ARTIFICIAL INDUCTION OF LUMPS IN THE FRUIT OF PAPAYA (*Carica papaya* L.)

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INTRODUCTION

Papaya (Carica papaya L.), is one of the most popular fruits cultivated in Hawaii and other tropical countries. The industry is more important in Hawaii than in any other country. Sizeable shipments are now being exported by air to metropolitan areas in the United States mainland.

Recently, the farmers have been faced with a problem involving quality of the marketed fruits. Hard portions, or islands of unripened tissue within the flesh of the papaya, hereafter referred to as lumps, are frequently encountered in the flesh of ripe fruits. This condition has been observed for some time, but is nowhere recorded in the literature. The problem is not one of great magnitude as yet, but investigation is needed as to its nature and causes so that it can be prevented or cured.

Lumps have been observed in fruits obtained from commercial orchards in Kapoho, Hawaii, and Waimanalo, Oahu and from the backyards in Honolulu (K. Kimura, G. Shigeura, and E. Morris, respectively, personal communications). A preliminary survey by R. M. Warner (unpublished data) confirmed the occurrence of lumpy fruits in a number of orchards on Oahu and Hawaii. He observed several types of lumps: large plate-like areas in the fleshy portion of the fruit, small grain-like lumps and rounded hemispherical lumps attached to the rind which frequently were associated with insect or disease injury.

This investigation was undertaken in an effort to provide some understanding of the causes of lumpiness and to serve as a basis for research which ultimately would provide control measures.

In attempts to determine the causes of lumpiness, the ability of injected substances to induce lumps was investigated. Theories as to possible physiological mechanisms involved were formulated.

REVIEW OF LITERATURE

The physiology of the ripening of fruit is indeed complex. In spite of its complexity, however, many investigations have already been undertaken and the ripening process has been shown to be associated with the degradation of pectic substances. This degradation is associated with the hydrolysis of protopectic, the water-insoluble parent pectic substance in plants, which upon restricted hydrolysis yields pectinic acids.

Postlmayr, et al., (15) showed that there was a definite transformation of protopectin into water-soluble pectin as fruit ripened. The increase in water-soluble pectin closely paralleled a decrease in the protopectin.

Kertesz (12), in his post-harvest study of apples found that the protopectin decreased during storage, first rapidly and later slowly. The level of the insoluble pectic constituent of the middle lamella was practically constant at first, but decreased rapidly as ripening progressed; the content in water-soluble pectic material was constant at a low level in growing fruit, and increased steadily until full maturity was attained. He further found that, in "over ripened" and mealy fruit, the proportion of soluble to insoluble pectic components decreased, perhaps because ultimately all pectic substances decomposed into non-pectic substances. With regard to the relationship of soluble pectic substances to firmness of fruit, it was shown that there was an increase of soluble pectic substances as firmness decreased,

approaching maturity. From other studies, he asserted that pectic substances were found principally in the middle lamellae of plant cells wherein they are believed to function as the "cementing" material which binds cells together.

Appleman and Conrad (2) indicated that transformation of protopectin into water-soluble pectin occurred during the ripening of freestone peaches. Relatively low storage temperatures retarded the rate of formation of water-soluble pectin and, thus, delayed the softening in freestone peaches. They confirmed the view that the degradation of pectic substances and other polysaccharides into soluble pectin was responsible for the ripening of apple, pears, and, in general, other fleshy fruits.

In the ripening of papaya, Jones & Kubota (10) observed that depolymerization of starch was not involved but some acid-hydrolyzable material decreased from 9 to 4 per cent, on a dry weight basis. This may mean that some substance such as hemicellulose and/or pectin in the cell wall was being changed to more soluble compounds.

Enzymes, not specifically known yet but presumably pectinesterase, are no doubt responsible for the change from protopectin to water-soluble pectin which is associated with ripening. Hamson (9), indicated that firmness and pectinesterase activity of tomato fruits were probably directly related: the strain with firm fruit showed a higher enzymatic activity than the soft strain. Hall and Dennison (7), however, contradicted the observation

by Hamson, because they found no significant relationship between firmness and pectinesterase activity in the tomato fruit they studied.

According to Yang, et al. (20) in the ripening of the brining cherries polygalacturonase was responsible for the degradation of pectin. The enzyme was inactivated under acid conditions. The firmness of cherries depended primarily on the natural pectin. When this was destroyed, the cherries softened. Their results confirmed the early work of McCready and McComb (14), who found that pectic enzymes were generally the cause of changes in texture during ripening of fruits. In avocado, peaches, and pears, pectic enzymes come in contact with pectic substance substrate during ripening and hydrolyze them to compounds of lower molecular weights. The degraded substances are then less effective in maintaining fruits in a firm state and are less important in determining the consistency of preserved fruits.

The firm "non-melting" texture of ripe clingstone peaches was attributed to the high retention of protopectin in thick, intact cell walls; whereas the soft "melting" texture of freestone was related to the reversed condition. The protopectin and water-soluble pectin were the constituents chiefly responsible for extreme differences in texture between ripe clingstone and freestone peaches. (Addoms et al., 1). These workers further considered that the development of thinner cell walls and actual physical breakdown of some of the mesocarp parenchyma cell walls in the ripe Elberta peach were the principal causes of the soft, "melting flesh" texture of the freestone

of the freestone in contrast to the thicker and completely intact cell walls of the ripe, firm textured clingstone peach.

Haller (8) reported that the softening of apples during ripening was associated with the conversion of water-insoluble pectin to soluble forms thereby rendering the cell wall less resistant to pressure.

MATERIALS AND METHODS

In order to artificially introduce liquid materials into the flesh of the papaya, methods of injection had to be developed. The use of hypodermic syringes was the best prospect. Several problems were encountered, however. The firmness of the papaya flesh did not yield to allow the liquid to enter, and the injected liquid tended to escape through the needle hole. The second problem was the plugging of the needles by the coagulation of the secreted latex resulting from the needle wound. The needle part of the hypodermic syringe, with a thin wire inserted through its channel, was inserted 3/4 inch into the green fruit. This wire prevented the obstruction of the channel by latex which exuded and otherwise became deposited within it. After five minutes, the latex had coagulated and the wire was removed. The needle was withdrawn 1/4 inch to make room for the liquid. The cartridge, containing the test solution was then attached to the needle, and 0.4 ml of the solution was deposited under the conditions of the experiment. The chemicals oozed out of the tissue if greater volume was used.

