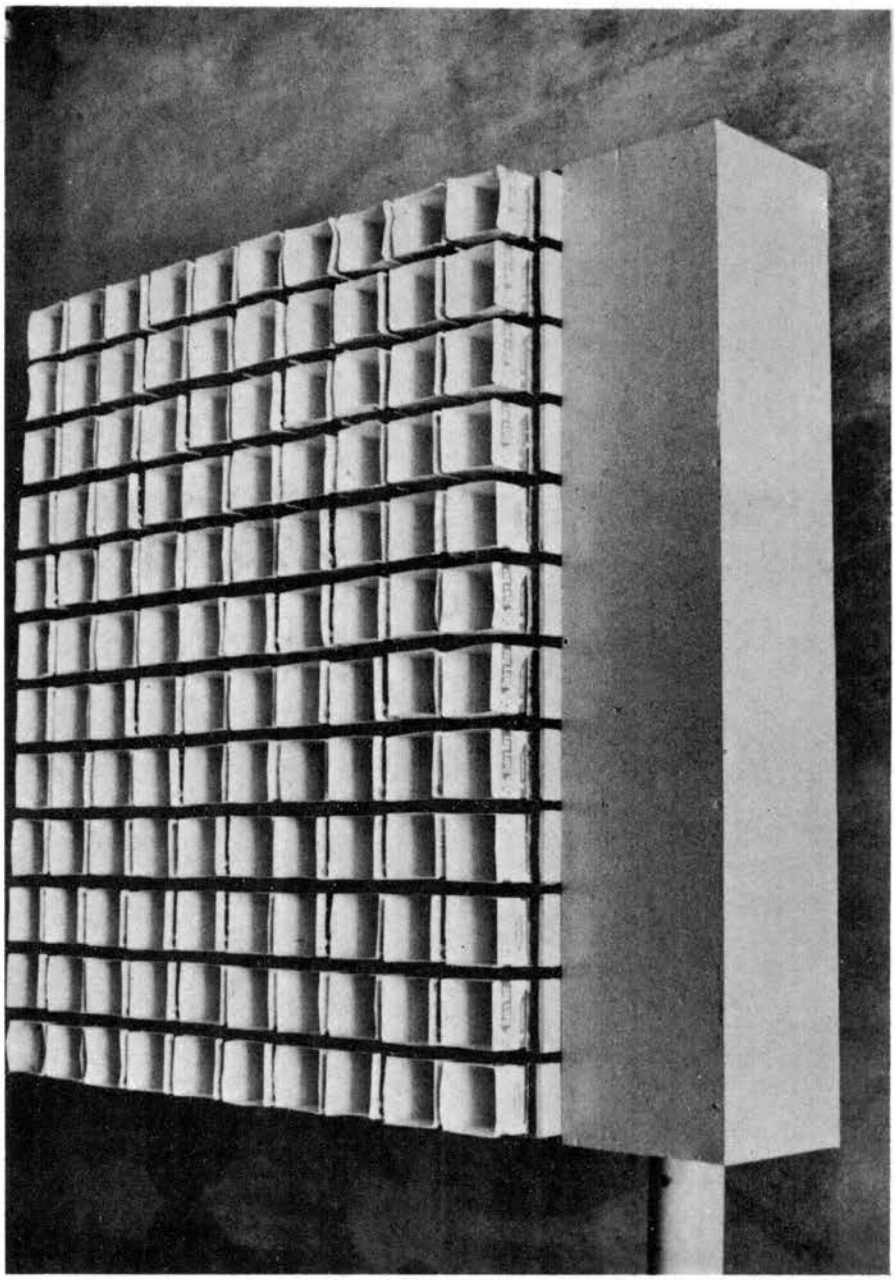

Figure 3. Peanut Dryer

CHAPTER V

DATA PRESENTATION AND ANALYSIS

Introduction

An oven drying technique was used to determine the moisture contents of the two treatments. This technique consisted of weighing a sample of peanuts and placing them in an oven at 162-163°F. After 48 hours they were reweighed. From this data the moisture contents were found to be 47% and 27% (wet basis).

Mold Production

A day-to-day record of visual observations of molding was kept. Mold production on those peanuts with acid appeared to start on one or two individual peanuts in the form of small white spots. After a period of time this peanut would cover with a white mold and a number of adjoining nuts would begin to mold. The nuts appeared to be joined by a mass of hair-like mold. From this point mold spread rapidly and various types were produced. Of the mold produced Trichoderma viride, Rhizopus stolonifer, and Fusarium moniliforme were visually identified.

The previous paragraph is intentionally general in nature. The period of time necessary for the mold to spread was dependent upon the acid application rate. For example, the peanuts with 0.50% acid spread so rapidly the steps involved were not noticeable while those at 1.25% or 1.50% may never have progressed beyond the initial moldy peanut. It

is felt that mold production at these higher rates was due to an individual peanut or small group of peanuts not being sufficiently covered with acid. Better coverage could probably have been accomplished by diluting the acid before application.

The time period that passed between application of the acid and initiation of first mold was dependent upon two factors, application rate and initial moisture content. Figure 4 shows a summary of the initiation of mold as recorded in the visual observations.

Those 47% m.c. peanuts with acid applications up to and including 0.75% molded badly; and those at 0.00 and 0.50% germinated extensively as well. At 1.25% acid the peanuts were at the "speckled with mold" stage on the 14th day; and those at 1.50% acid supported one moldy peanut.

The 27% peanuts behaved similarly except that the initiation of molding occurred after a longer period and no mold was observed after 14 days at acid application rates above and including 1.25%. Final molding as well as germination in the bins was not as profuse at the 27% m.c. In both cases an increase in temperature was noted in the cans supporting profuse molding.

These observations indicate that propionic acid is, in fact, a fungitoxic material. Mold production can be eliminated by application of a sufficient quantity of acid. Visual observations indicate that mold production is reduced as acid content increases; and less acid is required to achieve the desired effect when the moisture content is lower.

