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SOME FACTORS INFLUENCING CITRUS FRUIT DECAY EXPERIMENTS

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It has been known for many years that the principal storage decays of Florida citrus fruits are the stem-end rots and Penicillium molds. The taxonomy and morphology of the causal organisms *Diplodia natalensis* Evans, *Phomopsis citri* Fawc., *Penicillium digitatum* Sacc., and *Penicillium italicum* Wehmer have been very thoroughly studied. Some facts have been discovered about their physiological requirements in relation to their host but essentially nothing about their actual control from a commercial point of view. While much experimental work has been done in previous years, there appears to be at the present time a greater interest in the problem. This is perhaps due to better organization in the citrus industry and to a greater realization of the economic importance of losses caused by stem-end rot and Penicillium molds. High market values of citrus fruits may also be a factor. The recent discovery of Childs and Siegler (1, 2) that treatment of the fruit with a solution of thiourea is very effective in preventing rots and molds has served to further stimulate investigations in this field.

For the past 3½ years the authors have carried on extensive experiments for the purpose of developing practical methods of decay control for use in packinghouses. In the course of their investigations it became evident that before the effect of a treatment, either chemical, mechanical, or otherwise, could be evaluated, some means must be devised whereby the results of their experiments could be correctly judged. This was imperative because of the fact that fruits from the same tree varied in their response to decay. It was found that fruit from different sources, especially between magnesium-deficient trees and those well fertilized with this element, varied considerably in their natural amount of decay. Fruit from different varieties or from different groves having the same cultural treatment, all showed wide variation in the amounts of decay which developed when held at comparable storage conditions (3). Then, too, the temperature and humidity of the storage rooms appeared to be a factor in the amount of decay which developed. All of these factors tend to make it very difficult to interpret satisfactorily the results of any given experiment unless it is designed in such a way as to evaluate the degree of natural variation that is occurring in the fruit and over which there is no experimental control. Fortunately there are methods of designing experiments in such a manner that this variation may be estimated. One of these methods, known as the analysis of variance (7), has been used during the past two years or more to evaluate the results of the most of our experiments.

Analysis of variance for a typical experi-

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ment is given in Table 1. In each treatment 100 oranges divided into 4 lots of 25 were used and the number of decayed fruits in each lot recorded. In treatments 2, 4, and 6 thiourea was applied in different ways and the fruit processed in the packinghouse machine. Treatments 3, 5 and 7 correspond to 2, 4 and 6 respectively, but were given a more thorough rinsing with warm water after the washer. Number 1 is the untreated check. This analysis shows that there was a significant reduction in decay caused by all of the treatments with thiorea when compared with the check. It is also evident that more thorough rinsing after treatment 2 (i. e. treatment 3) did not increase the amount of decay, while for treatments 4 and 6 this was not true. The increases from 1% to 14% and from 3% to 13% are significant if the 5% level of probability is taken, but scarcely significant if as high a certainty as the 1% level is desired. The corresponding least difference necessary for significance at the 1% level would be 10.2% instead of 7.6%.

TABLE 1—Showing The Number Of Decayed Fruit Which Occurred In Each Lot Of 25 And The Values Found By Analysis Of Variances.

Trials	Treatment 1	2	3	4	5	6	7
A	10	0	0	0	5	1	4
B	9	1	1	0	2	1	3
C	5	0	0	1	5	1	2
D	10	1	2	0	2	0	4
Sum of decayed fruit	34	2	3	1	14	3	13

Source of variance	Degrees of freedom	Sum of Squares	Mean Squares	Calculated F value	Needed F value for significance
Total	27	245			
Treatments	6	211	35.2	21.7	2.55 (5% level
Error	21	34	1.62		3.76 (1% level

Least difference necessary for significance: 7.6 at 5% level, 10.2 at 1% level.

TABLE 2—Showing The Stimulating Effect Of Certain Chemicals On The Development of Stem-End Rot.*

Treatment	% of SER* after 3 wks.
Check	48
Copper sulphate 0.1%	56
Sodium hypochlorite 0.05%	78

From many tests during the past year the least difference necessary for significance at the 5% level of probability between lots of 100 fruit each in the amount of stem-end rot that developed in 3 weeks of storage has ranged from 5.8% to 15% with an average of 8.1%. From these observations it is apparent that if the experiments are not set up in such a way as to lend themselves to statistical analysis one must look for a difference of at least 12 to 15% between the percentages of stem-end rot which develops in three weeks among treatments applied to lots of 100 fruit each and randomized between themselves before that difference can be considered. If no randomization is possible even greater differences must be obtained before they can be definitely assumed to be significant.