Several types of chemicals were tested: Auxins (indoleacetic acid and 2, 4-dichlorophenoxyacetic acid), Kinin (kinetin-6-furfurylaminopurine), Gibberellin (gibberellic acid 3), Plant Growth Inhibitors (coumarin, maleic hydrazide, 2, 3, 5-triiodobenzoic acid [TIBA], and other chemicals (chloramphenicol, copper sulfate, 2, 4-dinitrophenol [DNP], DL-ethionine, mannitol).

Each chemical was tested in five concentrations. All the chemicals enumerated above were tested in the molar concentrations of 0, $3x10^{-5}$, 10^{-4}, $3x10^{-4}$, 10^{-3}. Because of its low solubility, kinetin was tested in 0, 10^{-5}, $3x10^{-5}$, 10^{-4} and $3x10^{-4}$ molar concentrations. Distilled water solvent was used in each case. Each fruit received these five concentrations of a given chemical randomly injected into the flesh (see Fig. 1). Essentially then each fruit served as a complete replicate. Twenty replicated fruits, one from each tree, were treated in testing a chemical. The fruits were allowed to ripen on the trees, and when harvested, they were examined for development of lumps within the treated region. The weight and volume of lumps were recorded. The former was easier to determine and more accurate; thus, only the weight of lumps is reported. The correlation between the two was very high (r = .977).

In January, 1963, the main experiments were conducted in an old orchard located at the Waimanalo Experiment Station. Fruits of Carica papaya L., variety solo (Fig. 2) were utilized throughout. The trees had been topped, and fruits developing on their several lateral branches served as test materials. Twenty trees of comparable size and vigor were used. The fruits to be treated with chemicals were further selected for reasonable similarity in age, as indicated by external color and size. Each fruit was allowed to ripen on the tree which was usually within two to six weeks following treatment.

FIGURE 1. RIPE PAPAYA FRUIT PREVIOUSLY INJECTED WITH FOUR LEVELS OF A CHEMICAL (NO. 2 TO 5) AND A DISTILLED WATER CONTROL (NO. 1). NOTE THE TISSUE SURROUNDING THE INJECTION SITE REMAINS GREEN AFTER THE REST OF THE FRUIT HAS TURNED YELLOW

FIGURE 2. THE OLD SOLO LINE PAPAYA AT WAIMANALO FARM EXPERIMENT STATION USED AS A TEST PLANT

Microscopic investigation was undertaken on the sections of normally ripe tissue, lumps induced by the distilled water control, and lumps induced by the higher concentrations of chemical treatments. The pectic substances were investigated in the sections of the fruit by the Ruthenium Red Method (11). The fresh tissue was sectioned and placed in aqueous ruthenium red (1:10, 000) for five to ten minutes. Then the stained section was mounted in water and examined under the microscope. Pectic substances appeared pink to red.

Statistical methods of data analysis as described by Snedecor (19) and LeClerg, Leonard and Clark (13) were followed.

RESULTS

The results here are the effect of each substance tested for the induction of lumps compared to the water control. The weights in grams of lumps induced by the five levels of each chemical injected were treated statistically by an analysis of variance.

Auxins

(a) Indoleacetic acid (IAA): In this experiment IAA induced lumps at all levels as shown in Fig. 3. In Table 1, the mean fresh weight and standard errors for each concentration are given. Levels 2 and 3 were significantly higher than the control and the increase of concentration brought about an increase in the weight of lumps. It is quite intriguing to note, however, that the mean weight of lumps decreased at level 4. The histogram in Fig. 4 presents these results graphically.

(b) 2,4-Dichlorophenoxyacetic acid (2,4-D): Another auxin, 2,4-D was used as one of the test chemicals injected because of its well-known effects upon plant cells. In this experiment, the mean weight of lumps produced from the molar concentrations of 0, $3x10^{-5}$, 10^{-4}, $3x10^{-4}$, and 10^{-3} was 11.71, 13.28, 16.27, 14.86, and 13.12 grams, respectively. Fairly large lumps were produced by all levels but no significant differences in weights were found.

FIGURE 3. THE LUMPS INDUCED BY INJECTION OF DIFFERENT MOLAR CONCENTRATIONS INDOLEACETIC ACID (IAA)

TABLE 1. MEAN FRESH WEIGHT OF LUMPS FROM FIVE LEVELS OF INDOLEACETIC ACID AND THE SIGNIFICANCE FROM THE COMPUTED F-VALUE

LEVELS	MOLAR CONCENTRATION	MEAN FRESH WT. IN GMS	SIGNIFICANCE
0	0	4.78 ± 1.23	---
1	3×10^{-5}	6.52 ± 1.0	---
2	10^{-4}	8.36 ± 0.93	*
3	3×10^{-4}	8.84 ± 1.06	*
4	10^{-3}	5.72 ± 1.12	---

* Significantly heavier than the control at the 5% level

--- Not significant

TABLE 2. MEAN FRESH WEIGHT OF LUMPS FROM FIVE LEVELS OF GIBBERELLIC ACID AND THE SIGNIFICANCE FROM THE COMPUTED F-VALUE

LEVELS	MOLAR CONCENTRATION	MEAN FRESH WT. IN GMS	SIGNIFICANCE
0	0	7.74 ± 0.92	
1	3×10^{-5}	12.84 ± 1.56	**
2	10^{-4}	13.64 ± 1.64	**
3	3×10^{-4}	14.69 ± 1.72	**
4	10^{-3}	18.46 ± 2.43	**