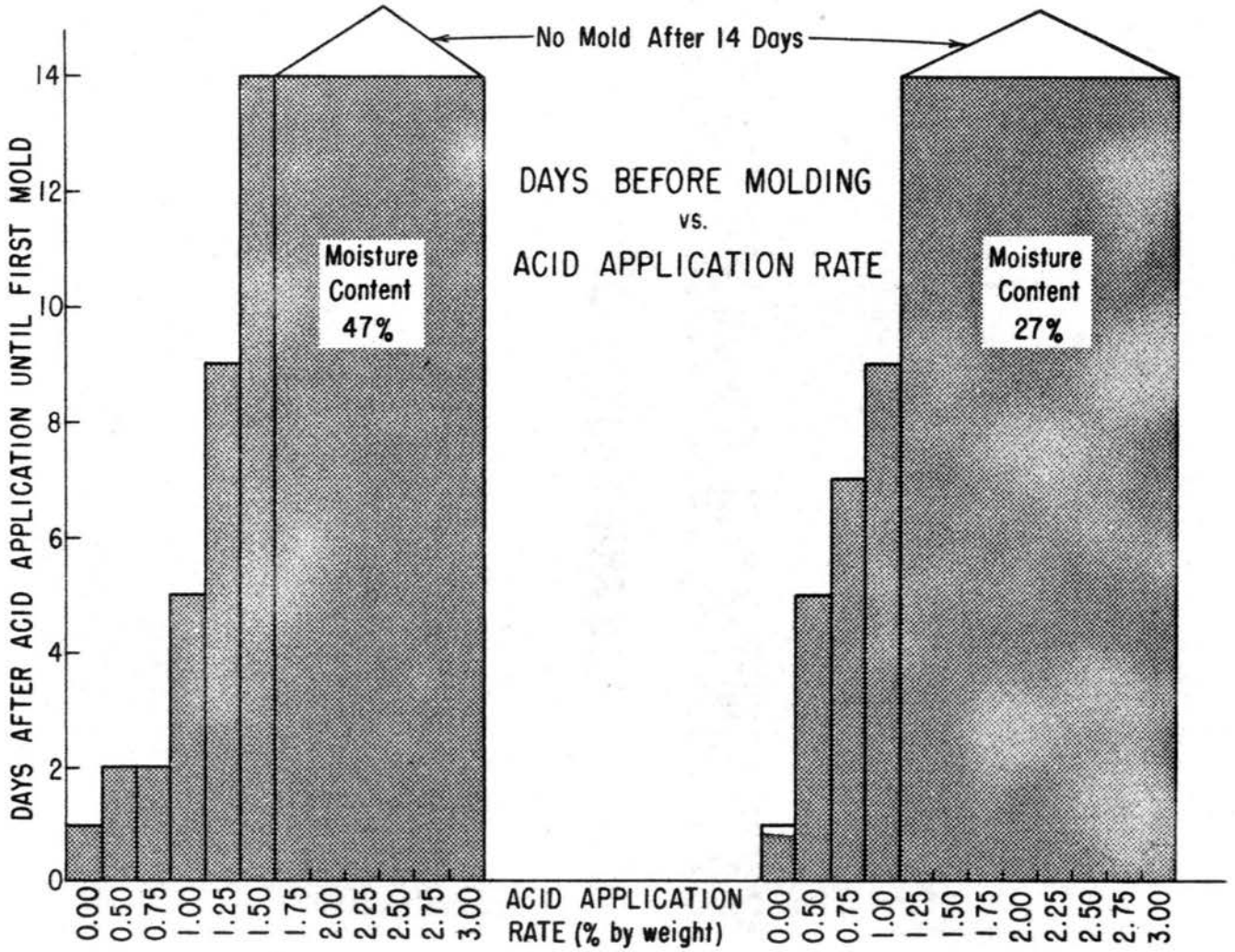

Figure 4. Summary of Mold Production

Aflatoxin Analysis

Selected samples were submitted to the Wisconsin Alumni Research Foundation for aflatoxin analyses. It was necessary to limit the number of analyses due to economic considerations. Therefore, those treated samples with mold at the 14-day storage level were pooled and submitted first. Those treatments exhibiting aflatoxin concentrations at the 14-day storage level were then submitted at the 8-day level. No aflatoxin was detected at this level indicating that peanuts were aflatoxin-free at storage levels less than 8 days.

Of the samples at the 14-day storage level, only those samples with visible mold growth were submitted. The defense of this decision rests on the fact that aflatoxin is produced by mold. So, if there is no mold there will be no aflatoxin produced.

Samples that were treated similarly but with no acid applied were analyzed for use as a check. These samples were submitted from the 5-day storage first. No aflatoxin was detected. So, samples were submitted at the 8-day storage level. Here, aflatoxin was detected.

Table II shows the results of the aflatoxin analyses. The data indicates a trend of aflatoxin reduction with increase in acid. This corresponds with an earlier visual observation that mold production decreased with increase in acid content. It is noted that the 47-0.50-(1,2,3)-14 sample did not have detectable aflatoxin. In fact, all samples at 47% m.c. had less aflatoxin than samples at 27% m.c. with corresponding acid contents. It was noted earlier that molding was more profuse at the higher moisture content. Based on this information, it is proposed that the profuse growth of more hardy molds may have prevented the healthy propagation of A. flavus.

TABLE II

AFLATOXIN ANALYSES OF INOCULATED PEANUTS

Sample Identification	PPB Aflatoxin			
	B_1	B_2	G_1	G_2
27-0.00-(1,2,3)- 5	0	0	0	0
27-0.00-(1,2,3)- 8	162	9	0	0
27-0.50-(1,2,3)- 8	0	0	0	0
27-0.50-(1,2,3)-14	2592	194	30	0
27-0.75-(1,2,3)- 8	0	0	0	0
27-0.75-(1,2,3)-14	130	12	2	0
27-1.00-(1,2,3)-14	0	0	0	0
27-1.25-(1,2,3)-14	0	0	0	0
47-0.00-(1,2,3)- 5	0	0	0	0
47-0.00-(1,2,3)- 8	8	0	0	0
47-0.50-(1,2,3)-14	0	0	0	0
47-0.75-(1,2,3)- 8	0	0	0	0
47-0.75-(1,2,3)-14	30	0	0	0
47-1.00-(1,2,3)-14	0	0	0	0
47-1.25-(1,2,3)-14	0	0	0	0

The notation (1,2,3) indicates the replications were pooled. Best Foods Method J.A.O.A.C. 53 p. 104 (1970). Sensitivity - 2ppb aflatoxin B_1.

At the 8-day storage level it is noted that the samples with no acid had detectable quantities of aflatoxin. At neither moisture content did any treated sample have detectable aflatoxin at this storage level. This suggests that propionic acid indeed has potential as an aflatoxin inhibitor.

It is also noted that aflatoxin content increased with storage time. Three treated samples exhibited aflatoxin on the 14th day. None of the treated samples had detectable aflatoxin on the 8th day. Both

check samples at the 8-day storage level had aflatoxin while none was detected at the 5-day level. This tends to reinforce a statement made earlier that aflatoxin is indeed cumulative.

Germination Test Analysis

A standard testing procedure as outlined in Appendix A was used to run the germination tests. Tests were run on individual replications at the lower rates of acid application and on pooled replications at the higher rates. In this way a complete set of germination test data was compiled with a minimum of tests. Propionic acid is known to kill germination (11, 25). So, it was anticipated that no germination would occur at the higher application rates. If excessive germination had occurred in these pooled samples, the individual replications would have been run.

One deviation from the normal testing procedure was in the germination count. Ordinarily, peanuts that have germinated by the 5-day count are removed. This sum is then added to the 10-day count for total germination. A germinated peanut as defined here is one with a healthy root and some visible root hairs. For this study peanuts were left from the 5-day count to the 10-day count. Also, all peanuts showing any sign of life in the form of a sprout of any size were counted as being germinated. Early indications were that true germination was severely impaired at even the lowest acid content. So, germination as reported here will be used as an indication of life in the peanut rather than true germination which would be considerably lower.

Complete germination test data are tabulated in Appendix B. Examination of the data showed the assumption of no germination at the

higher rates to be well founded. For all practical purposes germination was non-existent at rates above 1.00% and was severely impaired at all levels of acid application. The percent germination was found to be a function of the acid application rate, days of storage before drying, and moisture content.

Due to the vast amount of tabulated data, it was desired to present the data in a more compact form. Therefore, a least squares technique was used to fit a second order linear model in two variables to the data. A number of points were omitted for this analysis. The two points, 47-0.50-3-3 and 47-1.00-2-2, were omitted because they were unintentionally mixed with other treatments during the shelling process. Other points omitted were 27-1.50-1-1 and 27-0.50-2-2. These points showed values very inconsistent with surrounding points of the same treatment. Examination showed that they could be interchanged and fit very well. The labels must have been exchanged prior to testing germination. With the exception of one point, no treatment above 1.75% acid showed germination. Since the prime interest at this point was with the range of acid application showing germination, these points were omitted from the analysis.

Draper and Smith (14) describe several methods of selecting the "best" regression equation. The backward elimination method was selected and is outlined below:

1. A regression equation containing all variables is computed.
2. Every variable is treated as though it were the last variable to enter the regression equation and the partial F-test value is calculated.
3. The lowest partial F-test value, F_L, is then compared with a

preselected significance level, F_0.

(a) If $F_L < F_0$, the variable corresponding to F_L is removed and the procedure is repeated with this variable omitted.

(b) If $F_L > F_0$, adopt the regression equation as it is.