When it was found possible to interpret results from individual experiments, many tests were made to try to answer such questions as what, when and how certain chemicals might be applied to the fruit, or what mechanical means might be used to reduce decay. It has been pointed out by Hopkins and Loucks (3) that the packinghouse process as exemplified by tests from 6 different packinghouses did not affect the amount of decay that developed during three weeks in storage. Hopkins and Loucks (5) showed that picking of the fruit by pulling did not increase the amount of decay over that produced by clipping. It was found that the removal of the buttons after they had been loosened by treating the fruit with ethylene gas, removal of the calyx when pulling the fruit from the tree, or debuttoning by certain mechanical means decreased the amount of stem-end rot (4).

One of the paradoxes of these investigations is the fact that certain known fungicides actually increase the development of stem-end rot instead of reducing it. A typical result of soaking freshly picked fruit in solutions of $CuSO4$ or NaOCL is shown by the average of two experiments in Table 2.

Other chemicals which increased the development of stem-end rot are sodium thiosulphate 1% or 5%, ethylene gas 1/10,000 for 48 hours, and acetylene gas 1/10,000 for 48 to 72 hours.

A combination of copper sulphate and sodium thiosulphate increased the amount of stem-end rot in fruit obtained from two different locations even though the amount of copper used was very small. In Table 3 is shown the rot-stimulating effect of copper sulphate and sodium thiosulphate.

In order to test the antagonistic value of thiourea against the increase of stem-end rot by a mixture of 0.1% copper sulphate and 1.0% sodium thiosulphate a test was made using this mixture as a dip for 6 hours followed by a 5-minute dip in a 5% thiourea solution which was allowed to dry on without rinsing. The data in Table 4 show that the copper mixture greatly increased the amount of stem-end rot over that in the untreated check, 74% against 32% at 3 weeks. The combination of the copper mixture followed by thiourea prevented the development of any stem-end rot.

TABLE 3—THE STIMULATING EFFECT OF COPPER SULPHATE AND SODIUM THIOSULPHATE ON STEM-END ROT OF FRUIT FROM 2 DIFFERENT LOCATIONS

Solution	% SER after 3 weeks. Fruit from location.	
	1	2
0.1% $CuS04$ $5H_2O$+1.0% $Na_2S_2O_3$	72	54
0.05% $CuSO_4$, $5H_2O$+1.0% $Na_2S_2O_3$	64	42
Check, No treatment	14	10

The results from certain experiments indicate that some of the natural products of metabolism of stored citrus fruits increased the amount of stem-end rot. Since it is known that ethylene gas increases stem-end rot and ethylene is one of the products of metabolism, an experiment was set up to determine if the removal of this or some other gas would be effective in reducing the rots.

TABLE 4—SHOWING THE ANTAGONISTIC EFFECT OF THIOUREA AGAINST COPPER FOR INCREASING THE DEVELOPMENT OF STEM-END ROT.

Treatment	Amt. of SER after 1 wk.	2 wks.	3 wks.	4 wks.
Check	0	18	32	36
0.5% $CuSO4$ - 1.0% $Na_2S_2O_3$	34	68	74	80
Same followed by 5% thiourea	0	0	0	0

One lot of fruit was sealed in a 50-gallon drum, another was placed in a drum in which the air was circulated over potassium permanganate and potassium hydroxide to absorb the ethylene, carbon dioxide and perhaps other gases. A third lot of fruit was held in ordinary storage. The results are shown in Table 5 which indicates that the removal of metabolic products is important in controlling stem-end rot.

TABLE 5—EFFECT OF REMOVING METABOLIC PRODUCTS

Treatment	% SER after 3 weeks
Closed drum	54
Closed drum + $KMnO_4$ + KOH	10
Check ordinary storage	12

Various methods of storing fruit that would naturally allow varying accumulations of ethylene or other metabolic products were tested and Table 6 shows that the type of storage providing the most ventilation and which most effectively removed these products is the best from a control standpoint. In this connection it should be mentioned that by immediately sealing the stem of clipped fruit by paraffin, which would retard or prevent the exchange of gases through the stem tissues, the development of stem-end rot was greatly increased.

Another experiment was set up to test the suggestion that fruit picked by clipping with dull clippers developed more rot than fruit which is picked by cutting the stem with a very sharp knife. The results showed that the fruit picked by cutting developed enough less stem-end rot than those picked by the dull clippers to be just on the borderline of significance. The stem tissue crushed by the clippers may have formed more of a blockage to the free passing of gases through the stem end than did the tissue which was cut by the sharp knife and not crushed.