** Significantly heavier than the control at the 1% level

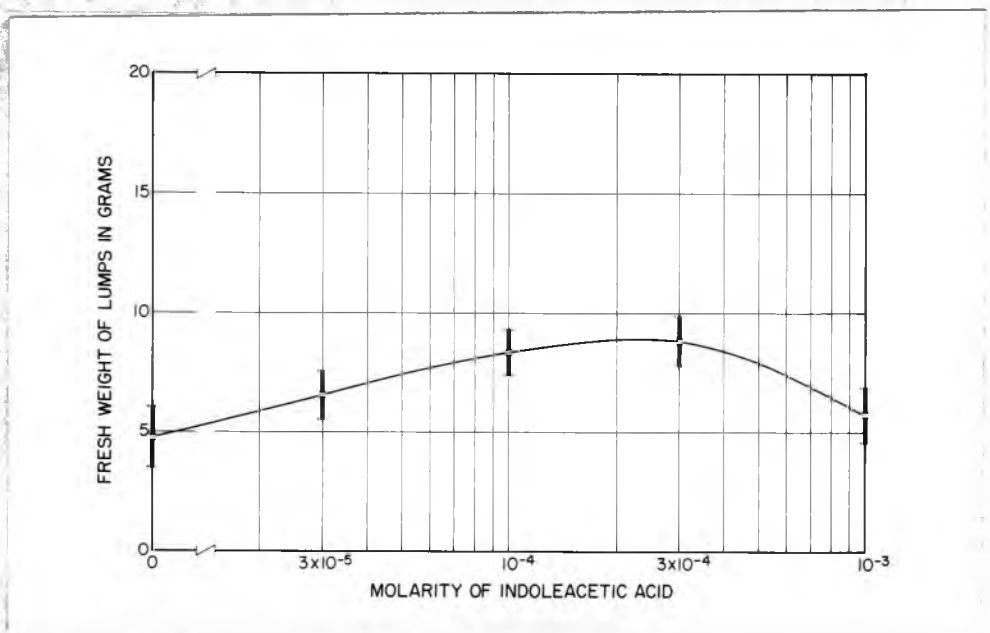

FIGURE 4. THE EFFECT OF FIVE LEVELS OF INDOLEACETIC ACID UPON THE WEIGHT OF LUMPS FORMED IN THE TREATED PAPAYA FRUIT. STANDARD ERRORS ARE INDICATED BY THE LINE SYMBOLS AT EACH POINT ON THE CURVE

Gibberellins

Gibberellic acid (GA_3): The mean weight of lumps induced by the gibberellic acid treatment showed a significant difference over the control as indicated in Table 2. These results showed that gibberellic acid had a profound effect upon the formation of lumps. Even at the lowest concentration of the chemical (level 1), the increase in weight was highly significant. The histogram in Fig. 5 shows that the mean weight at level 4 was significantly greater than levels 1 and 2. The size of the lumps increased with the concentration of the chemical injected.

Kinins

Kinetin: Injections of kinetin at molar concentrations of 0, $1x10^{-5}$, $3x10^{-5}$, 10^{-4}, and $3x10^{-4}$ resulted in the formation of lumps the mean weights of which were 11.04, 11.36, 11.54, 12.53, and 10.4 grams, respectively. The analysis of variance showed no differences between any of these levels, although the lumps were all moderately large.

Inhibitors

In this group, three compounds known to be plant growth inhibitors were injected.

(a) Coumarin: From the results represented in Table 3, it is clear that level 1 did not give a significant increase in the formation of lumps over the control. Nevertheless, with the increase of concentration, the means

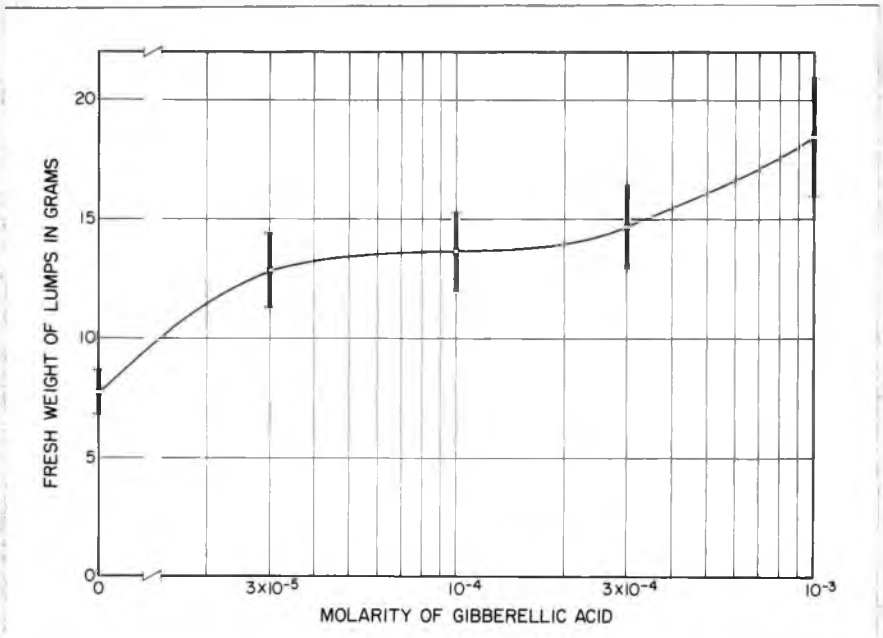

FIGURE 5. THE EFFECT OF FIVE LEVELS OF GIBBERELLIC ACID UPON THE WEIGHT OF LUMPS FORMED IN THE TREATED PAPAYA FRUIT. STANDARD ERRORS ARE INDICATED BY THE LINE SYMBOLS AT EACH POINT ON THE CURVE

TABLE 3. MEAN FRESH WEIGHT OF LUMPS FROM FIVE LEVELS OF COUMARIN AND THE SIGNIFICANCE FROM THE COMPUTED F-VALUE

LEVELS	MOLAR CONCENTRATION	MEAN FRESH WT. IN GMS	SIGNIFICANCE
0	0	10.42 ± 1.27	
1	3×10^{-5}	10.98 ± 0.96	---
2	10^{-4}	12.81 ± 1.25	*
3	3×10^{-4}	13.23 ± 0.96	**
4	10^{-3}	12.45 ± 0.96	*

* Significantly heavier than the control at the 5% level

** Significantly heavier than the control at the 1% level

--- Not significant

became significant over the water control. This increase of mean due to increase of concentration effect is shown further in the histogram of Fig. 6. It should be noted that the highest concentration declined in its ability to induce lumps.