Appendix C contains the analyses of variance leading to the "best" regression equation of percent germination (PG) on days of storage (DS) and propionic acid content (PA). Four equations were found and are summarized in Table III. An equation was found for the 5-day and 10-day counts for each moisture content. It was felt that by developing an equation for each count the fact that peanuts died between the 5th day and the 10th day could be demonstrated graphically. Had there been more moisture contents, they would have been added to the regression equation as a third variable.

TABLE III

COEFFICIENTS OF REGRESSION EQUATIONS

Moisture Content %	5-Day Count	10-Day Count	Coefficients					
			b_0	b_1	b_2	b_3	b_4	b_5
27	X		102.3	-130.6	40.6	-	-	-
27		X	94.6	-125.4	40.6	-	-	-
47	X		47.9	- 93.3	38.4	7.2	-0.16	-3.3
47		X	44.2	- 89.1	36.9	7.5	-0.19	-3.2

$PG = b_0 + b_1 \times (PA) + b_2 \times (PA)^2 + b_3 \times (DS) + b_4 \times (DS)^2 + b_5 \times (PA)(DS)$

PG = Percent germination.
PA = Propionic acid application rate.
DS = Days of storage before drying.

It must be kept in mind that the percent germination referred to in this discussion is actually a measure of the percent of peanuts

showing signs of life and not germination in its truest sense. Also, the regression equations referred to in Table III are for comparison purposes only.

The analyses of variance in Appendix C indicate that storage time is not statistically significant as a predictor of germination at the 27% moisture content. Storage time was significant at the 47% m.c., however. In both cases the level of rejection was 0.05. Plots of these regression equations are shown in Figures 5, 6, and 7. The plots are generally valid in the range 0.50 to 1.50%. General trends that should be noted from these plots are: (1) germination decreased from the 5-day count to the 10-day count; (2) germination increased as the storage time before drying increased; and (3) in general, germination was higher at the lower moisture content.

The first trend indicates that many of the peanuts that attempted to germinate by the fifth day died by the tenth day. Apparently the acid in these cases did not completely kill the peanut immediately, but weakened it sufficiently to prevent healthy growth. In nearly all cases over half of the peanuts showed no sign of life. So, to say the least, germination was severely impaired by the propionic acid.

The second trend demands an explanation. It is in complete opposition to expectation. However, no absolute explanation is apparent. The trend is only present in the peanuts at the higher moisture content. Storage time was found to not be statistically significant at the lower moisture peanuts. This suggests that maturity of the peanuts may be the cause of the increase in germination with storage time. The 47% m.c. peanuts were dug one afternoon and combined the following morning. The 27% m.c. peanuts had four extra days for maturing before

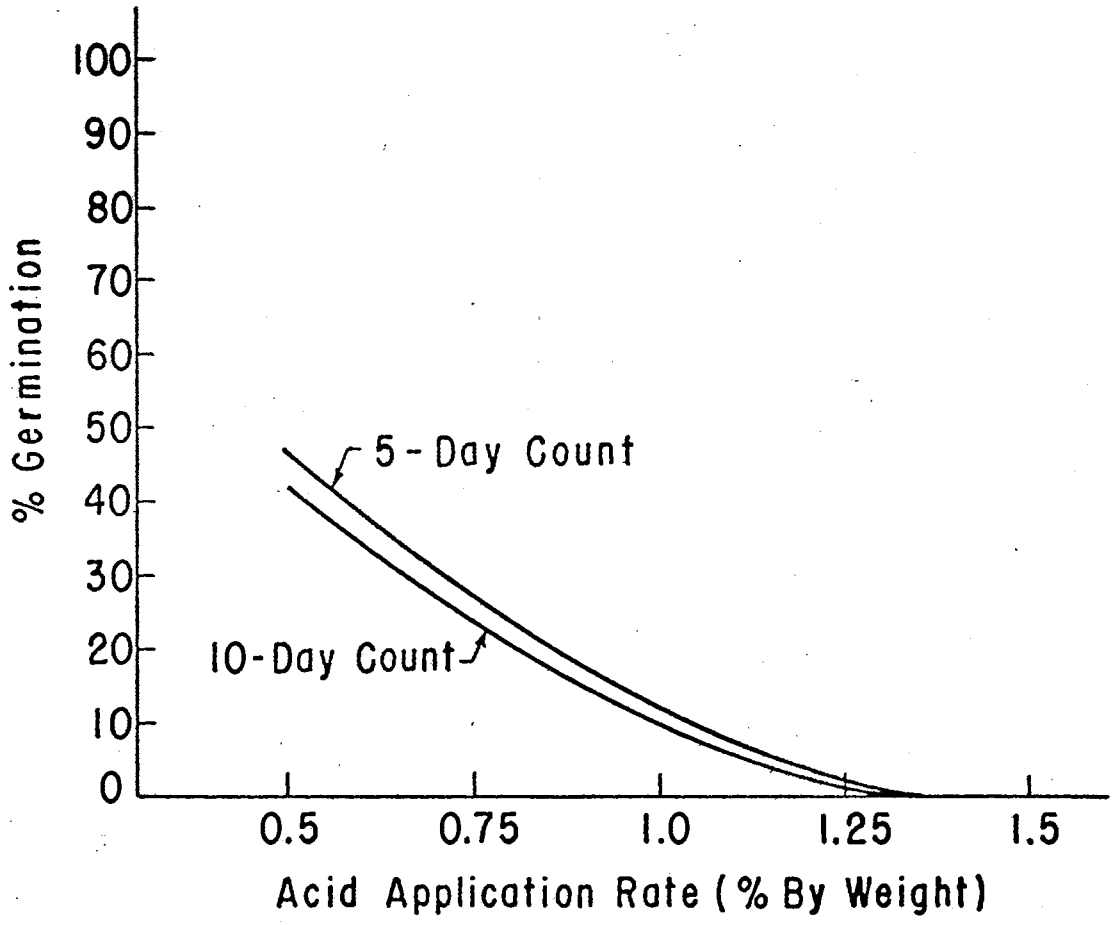

Figure 5. Percent Germination vs. Acid Application Rate, 27% m.c.