TABLE 6—RESULTS OBTAINED BY VARYING THE DEGREE OF VENTILATION IN STORAGE SPACE

Type of Storage	% SER after three weeks
Sealed in drum	80
Drum covered with cheesecloth	54
Commercial storage, with air circulated	36
Ventilated drum	36
On trays in large open room	24

Activated carbon which was saturated with bromine was very effective in controlling stem-end rot when placed in cloth pads and enclosed in packed boxes of oranges. Under the circumstances of the tests, the molds were slightly increased, but it is believed that by more refined methods this harmful effect could be eliminated. The results from one experiment are shown in Table 7 where stem-end rot was reduced by activated carbon saturated with either bromine or chlorine.

TABLE 7—DECREASE IN STEM-END ROT CAUSED BY ACTIVATED CARBON FORTIFIED WITH BROMINE OR CHLORINE

Treatment	% SER during 2 wks. storage
Check	10
Activated carbon	17
Activated carbon + bromine	0.8
Activated carbon + chlorine	6

Results from the above mentioned experiments uphold the theory that something in the metabolic process hastens the development of stem-end rot and that anything which reduces the accumulation of metabolic products, possibly ethylene, will retard the development of rots. In this connection, since the movement of oxygen into and from the stem tissue was modified by all of these tests, the possibility of these results being associated with an oxidation-reduction interaction was considered. However, oranges treated with various oxidizing agents developed as much rot as untreated. Attempts at toning the rind physiologically to make the fruit more resistant to rots by the use of various ions such as Ca, H, Fe, Mn, Al, and Zn were unsuccessful.

It has been known for a long time that if the buttons can be removed from the citrus fruits their predilection to stem-end rot was greatly reduced. Because the removal of buttons involves a great amount of labor, methods were tested that might loosen the buttons enough that they would be rubbed off by the regular packinghouse machinery brushes. Fruits were soaked with various hormones and other chemicals to no avail. Gassing with various materials seemed to give some promise, so a tensiometer was used to record the relative tension necessary to pull the buttons from oranges after they had been treated with various gases. It was found that these gases were effective in causing the buttons to loosen in the following order; ethylene the most effective and ether the least; ethylene 1:5000, acetylene 1:5000, chloroform 1:100; benzene 1:123, carbon tetrachloride 1:138, toluene 1:150 and ether 1:146. Approximately six times as much pull was required to remove the buttons from fruit treated with ether as from those treated with ethylene. So long as the fruit is picked by pulling as it is at present, no means of removing the buttons by the brushes will be possible. In fact, a sufficient number of buttons clipped with short stems and gassed with ethylene were not removed by the brushes to give a commercial control of stem-end rot. It would be necessary to cut the stems longer than is best from the stem-puncture injury standpoint. Until some means is devised for economical removal of the buttons this method of control appears to be ruled out.

Approximately 75 chemicals were tested for their fungicidal action against the four organisms involved in this discussion by incorporating them in agar plates inoculated with the fungi. Among them the following were found to be effective in controlling one or more of the organisms in culture: 8-hydroxyquinoline, chlorothymol, thymol, copper sulphate, allyl isothiocyanate, cupferron, and 8-hydroxyquinoline sulphate when used at concentrations of 500 p.p.m. Thiourea was effective at a concentration of 3%. When these chemicals were used as a dip or in conjunction with wax, thiourea was the only one which was effective in controlling the fruit rots. As mentioned before, copper sulphate actually increased the amount of rot.

The above results indicate that culture tests to determine the effectiveness of chemicals in controlling rots on citrus fruits are not practical. It was discovered that a drop of a chemical in solution placed on the buttons of the fruit is a more reliable means of quickly and cheaply testing the efficacy of the chemical (6). Approximately 50 chemicals have been so tested and the following have given good results: merthiolate, thiourea, 8-hydroxyquinoline sulphate, 2, 4, diamino-diphenylamine, 8-