(b) Maleic Hydrazide: The injection of maleic hydrazide produced an array of lumps such as shown in Fig. 7. The mean weights of this treatment are given in Table 4. All levels of maleic hydrazide (1, 2, 3, 4) gave a significant increase over the control. However, no significant differences among levels 1, 2, and 3 were observed. A difference, significant at the 1% level, was found between level 4 and levels 1, 2, and 3. Here again, the increase in concentration had a profound effect upon the induction of lumps. The upward trend of lump formation as the concentration of maleic hydrazide increased to level 4, is further illustrated in Fig. 8.

(c) Triiodobenzoic acid (TIBA): The effect of the triiodobenzoic acid application on the lump formation is shown in Table 5. All levels produced a mean weight of lumps significantly heavier than the control at 1% level. There was no difference in weight of lumps among the TIBA treatments as the concentration was increased. The histogram in Fig. 9 shows this increase graphically.

Other Substances

(a) Dinitrophenol (DNP): The dinitrophenol treatment produced a mild

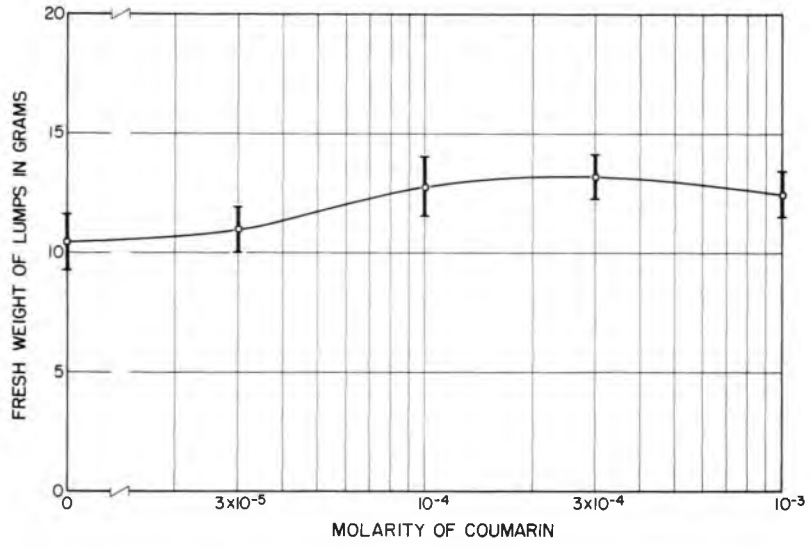

FIGURE 6. THE EFFECT OF FIVE LEVELS OF COUMARIN UPON THE WEIGHT OF LUMPS FORMED IN THE TREATED PAPAYA FRUIT. STANDARD ERRORS ARE INDICATED BY THE LINE SYMBOLS AT EACH POINT ON THE CURVE

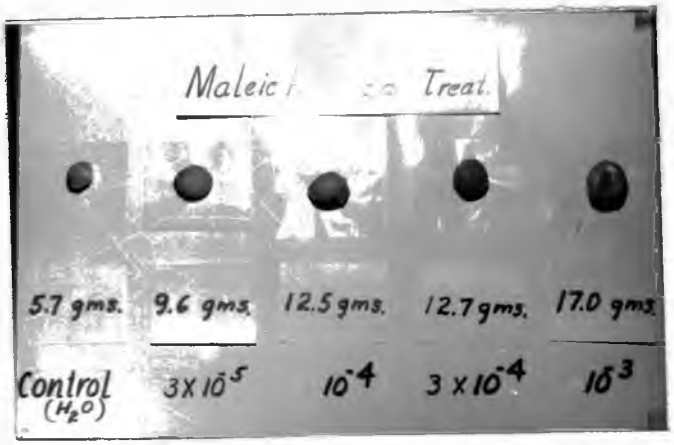

FIGURE 7. THE LUMPS INDUCED BY INJECTION OF DIFFERENT MOLAR CONCENTRATIONS OF MALEIC HYDRAZIDE

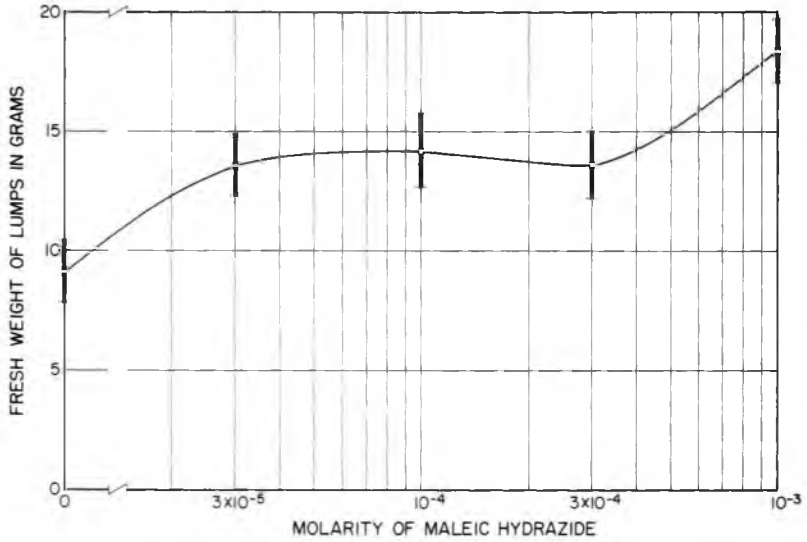

FIGURE 8. THE EFFECT OF FIVE LEVELS OF MALEIC HYDRAZIDE UPON THE WEIGHT OF LUMPS FORMED IN THE TREATED PAPAYA FRUIT. STANDARD ERRORS ARE INDICATED BY THE LINE SYMBOLS AT EACH POINT ON THE CURVE