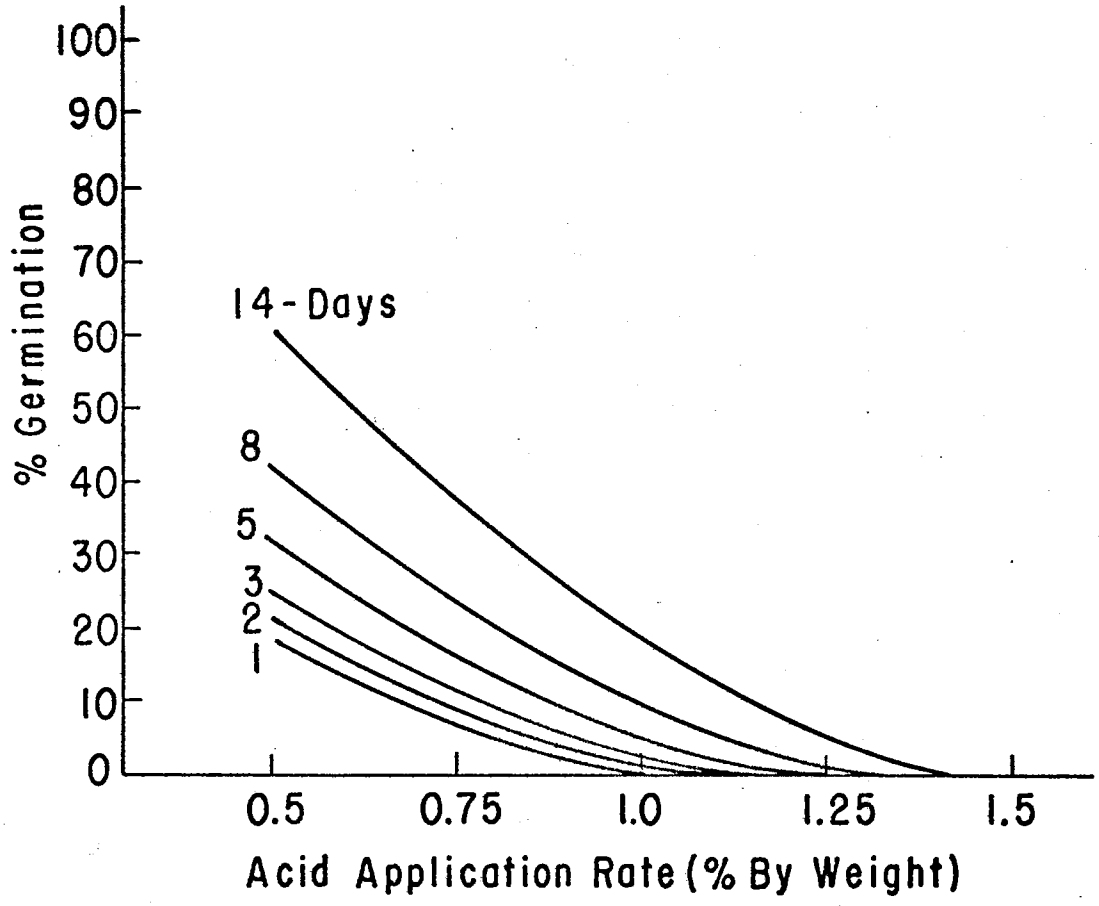

Figure 6. Percent Germination vs. Acid Application Rate, 47% m.c., 5-Day Count

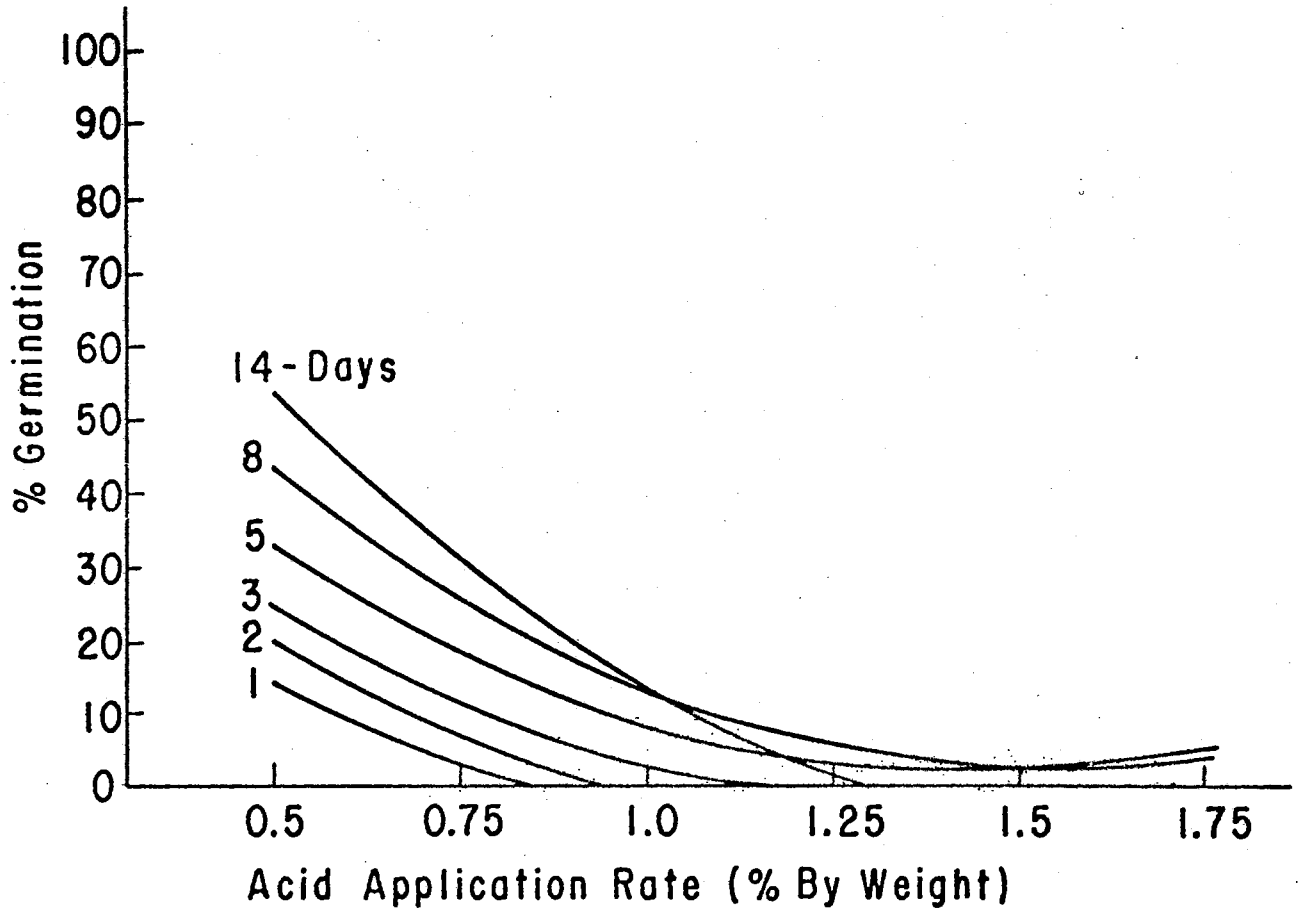

Figure 7. Percent Germination vs. Acid Application Rate, 47% m.c., 10-Day Count

digging and three days of field curing before combining. It is possible that the higher moisture peanuts matured while in storage. The earlier samples were dried before any significant maturing could occur. It was noted that the excessive molding produced significant heat and had a drying effect on the peanuts. It is proposed here that the drying process may have allowed the peanuts to mature sufficiently during storage to increase the germination.

Other observations recorded were profuse molding and rotting of the peanuts. The molding was present in most samples, particularly those with little germination. At the 2.50 to 3.00% range not so much molding was noted and many of the peanuts merely rotted.

Organoleptic (Taste) Evaluation

Originally, it was desired to develop an experimental design based on statistics to evaluate the effects of propionic acid on the flavor of peanuts. From this design conclusions could be drawn and statements could be made at some given significance level. However, further investigation showed this to be impractical (20). Also, preliminary tasting of the raw peanuts showed the flavor to be severely impaired. So, prior to setting up a complete set of taste tests, one treatment was submitted to a taste panel.

The one treatment, 27-0.50-4-1, was compared to a check sample and a standard by the five-member taste panel. The check sample was a sample brought in at the same time as the test treatment but dried immediately with no acid treatment. The standard was provided by the taste panel. This taste test was conducted by the Oklahoma State University Peanut Quality Laboratory to evaluate odor, flavor, roast, and

preference of roasted peanuts and peanut butter.

Prior to submitting this sample and in anticipation of running a complete set of tests, samples were submitted to the WARF Institute, Inc. for aflatoxin analyses. This was to insure that peanuts submitted to the taste panel would be safe for consumption. Samples were submitted from the test day where molding was first deemed excessive. Peanuts with no molding by the 14th day of storage were not submitted and were assumed to be free of aflatoxin. Table IV summarizes the results of these analyses. No aflatoxin was detected. So, all treatments with storage times less than that of the treatment analyzed were deemed safe for submission to the taste panel.