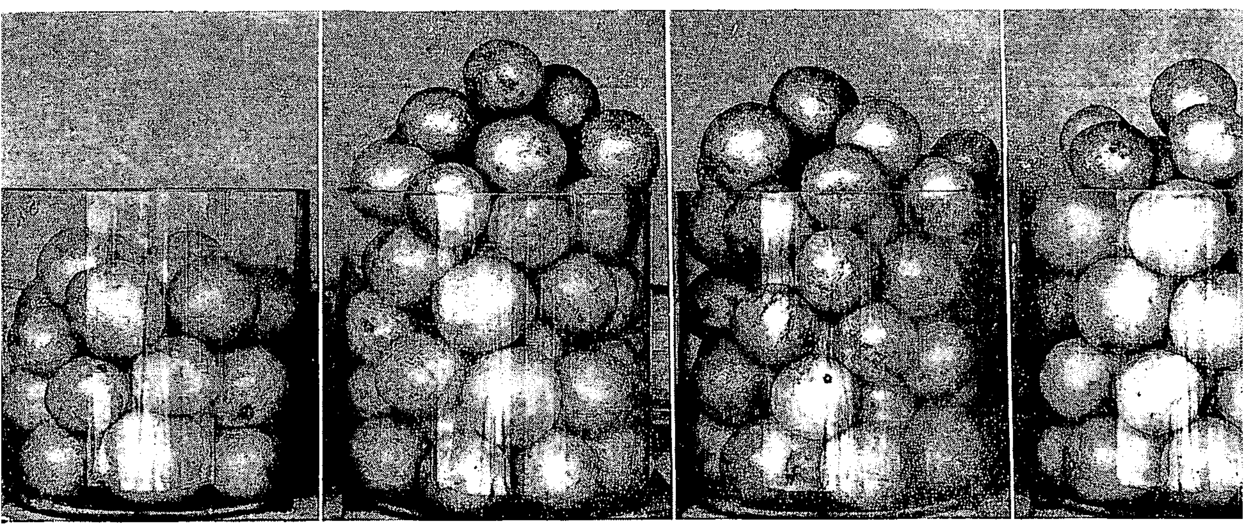

Figure 1. Reduction of decay obtained by painting the buttons with various chemicals. Sound fruit remaining after 5 weeks in storage. Left to right: Check (no treatment), merthiolate, 8-hydroxylquinoline sulfate, thiourea.

hydroxyquinoline base in oil, 2 methyl-1, 4-napthoquinone, naphthol, phenyl-mercuric acetate, phenyl-mercuri-triethanol ammonium lactate, and thiosemicarbazide. In Figure 1 is shown the amount of sound fruit remaining after 5 weeks, when comparing those having buttons painted with certain chemicals against those that were not painted.

Not all of these compounds have been further tested to discover their effectiveness when applied in some manner which is commercially possible. Many of the 8-hydroxyquinoline derivatives have been tried but consistently good results have not been obtained by any method used so far. It has been used mostly in the sulphate form because it is more soluble. Thiourea has been tested very extensively and has been found to control the rots effectively when the fruit was subjected to a 10% solution for 6 to 10 minutes. An increase in temperature of the treating bath to 55°C improved the efficiency of the treatment as did also a hot treatment in a 25% solution for 2 minutes or a 30-second submersion in a 10% solution at room temperature and the fruit allowed to stand for 1 hour before the thiourea was washed off. The use of thiourea

Figure 2. Effect of 10% thiourea in soaking, scrubbing, and color-add sections of the packing-house process. Amount of decay during 2 weeks in storage from treated box is shown on the left and from untreated regular packed box on the right

is prohibited at the present time because its toxic effect on humans is not yet known.

A few proprietary materials that have been supplied by their distributors have been given very thorough tests for their ability to control rots. Paper mats containing some ammonium compound were found to give no control over stem-end rot or molds when 8 to 24 wafers were placed in a packed box of oranges. Various green paper wraps were extensively tested on oranges and grapefruit for their ability to control storage diseases in lighted and darkened storage rooms. They were found to have no effect on the diseases and shrinkage was just as great in the green wraps as it was in the light orange colored paper wraps that are commonly used for wrapping citrus.

Two epoxides, ethylene oxide and propylene oxide, which were tried as fumigants proved ineffective at any concentration that was low enough to be harmless to the fruit rind.

Although at the present time no usable chemical has been found that is highly effective against citrus storage rots by any of the methods so far used, that is cheap enough to be profitable, and whose toxicity to humans is known, the results reported here seem very hopeful that such a compound can be discovered.

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PROBLEMS IN THE DEHYDRATION OF ORANGE JUICE[1]

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A process has been developed employing vacuum diffusion for the dehydration of orange juice to a virtually anhydrous powder. The powder is extremely palatable and should go far in increasing the distribution of orange juice to segments of the world's population now deprived of this valuable food. The dehydration can be stopped short of the powder state to make a concentrate which can be sold as a frozen food. This frozen concentrate when reconstituted with three times its volume of water closely approximates fresh juice.

The purpose of the present paper is to discuss the process and describe some of the problems involved, as well as to present data in the hope that some duplication of effort by other workers may be avoided.

Orange juice is screened and circulated at a temperature of 55°F. through concentrators where the water is evaporated under vacuum until the concentration reaches 50 to 60%

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