TABLE 4. MEAN FRESH WEIGHT OF LUMPS FROM FIVE LEVELS OF MALEIC HYDRAZIDE AND THE SIGNIFICANCE FROM THE COMPUTED F-VALUE

LEVELS	MOLAR CONCENTRATION	MEAN FRESH WT. IN GMS	SIGNIFICANCE
0	0	9.11 ± 1.29	
1	3×10^{-5}	13.59 ± 1.28	**
2	10^{-4}	14.19 ± 1.56	**
3	3×10^{-4}	13.6 ± 1.39	**
4	10^{-3}	13.35 ± 1.36	**

** Significantly heavier than the control at the 1% level

TABLE 5. MEAN FRESH WEIGHT OF LUMPS FROM FIVE LEVELS OF TRIIODOBENZOIC ACID AND THE SIGNIFICANCE FROM THE COMPUTED F-VALUE

LEVELS	MOLAR CONCENTRATION	MEAN FRESH WT. IN GMS	SIGNIFICANCE
0	0	7.62 ± 1.09	
1	3×10^{-5}	11.40 ± 1.06	**
2	10^{-4}	11.45 ± 0.95	**
3	3×10^{-4}	11.25 ± 1.05	**
4	10^{-3}	11.66 ± 0.87	**

** Significantly heavier than the control at the 1% level

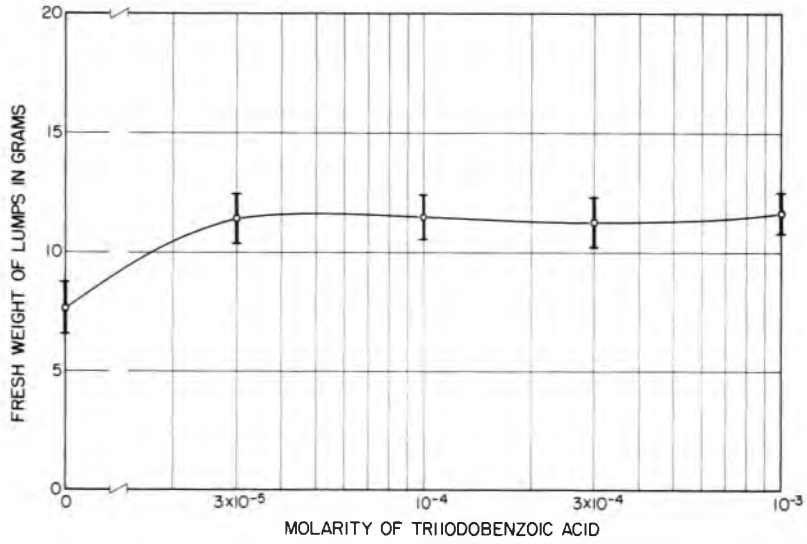

FIGURE 9. THE EFFECT OF FIVE LEVELS OF TRIIODOBENZOIC ACID UPON THE WEIGHT OF LUMPS FORMED IN THE TREATED PAPAYA FRUIT. STANDARD ERRORS ARE INDICATED BY THE LINE SYMBOLS AT EACH POINT ON THE CURVE

increase in the weight of lumps as shown in Fig. 10. Table 6 shows that levels 3 and 4 gave a significant increase in weight of lumps over the control at the 5% level. These means and their standard errors are shown graphically in Fig. 11.

(b) Chloramphenicol: This chemical was injected into the papaya fruit at molar concentrations of 0, $3x10^{-5}$, 10^{-4}, $3x10^{-4}$, and 10^{-3}. The lumps produced had mean weights of 9.75, 11.53, 12.44, 10.87, and 12.78 grams, respectively. There was no significant difference found among these means as determined by the analysis of variance. The lumps formed in one fruit replicate is shown in Fig. 12.

(c) Copper Sulfate: Copper sulfate was injected into the papaya fruit in the following concentrations: 0, $3x10^{-5}$, 10^{-4}, $3x10^{-4}$, and 10^{-3}. These five levels produced the mean weights of lumps of 3.93, 6.80, 4.76, 5.33 and 5.98 grams, respectively. Analysis of variance showed no significant differences. These means seem to be low compared with the means of the other chemicals injected at the same molar concentrations. Fig. 13 shows a photograph of the lumps from one fruit replicate.

(d) Ethionine: Papaya fruits injected with ethionine at molar strengths of 0, $3x10^{-5}$, 10^{-4}, $3x10^{-4}$, and 10^{-3} resulted in mean lump weights of 7.25, 9.76, 9.99, 10.16, and 10.56 grams, respectively. No significant differences were found by analysis of variance.