TABLE IV

AFLATOXIN ANALYSES OF PEANUTS FOR ORGANOLEPTIC EVALUATION

Sample Identification	PPB Aflatoxin			
	B_1	B_2	G_1	G_2
47-0.50-4- 3	0	0	0	0
47-0.75-4- 8	0	0	0	0
47-1.00-4- 8	0	0	0	0
47-1.25-4-14	0	0	0	0
27-0.50-4- 5	0	0	0	0
27-0.75-4- 8	0	0	0	0
27-1.00-4-14	0	0	0	0
27-1.25-4-14	0	0	0	0

Best Foods Method J.A.O.A.C. 53 p. 104 (1970). Sensitivity - 2 ppb aflatoxin B_1.

Results of the taste panel tests on roasted peanuts are tabulated in Table V. The CLER score summary of roasted peanuts refers to the flavor of the roasted peanuts. Each panel member rated each sample on a numerical scale which increased with quality increase. The highest possible score was 100. The taste panel rated the flavor of the acid-treated peanuts considerably lower than either the check sample or the standard.

The roast portion refers to a visual comparison of the test samples with a visual standard. Each nut of a sample of 20 is rated by the taste panel on a scale of 1 through 4. The lower rating indicates the higher quality roast. Again, the acid-treated peanuts rated poorly. Visual observations during the roasting process indicated that the acid-treated peanuts tended to roast unevenly.

The preference rank is compared to the laboratory standard and indicates the personal preferences of the taste panel. In this case the higher the number the lower the rating. Again, the acid-treated peanuts received the lowest rating.

Results of the taste panel tests on peanut butter samples prepared from the treated peanuts are tabulated in Table VI. The personal preference mean rank showed results similar to the roasted peanuts. The acid-treated sample again received the poorest rating.

The second portion of the peanut butter summary ranks odor and flavor either superior to, equal to, or inferior to the standard. The score recorded is the percent of the panel members rating the peanut butter at a particular level. It is seen that the acid-treated sample was ranked inferior to the standard for both odor and flavor by all panel members. The check sample also rated rather low. It was observed

TABLE V

CLER SCORE SUMMARY OF ROASTED PEANUTS

Sample Identity	Date Tasted	Flavor						
		A	B	C	D	E	Total	Mean
27-0.50-4-1	2/25/71	0	0	0	15	0	15	3.0
check	2/25/71	54	72	60	94	90	370	74.0
standard	2/25/71	66	52	62	100	96	376	75.2

Sample Identity	Date Tasted	Roast						
		A	B	C	D	E	Total	Mean
27-0.50-4-1	2/25/71	2.0	2.5	2.5	2.4	3.0	12.4	2.5
check	2/25/71	2.0	1.6	1.4	1.2	1.2	7.4	1.5
standard	2/25/71	2.0	1.6	1.3	1.0	1.0	6.9	1.4

Sample Identity	Date Tasted	Pref. Rank						
		A	B	C	D	E	Total	Mean
27-0.50-4-1	2/25/71	4	4	4	4	4	20	4.0
check	2/25/71	4	1	1	2	2.5	10.5	2.1
standard	2/25/71	2	2	2	1	1	8	1.6

TABLE VI

PEANUT BUTTER SUMMARY

Sample Identity	P.B. No.	Date Tasted	Mean Rank	Peanut Butter %	Gms/ 100 Seed
27-0.50-4-1	3826	2/25/71	4.0	95.46	37.47
check	3827	2/25/71	2.5	88.71	38.00
standard	3829	2/25/71	1.3	86.66	38.90

Sample Identity	P.B. No.	Date Tasted	Superior to Standard		Equal to Standard		Inferior to Standard	
			Odor	Flavor	Odor	Flavor	Odor	Flavor
27-0.50-4-1	3826	2/25/71	0	0	0	0	100	100
check	3827	2/25/71	0	20	20	20	80	60
standard	3829	2/25/71	0	0	100	100	0	0

Sample Identity	P.B. No.	Date Tasted	Mean Rating of:					
			Odor	Flavor	Taste	Roast	Tex-ture	Dryness
27-0.50-4-1	3826	2/25/71	4.0	4.0	3.6	4.0	1.2	1.4
check	3827	2/25/71	3.4	3.4	2.4	1.8	1.4	1.6
standard	3829	2/25/71	3.0	2.2	1.8	2.2	1.8	1.8

that the check sample had an odor and flavor suggesting acid content. Apparently, storage of the check sample in the presence of treated samples was sufficient to affect the odor and flavor of the check samples.

Mean ratings of odor, flavor, taste, and roast showed the acid-treated sample to be decidedly inferior. In texture and dryness the treated sample was rated fairly smooth and moist. Visual observations indicated that the acid caused the treated samples to appear much more oily than the standard sample.

Because the low rating of the treated sample was anticipated, the taste panel members were instructed to record their comments related to these samples. A summary of their comments is recorded in Table VII. Based on these comments and the taste panel results, the decision was made to curtail the taste panel tests. It was felt that the taste and odor were so adversely affected by the acid that no valid degree of difference between the samples would be detected. It was also questionable whether the taste panel members would be willing to taste them or whether the director of the lab would ask them to. Based on the available evidence, it can be stated that propionic acid definitely has an adverse effect on the flavor of peanuts and peanut butter.

TABLE VII

TASTE PANEL COMMENTS

Roasted Peanuts
1. Undoubtedly the worst thing I've ever tasted in my life.
2. Pucker bitter. Tastes a bit like something at a dentist's office. Some are one-half OK and one-half over roasted.
3. Terrible, sour, bitter.
4. Too bitter and over roasted almost to the point of being sour.
5. #1 did not have any peanut flavor.

Peanut Butter
1. Terrible! Very bitter, and such a bad flavor that you can hardly distinguish what it tastes like.
2. Awful, too off, off color also. Smell fits taste--BAD.
3. It is terrible--tastes like old butter--the odor hurt my nose.
4. Awful--nothing at all like peanut butter.
5. Hurt my nose.

CHAPTER VI

SUMMARY AND CONCLUSIONS

Summary

Various methods have been attempted to control aflatoxin in wet peanuts. However, none have been successful to date. This research was an attempt to solve the aflatoxin problem by applying propionic acid to the wet peanuts.

The specific objective of this study was to evaluate the effects of various application rates of propionic acid on: (1) aflatoxin production; (2) germination; and (3) organoleptic properties of peanuts as related to the acceptability of propionic acid for storage of high moisture peanuts.

Two field treatments used were harvest immediately after digging and harvest after three days of field curing. The moisture contents were found to be 27% and 47% w.b., respectively. Ten application rates of propionic acid were tested. They were 0.00, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00, 2.50 and 3.00% by weight. Four replications of each application were tested. Three were inoculated with A. flavus spores and the fourth was kept for taste panel evaluation.

Conclusions

It was found that the propionic acid is indeed effective in the inhibition of mold growth on high moisture peanuts. The time period

that passed between application of the acid and initiation of first mold increased with an increase in acid content. Mold production was eliminated up to 14 days by application of sufficient acid quantities. The amount of acid necessary to control mold decreased with decreasing moisture content.

Propionic acid also showed potential as an aflatoxin inhibitor. Aflatoxin production tended to decrease with increased acid applications and to increase with storage time. At both moisture contents no aflatoxin was detected after 14 days on peanuts treated with a minimum of 1.00% acid.

Adverse effects were noted both on germination and on flavor. Germination was severely impaired at all levels of propionic acid application. No germination was recorded at the higher application rates. The flavor of roasted peanuts and peanut butter was also rendered very undesirable by the acid.

Future Studies

It is recommended that further studies be made in the following areas:

1. The efficacy of propionic acid for aflatoxin inhibition on agricultural products destined for livestock consumption.
2. The possibility of removal of the propionic acid from treated peanuts prior to processing for human consumption.

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APPENDIX A

STANDARD GERMINATION TEST FOR PEANUTS

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STANDARD GERMINATION TEST FOR PEANUTS

The germination test is based on 200 peanuts. General procedure consists of:

(1) Soak 16 paper towels rolled into a cylinder.

(2) Break down into four groups of 50 peanuts.

  (a) Lay down 1 sheet of wax paper.

  (b) Place two wet paper towels on wax paper.

  (c) Place 50 peanuts on towels.

  (d) Place two more wet paper towels on top.

  (e) Roll tightly and tuck in at bottom.

(3) Place complete group of 200 peanuts in an environment of 60-86°F with a continuous supply of water circulating through the chamber to keep a fairly high relative humidity.

(4) Count at each of 5 and 10 days and calculate the percent germination.

APPENDIX B

GERMINATION TEST DATA

TABLE VIII

GERMINATION TEST DATA--27% M.C.

(Germination Tests are Based on 200 Count)

Identification	5-Day Count	% Germination	10-Day Count	% Germination
27-0.00-1-0	164.	82.0	153.	76.5
27-0.00-2-0	168.	84.0	154.	77.0
27-0.00-(1,2,3)-1	198.	99.0	198.	99.0
27-0.50-1-1	118.	59.0	100.	50.0
27-0.50-2-1	119.	59.5	117.	58.5
27-0.50-3-1	135.	67.5	120.	60.0
27-0.75-1-1	53.	26.5	48.	24.0
27-0.75-2-1	47.	23.5	47.	23.5
27-0.75-3-1	80.	40.0	72.	36.0
27-1.00-1-1	25.	12.5	20.	10.0
27-1.00-2-1	28.	14.0	25.	12.5
27-1.00-3-1	18.	9.0	13.	6.5
27-1.25-1-1	4.	2.0	3.	1.5
27-1.25-2-1	6.	3.0	4.	2.0
27-1.25-3-1	10.	5.0	5.	2.5
27-1.50-1-1	139.	69.5	124.	62.0
27-1.50-1-1	130.	65.0	108.	54.0
27-1.50-2-1	0.	0.0	2.	1.0
27-1.50-3-1	2.	1.0	1.	0.5
27-1.75-(1,2,3)-1	0.	0.0	0.	0.0
27-2.00-(1,2,3)-1	0.	0.0	0.	0.0
27-2.50-(1,2,3)-1	0.	0.0	0.	0.0
27-3.00-(1,2,3)-1	0.	0.0	0.	0.0

TABLE VIII (CONTINUED)

Identification	5-Day Count	% Germination	10-Day Count	% Germination
27-0.00-(1,2,3)-2	160.	80.0	154.	77.0
27-0.50-1-2	133.	66.5	97.	48.5
27-0.50-2-2	0.	0.0	0.	0.0
27-0.50-2-2	0.	0.0	0.	0.0
27-0.50-3-2	137.	68.5	106.	53.0
27-0.75-1-2	43.	21.5	33.	16.5
27-0.75-2-2	34.	17.0	27.	13.5
27-0.75-3-2	91.	45.5	60.	30.0
27-1.00-1-2	10.	5.0	12.	6.0
27-1.00-2-2	15.	7.5	11.	5.5
27-1.00-3-2	8.	4.0	8.	4.0
27-1.25-1-2	3.	1.5	2.	1.0
27-1.25-2-2	6.	3.0	3.	1.5
27-1.25-3-2	7.	3.5	4.	2.0
27-1.50-1-2	2.	1.0	2.	1.0
27-1.50-2-2	1.	0.5	3.	1.5
27-1.50-3-2	2.	1.0	2.	1.0
27-1.75-(1,2,3)-2	1.	0.5	0.	0.0
27-2.00-(1,2,3)-2	0.	0.0	0.	0.0
27-2.50-(1,2,3)-2	0.	0.0	0.	0.0
27-3.00-(1,2,3)-2	0.	0.0	0.	0.0
27-0.50-1-3	121.	60.5	98.	49.0
27-0.50-2-3	83.	41.5	68.	34.0
27-0.50-3-3	136.	68.0	120.	60.0
27-0.75-1-3	7.	3.5	5.	2.5

TABLE VIII (CONTINUED)

Identification	5-Day Count	% Germination	10-Day Count	% Germination
27-0.75-2-3	25.	12.5	23.	11.5
27-0.75-3-3	75.	37.5	60.	30.0
27-1.00-1-3	6.	3.0	7.	3.5
27-1.00-2-3	11.	5.5	7.	3.5
27-1.00-3-3	4.	2.0	5.	2.5
27-1.25-1-3	2.	1.0	2.	1.0
27-1.25-2-3	0.	0.0	0.	0.0
27-1.25-3-3	3.	1.5	2.	1.0
27-1.50-1-3	0.	0.0	0.	0.0
27-1.50-2-3	2.	1.0	2.	1.0
27-1.50-3-3	1.	0.5	2.	1.0
27-1.75-(1,2,3)-3	0.	0.0	0.	0.0
27-2.00-(1,2,3)-3	0.	0.0	0.	0.0
27-2.50-(1,2,3)-3	0.	0.0	0.	0.0
27-3.00-(1,2,3)-3	0.	0.0	0.	0.0
27-0.50-1-5	148.	74.0	140.	70.0
27-0.50-2-5	90.	45.0	78.	39.0
27-0.50-3-5	151.	75.5	143.	71.5
27-0.75-1-5	15.	7.5	17.	8.5
27-0.75-2-5	21.	10.5	21.	10.5
27-0.75-3-5	79.	39.5	71.	35.5
27-1.00-(1,2,3)-5	6.	3.0	3.	1.5
27-1.25-(1,2,3)-5	2.	1.0	3.	1.5
27-1.50-(1,2,3)-5	0.	0.0	0.	0.0

TABLE VIII (CONTINUED)

Identification	5-Day Count	% Germination	10-Day Count	% Germination
27-1.75-(1,2,3)-5	0.	0.0	0.	0.0
27-2.00-(1,2,3)-5	0.	0.0	0.	0.0
27-2.50-(1,2,3)-5	0.	0.0	0.	0.0
27-3.00-(1,2,3)-5	0.	0.0	0.	0.0
27-0.50-1-8	137.	68.5	127.	63.5
27-0.50-2-8	112.	56.0	108.	54.0
27-0.50-3-8	124.	62.0	116.	58.0
27-0.75-1-8	6.	3.0	7.	3.5
27-0.75-2-8	16.	8.0	14.	7.0
27-0.75-3-8	35.	17.5	28.	14.0
27-1.00-(1,2,3)-8	0.	0.0	0.	0.0
27-1.25-(1,2,3)-8	0.	0.0	0.	0.0
27-1.50-(1,2,3)-8	0.	0.0	0.	0.0
27-1.75-(1,2,3)-8	1.	0.5	1.	0.5
27-2.00-(1,2,3)-8	0.	0.0	0.	0.0
27-2.50-(1,2,3)-8	0.	0.0	0.	0.0
27-3.00-(1,2,3)-8	0.	0.0	0.	0.0
27-0.50-(1,2,3)-14	131.	65.5	95.	47.5
27-0.75-(1,2,3)-14	7.	3.5	5.	2.5
27-1.00-(1,2,3)-14	0.	0.0	0.	0.0
27-1.25-(1,2,3)-14	0.	0.0	0.	0.0
27-1.50-(1,2,3)-14	0.	0.0	0.	0.0
27-1.75-(1,2,3)-14	0.	0.0	0.	0.0
27-2.00-(1,2,3)-14	0.	0.0	0.	0.0
27-2.50-(1,2,3)-14	0.	0.0	0.	0.0
27-3.00-(1,2,3)-14	0.	0.0	0.	0.0

TABLE IX

GERMINATION TEST DATA--47% M.C.