(e) Mannitol: Molar concentrations of mannitol at the following levels

FIGURE 10. THE LUMPS INDUCED BY INJECTION OF DIFFERENT MOLAR CONCENTRATIONS OF DINITROPHENOL

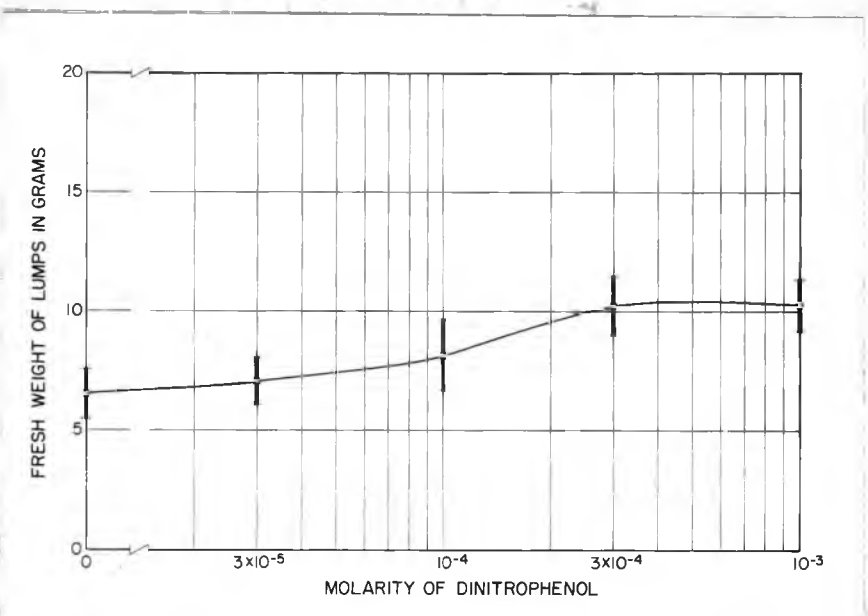

FIGURE 11. THE EFFECT OF FIVE LEVELS OF DINITROPHENOL UPON THE WEIGHT OF LUMPS FORMED IN THE TREATED PAPAYA FRUIT. STANDARD ERRORS ARE INDICATED BY THE LINE SYMBOLS AT EACH POINT ON THE CURVE

TABLE 6. MEAN FRESH WEIGHT OF LUMPS FROM FIVE LEVELS OF DINITROPHENOL AND THE SIGNIFICANCE FROM THE COMPUTED F-VALUE

LEVELS	MOLAR CONCENTRATION	MEAN FRESH WT. IN GMS	SIGNIFICANCE
0	0	6.52 ± 1.06	
1	3×10^{-5}	7.09 ± 0.99	---
2	10^{-4}	8.13 ± 1.47	---
3	3×10^{-4}	10.23 ± 1.24	*
4	10^{-3}	10.25 ± 1.05	*

* Significantly heavier than the control at the 5% level

--- Not significant

FIGURE 12. THE LUMPS INDUCED BY INJECTION OF DIFFERENT MOLAR CONCENTRATIONS OF CHLORAMPHENICOL

FIGURE 13. THE LUMPS INDUCED BY INJECTION OF DIFFERENT MOLAR CONCENTRATIONS OF COPPER SULFATE

0, $3x10^{-5}$, 10^{-4}, $3x10^{-4}$, and 10^{-3} resulted in the induction of lumps with mean weight of 8.49, 8.84, 9.13, 9.38, and 10.46 grams, respectively. Analysis of variance showed no significant differences betweenlevels. The lumps induced in one replicate are shown in Fig. 14.

The enhancing capacity of each tested chemical to induce lumps was found to be varied. Some chemicals were significantly higher in mean weight of lumps at the higher concentrations over the control. Some were found significant over the control at lower concentrations. Others were found to be not significant at all in the mean weight of lumps over the control. Table 7 shows the minimum molar concentrations of substances injected and significantly enhanced the lump weight over the control: 10^{-4} for indoleacetic acid, $3x10^{-5}$ for gibberellin, 10^{-4} for coumarin, $3x10^{-5}$ for maleic hydrazide, $3x10^{-5}$ for triiodobenzoic acid and $3x10^{-4}$ for dinitrophenol. Those that did not give significant differences over the mean weight of lumps of the control were 2, 4-dichlorophenoxyacetic acid, kinetin, chloramphenicol, copper sulfate, dl-ethionine and mannitol.

Effect of Age Upon Lump Formation

It was noted as the injected papaya were harvested that some took a much longer time to ripen than expected. Nine out of 20 fruits injected with maleic hydrazide ripened 8 to 10 weeks after injection. The average time of ripening was about four weeks. When the injections were made, fruits

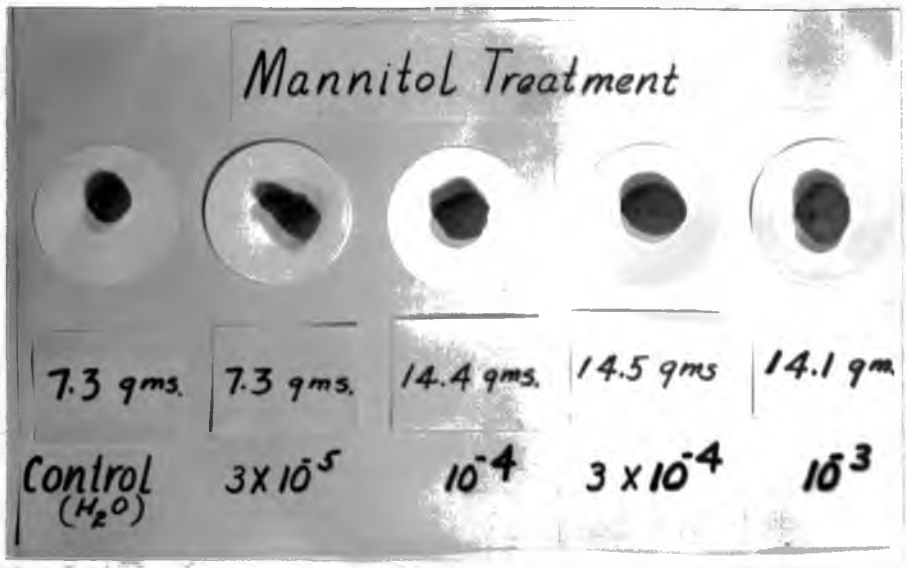

FIGURE 14. THE LUMPS INDUCED BY INJECTION OF DIFFERENT MOLAR CONCENTRATIONS OF MANNITOL

TABLE 7. MINIMUM MOLAR CONCENTRATIONS OF INJECTED SUBSTANCES SIGNIFICANTLY ENHANCING LUMP WEIGHT OVER THE WATER CONTROL

SUBSTANCES	MINIMUM MOLAR CONCENTRATION
Auxins	
IAA	10^{-4}
2, 4-D	None *
Gibberellin	
GA_3	3×10^{-5}
Kinin	
Kinetin	None *
Plant Growth Inhibitors	
Coumarin	10^{-4}
Maleic Hydrazide	3×10^{-5}
Triiodobenzoic acid	3×10^{-5}
Others	
Chloramphenicol	None *
Copper Sulfate	None *
Dinitrophenol	3×10^{-4}
Ethionine	None *
Mannitol	None *

* Not significantly heavier than the control

were selected about the same age so far as it was possible to determine. There seems to be a strong possibility that ripening of some fruits was delayed.