(Germination Tests are Based on 200 Count)

Identification	5-Day Count	% Germination	10-Day Count	% Germination
47-0.00-(1,2,3)-1	122.	61.0	106.	53.0
47-0.00-4-1	146.	73.0	130.	65.0
47-0.50-1-1	18.	9.0	17.	8.5
47-0.50-2-1	11.	5.5	9.	4.5
47-0.50-3-1	40.	20.0	37.	18.5
47-0.75-1-1	7.	3.5	8.	4.0
47-0.75-2-1	28.	14.0	19.	9.5
47-0.75-3-1	23.	11.5	19.	9.5
47-1.00-1-1	1.	0.5	1.	0.5
47-1.00-2-1	0.	0.0	1.	0.5
47-1.00-3-1	2.	1.0	2.	1.0
47-1.25-1-1	1.	0.5	1.	0.5
47-1.25-2-1	0.	0.0	0.	0.0
47-1.25-3-1	0.	0.0	0.	0.0
47-1.50-(1,2,3)-1	0.	0.0	0.	0.0
47-1.75-(1,2,3)-1	0.	0.0	0.	0.0
47-2.00-(1,2,3)-1	0.	0.0	9.	4.5
47-2.50-(1,2,3)-1	0.	0.0	0.	0.0
47-3.00-(1,2,3)-1	0.	0.0	0.	0.0
47-0.00-(1,2,3)-2	131.	65.5	133.	66.5
47-0.50-1-2	26.	13.0	18.	9.0
47-0.50-2-2	25.	12.5	19.	9.5
47-0.50-3-2	29.	14.5	21.	10.5
47-0.75-1-2	6.	3.0	6.	3.0
47-0.75-2-2	47.	23.5	41.	20.5

TABLE IX (CONTINUED)

Identification	5-Day Count	% Germination	10-Day Count	% Germination
47-0.75-3-2	39.	19.5	34.	17.0
47-1.00-1-2	0.	0.0	0.	0.0
47-1.00-2-2	18.	9.0	18.	9.0
47-1.00-3-2	1.	0.5	2.	1.0
47-1.25-1-2	1.	0.5	1.	0.5
47-1.25-2-2	0.	0.0	0.	0.0
47-1.25-3-2	0.	0.0	0.	0.0
47-1.50-(1,2,3)-2	0.	0.0	0.	0.0
47-1.75-(1,2,3)-2	0.	0.0	0.	0.0
47-2.00-(1,2,3)-2	0.	0.0	0.	0.0
47-2.50-(1,2,3)-2	0.	0.0	0.	0.0
47-3.00-(1,2,3)-2	0.	0.0	0.	0.0
47-0.50-1-3	5.	2.5	5.	2.5
47-0.50-2-3	3.	1.5	3.	1.5
47-0.50-3-3	25.	12.5	21.	10.5
47-0.75-1-3	0.	0.0	0.	0.0
47-0.75-2-3	7.	3.5	8.	4.0
47-0.75-3-3	8.	4.0	8.	4.0
47-1.00-1-3	0.	0.0	0.	0.0
47-1.00-2-3	0.	0.0	0.	0.0
47-1.00-3-3	0.	0.0	0.	0.0
47-1.25-1-3	0.	0.0	0.	0.0
47-1.25-2-3	0.	0.0	0.	0.0
47-1.25-3-3	0.	0.0	0.	0.0
47-1.50-(1,2,3)-3	0.	0.0	0.	0.0
47-1.75-(1,2,3)-3	0.	0.0	0.	0.0

TABLE IX (CONTINUED)

Identification	5-Day Count	% Germination	10-Day Count	% Germination
47-2.00-(1,2,3)-3	0.	0.0	0.	0.0
47-2.50-(1,2,3)-3	0.	0.0	0.	0.0
47-3.00-(1,2,3)-3	0.	0.0	0.	0.0
47-0.50-1-5	95.	47.5	95.	47.5
47-0.50-2-5	87.	43.5	88.	44.0
47-0.50-3-5	106.	53.0	111.	55.5
47-0.75-1-5	25.	12.5	24.	12.0
47-0.75-2-5	82.	41.0	76.	38.0
47-0.75-3-5	29.	14.5	28.	14.0
47-1.00-(1,2,3)-5	11.	5.5	11.	5.5
47-1.25-(1,2,3)-5	1.	0.5	2.	1.0
47-1.50-(1,2,3)-5	0.	0.0	0.	0.0
47-1.75-(1,2,3)-5	0.	0.0	0.	0.0
47-2.00-(1,2,3)-5	0.	0.0	0.	0.0
47-2.50-(1,2,3)-5	0.	0.0	0.	0.0
47-3.00-(1,2,3)-5	0.	0.0	0.	0.0
47-0.50-1-8	110.	55.0	111.	55.5
47-0.50-2-8	81.	40.5	78.	39.0
47-0.50-3-8	119.	59.5	118.	59.0
47-0.75-1-8	32.	16.0	30.	15.0
47-0.75-2-8	89.	44.5	85.	42.5
47-0.75-3-8	58.	29.0	53.	26.5
47-1.00-(1,2,3)-8	14.	7.0	13.	6.5
47-1.25-(1,2,3)-8	1.	0.5	1.	0.5

TABLE IX (CONTINUED)

Identification	5-Day Count	% Germination	10-Day Count	% Germination
47-1.50-(1,2,3)-8	0.	0.0	0.	0.0
47-1.75-(1,2,3)-8	0.	0.0	0.	0.0
47-2.00-(1,2,3)-8	0.	0.0	0.	0.0
47-2.50-(1,2,3)-8	0.	0.0	0.	0.0
47-3.00-(1,2,3)-8	0.	0.0	0.	0.0
47-0.50-(1,2,3)-14	129.	64.5	113.	56.5
47-0.50-(1,2,3)-14	90.	45.0	87.	43.5
47-0.75-(1,2,3)-14	58.	29.0	52.	26.0
47-0.75-(1,2,3)-14	65.	32.5	61.	30.5
47-1.00-(1,2,3)-14	21.	10.5	18.	9.0
47-1.00-(1,2,3)-14	25.	12.5	24.	12.0
47-1.25-(1,2,3)-14	4.	2.0	2.	1.0
47-1.25-(1,2,3)-14	1.	0.5	1.	0.5
47-1.50-(1,2,3)-14	0.	0.0	0.	0.0
47-1.75-(1,2,3)-14	0.	0.0	0.	0.0
47-2.00-(1,2,3)-14	0.	0.0	0.	0.0
47-2.50-(1,2,3)-14	0.	0.0	0.	0.0
47-3.00-(1,2,3)-14	0.	0.0	0.	0.0

APPENDIX C

GERMINATION TEST ANALYSES OF VARIANCE

TABLE X

ANALYSES OF VARIANCE, 27% M.C., 5-DAY COUNT

Source	df	SS	MS	F
Total	76	99865.8	1314.0	
Mean	1	37691.3	37691.3	45.47
Residual	75	62174.4	829.0	
PA	1	44184.2	44184.2	181.74
Residual	74	17990.3	243.1	
$(PA)^2$	1	7044.5	7044.5	46.98
Residual	73	10945.8	149.9	
DS	1	54.9	54.9	0.36
Residual	72	10890.9	151.3	
$(DS)^2$	1	19.2	19.2	0.13
Residual	71	10871.7	153.1	
(PA)(DS)	1	136.1	136.1	0.89
Residual	70	10735.6	153.4	
Total Reduction	6	89130.2	14855.0	96.86

PA = Propionic Acid Application Rate
DS = Days of Storage Before Drying
Level of Rejection = 0.05
F_L = 0.13. So, reject $(DS)^2$ from the model.

TABLE X (CONTINUED)

Source	df	SS	MS	F
Total	76	99865.8	1314.0	
Mean	1	37691.3	37691.3	45.47
Residual	75	62174.4	829.0	
PA	1	44184.2	44184.2	181.74
Residual	74	17990.3	243.1	
$(PA)^2$	1	7044.5	7044.5	46.98
Residual	73	10945.8	149.9	
DS	1	54.9	54.9	0.36
Residual	72	10890.9	151.3	
(PA)(DS)	1	152.3	152.3	1.01
Residual	71	10738.6	151.2	
Total Reduction	5	89127.1	17825.4	117.86

PA = Propionic Acid Application Rate
DS = Days of Storage Before Drying
Level of Rejection = 0.05
F_L = 0.36. So, reject DS from the model.

TABLE X (CONTINUED)

Source	df	SS	MS	F
Total	76	99865.8	1314.0	
Mean	1	37691.3	37691.3	45.47
Residual	75	62174.4	829.0	
PA	1	44184.2	44184.2	181.74
Residual	76	17990.3	243.1	
$(PA)^2$	1	7044.5	7044.5	46.98
Residual	73	10945.8	149.9	
(PA)(DS)	1	132.9	132.9	0.89
Residual	72	10812.8	150.2	
Total Reduction	4	89052.9	22263.2	148.24

PA = Propionic Acid Application Rate
DS = Days of Storage Before Drying
Level of Rejection = 0.05
F_L = 0.89. So, reject (PA)(DS) from the model.

TABLE X (CONTINUED)

Source	df	SS	MS	F
Total	76	99865.8	1314.0	
Mean	1	37691.3	37691.3	45.47
Residual	75	62174.4	829.0	
PA	1	44184.2	44184.2	181.74
Residual	74	17990.3	243.1	
$(PA)^2$	1	7044.5	7044.5	46.98
Residual	73	10945.8	149.9	
Total Reduction	3	88919.9	29640.0	197.68

PA = Propionic Acid Application Rate
Level of Rejection = 0.05
F_L = 45.47. So, keep the model as is.

Final Model: PG = 102.3 - 130.6 x (PA) + 40.6 x $(PA)^2$
PG = Percent Germination
Standard error of estimate = 12.24
R^2 = 0.824

TABLE XI

ANALYSES OF VARIANCE, 27% M.C., 10-DAY COUNT

Source	df	SS	MS	F
Total	76	80422.5	1058.2	
Mean	1	29290.1	29290.1	42.96
Residual	75	51132.4	681.8	
PA	1	36050.3	36050.3	176.88
Residual	74	15082.1	203.8	
$(PA)^2$	1	7048.2	7048.2	64.04
Residual	73	8033.9	110.1	
DS	1	55.1	55.1	0.50
Residual	72	7978.8	110.8	
$(DS)^2$	1	75.4	75.4	0.68
Residual	71	7903.4	111.3	
(PA)(DS)	1	58.1	58.1	0.52
Residual	70	7845.3	112.1	
Total Reduction	6	72577.1	12096.2	107.93

PA = Propionic Acid Application Rate
DS = Days of Storage Before Drying
Level of Rejection = 0.05
F_L = 0.50. So, reject DS from the model.

TABLE XI (CONTINUED)

Source	df	SS	MS	F
Total	76	80422.5	1058.2	
Mean	1	29290.1	29290.1	42.96
Residual	75	51132.4	681.8	
PA	1	36050.3	36050.3	176.88
Residual	74	15082.1	203.8	
$(PA)^2$	1	7048.2	7048.2	64.04
Residual	73	8033.9	110.1	
$(DS)^2$	1	91.6	91.6	0.83
Residual	72	7942.3	110.3	
(PA)(DS)	1	16.6	16.6	0.15
Residual	71	7925.7	111.6	
Total Reduction	5	72496.8	14499.3	129.89

PA = Propionic Acid Application Rate
DS = Days of Storage Before Drying
Level of Rejection = 0.05
F_L = 0.15. So, reject (PA)(DS) from the model.

TABLE XI (CONTINUED)

Source	df	SS	MS	F
Total	76	80422.5	1058.2	
Mean	1	29290.1	29290.1	42.96
Residual	75	51132.4	681.8	
PA	1	36050.3	36050.3	176.88
Residual	74	15082.1	203.8	
$(PA)^2$	1	7048.2	7048.2	64.04
Residual	73	8033.9	110.1	
$(DS)^2$	1	91.6	91.6	0.83
Residual	72	7942.3	110.3	
Total Reduction	4	72480.2	18120.0	164.27

PA = Propionic Acid Application Rate
DS = Days of Storage Before Drying
Level of Rejection = 0.05
F_L = 0.83. So, reject $(DS)^2$ from the model.

TABLE XI (CONTINUED)

Soucre	df	SS	MS	F
Total	76	80422.5	1058.2	
Mean	1	29290.1	29290.1	42.96
Residual	75	51132.4	681.8	
PA	1	36050.3	36050.3	176.88
Residual	74	15082.1	203.8	
$(PA)^2$	1	7048.2	7048.2	64.04
Residual	73	8033.9	110.1	
Total Reduction	3	72388.6	24129.5	219.25

PA = Propionic Acid Application Rate
Level of Rejection = 0.05
F_L = 42.96. So, keep the model as is.

Final Model: $PG = 94.6 - 125.4 \times (PA) + 40.6 \times (PA)^2$
PG = Percent Germination
Standard error of estimate = 10.49
R^2 = 0.843

TABLE XII

ANALYSIS OF VARIANCE, 47% M.C., 5-DAY COUNT

Source	df	SS	MS	F
Total	72	39099.5	543.0	
Mean	1	12720.0	12720.0	34.24
Residual	71	26379.5	371.5	
PA	1	12574.7	12574.7	63.76
Residual	70	13804.7	197.2	
$(PA)^2$	1	2750.9	2750.9	17.17
Residual	69	11053.9	160.2	
DS	1	2992.1	2992.1	25.24
Residual	68	8061.7	118.6	
$(DS)^2$	1	700.6	700.6	6.38
Residual	67	7361.2	109.9	
(PA)(DS)	1	2229.7	2229.7	28.68
Residual	66	5131.5	77.7	
Total Reduction	6	33968.0	5661.3	72.82

PA = Propionic Acid Application Rate
DS = Days of Storage Before Drying
Level of Rejection = 0.05
F_L = 6.38. So, keep the model as is.

Final Model: $PG = 47.9 - 93.3 \times (PA) + 38.4 \times (PA)^2 + 7.2 \times (DS) - 0.16 \times (DS)^2 - 3.3 \times (PA)(DS)$

PG = Percent Germination

Standard error of estimate = 9.14

R^2 = 0.788

TABLE XIII

ANALYSIS OF VARIANCE, 47% M.C., 10-DAY COUNT

Source	df	SS	MS	F
Total	72	35768.8	496.8	
Mean	1	11262.4	11262.4	32.63
Residual	71	24506.3	345.2	
PA	1	11251.8	11251.8	59.42
Residual	70	13254.6	189.4	
$(PA)^2$	1	2527.7	2527.7	16.26
Residual	69	10726.9	155.5	
DS	1	2716.7	2716.7	23.06
Residual	68	8010.2	117.8	
$(DS)^2$	1	912.5	912.5	8.61
Residual	67	7097.7	105.9	
(PA)(DS)	1	2077.9	2077.9	27.32
Residual	66	5019.8	76.1	
Total Reduction	6	30748.9	5124.8	67.38

PA = Propionic Acid Application Rate
DS = Days of Storage Before Drying
Level of Rejection = 0.50
F_L = 8.61. So, keep the model as is.

Final Model: $PG = 44.2 - 89.1 \times (PA) + 36.9 \times (PA)^2 + 7.5 \times (DS) - 0.19 \times (DS)^2 - 3.2 \times (PA)(DS)$

PG = Percent Germination

Standard error of estimate = 8.72

R^2 = 0.795

VITA[2]

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