It was also noted that, in the later ripening fruits, the size of the lumps was larger and heavier. The correlation of the weight of lumps produced and the number of days from injection to harvest for each chemical is presented in Table 8. The sum of the weights of lumps for all levels, 0 to 4, was used for the Y value in computing the correlation. All of the correlations except two were significant at the 1% level. The highest correlations were gibberellin + 0.939, TIBA + 0.867, 2,4-D + 0.851 and mannitol + 0.847.

When the weight of lumps of all the fruit replicates were correlated with the number of days, regardless of the chemical, the coefficient was found to be +0.992. This is almost a perfect correlation. The regression line for these calculations is shown in Fig. 15. This indicates that the nearer to ripening the treatment was applied, the less the weight of lumps produced. Actual observations of the treated fruits confirmed that some of those which ripened 10 days after the injection, produced no lumps. It is likely that after the onset of ripening, lumps probably cannot be induced no matter what lump-inducing agent is used into the tissues of the fruit.

Microscopic Investigations

Microscopic investigations were made on pectic substances in the

TABLE 8. THE CORRELATION COEFFICIENTS FOR NUMBER OF DAYS FROM DATE OF INJECTION TO HARVESTING, WITH WEIGHT OF LUMPS PRODUCED

SUBSTANCES	CORRELATION COEFFICIENT
Auxins	
IAA	+0.487 *
2,4-D	+0.851 **
Gibberellin	
GA_3	+0.939 **
Kinin	
Kinetin	+0.775 **
Plant Growth Inhibitor	
Coumarin	+0.593 **
Maleic Hydrazide	+0.676 **
Triiodobenzoic acid	+0.867 **
Others	
Chloramphenicol	+0.756 **
Copper Sulfate	+0.721 **
Dinitrophenol	+0.405 ns
Ethionine	+0.644 **
Mannitol	+0.847 **

* Significant at 5% level

** Significant at 1% level

ns Not significant

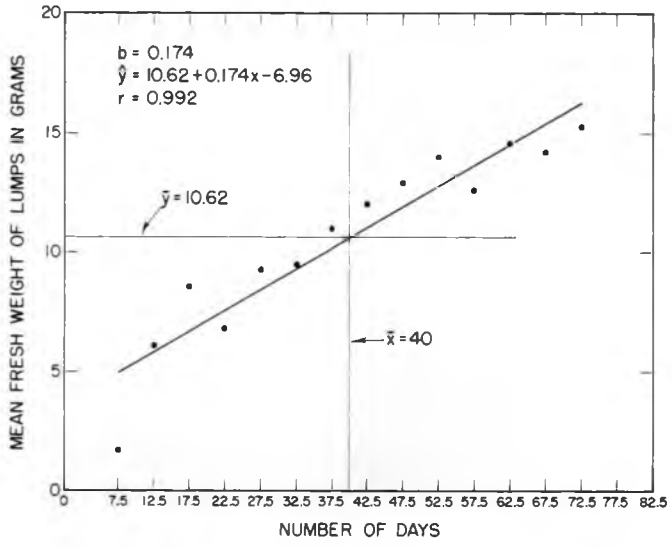

FIGURE 15. RELATIONSHIP OF FRESH WEIGHT OF INDUCED LUMPS TO THE NUMBER OF DAYS FROM DATE OF INJECTION TO HARVEST

cell walls by the Ruthenium Red Method. Pectic materials appear pink to dark red in color when stained by this method.

Fig. 16 shows a photomicrograph of representative tissue of a lump induced by one of the higher concentrations of TIBA (10^{-4}). Note the heavy staining found in the middle lamella between adjoining cells. This indicates a concentration of pectic substances as predicted by Kertesz (11). These substances apparently have not undergone degradation, otherwise, there should have been physical breakdown and cellular dissociation which accompanies ripening (1).

Fig. 17 shows the lumpy tissue induced by the control (distilled water). The concentration of the stains in this photomicrograph shows that most of the pectic substances were found in the middle lamella also. Physical breakdown of the tissue due to cellular dissociation probably had not taken place.

Tissue from a normally ripened, non-lumpy portion of the same fruit stained with ruthenium red is shown in Fig. 18. In contrast to Figs. 16 and 17, pectic substances were diffused throughout the cells. Perhaps it had undergone the transformation from water-insoluble pectin to the water-soluble form. The pectic substances were no longer found exclusively in the middle lamella. The physical breakdown of the tissue due to cellular dissociation as a result of the protopectin conversion must have taken place. Advanced cellular dissociation may be indicated by less

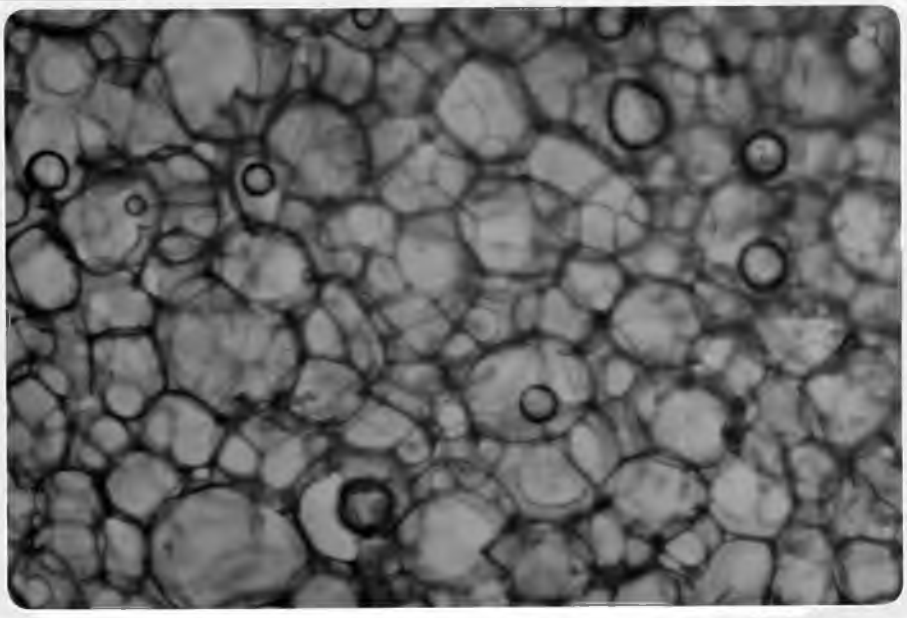

FIGURE 16. THE TISSUE OF INDUCED LUMP WITH THE CELLS RIGIDLY CEMENTED BY THE PECTIC SUBSTANCES (PROBABLY PROTOPECTIN) AS INDICATED BY THE HEAVILY STAINED PORTION BETWEEN THE CELL WALLS OF THE ADJACENT CELLS. NOTE THE PECTIC SUBSTANCES ARE HIGHLY CONCENTRATED IN THE MIDDLE LAMELLA. THERE SEEM TO BE NO SIGN OF CELLULAR DISSOCIATION AS WITH RIPENING

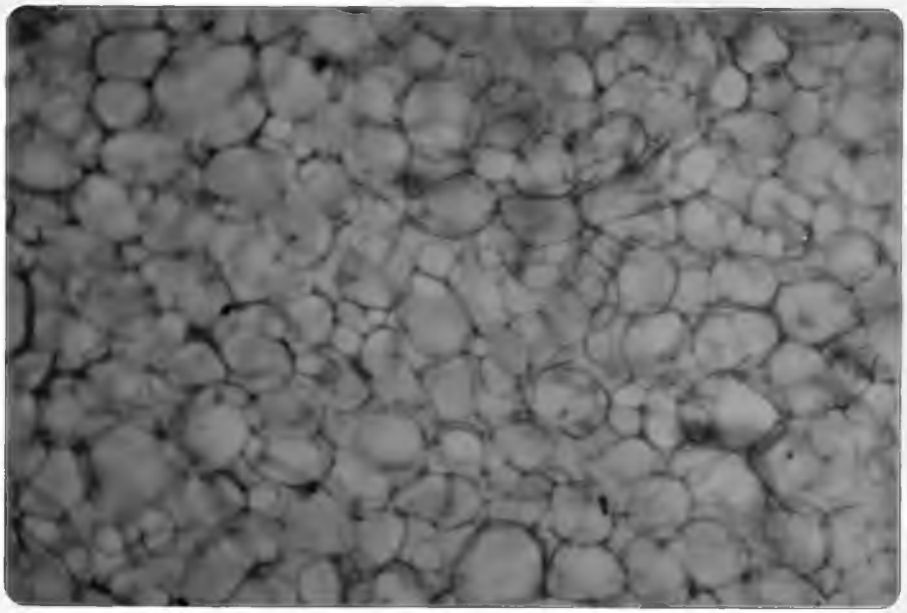

FIGURE 17. THE TISSUE OF AN INDUCED LUMP (DISTILLED WATER CONTROL) SHOWING THE APPARENT PRESENCE OF PECTIC SUBSTANCES IN THE MIDDLE LAMELLA. NO INDICATION OF CELLULAR DISSOCIATION COULD BE OBSERVED AT THIS STAGE

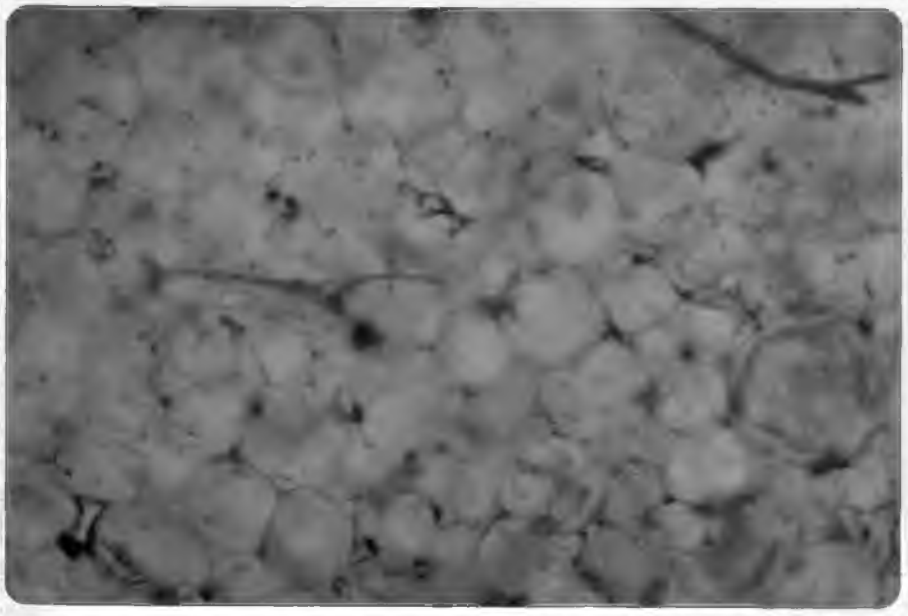

FIGURE 18. THE NORMAL TISSUE OF RIPE FRUIT SHOWING THIN CELL WALLS AND THE CELLULAR DISSOCIATION WHICH ACCOMPANIES RIPENING. NOTE THE LIGHT PINK STAINED PECTIC SUBSTANCES DISTRIBUTED THROUGH THE TISSUE, AN INDICATION THAT PECTIC SUBSTANCES ARE NO LONGER CONCENTRATED IN THE MIDDLE LAMELLA

pink or red stain, in some parts of the section, and would indicate less of the pectic substances present. These observations appear to corroborate the findings of Kertesz (11) that in "over ripened" or mealy fruit, the proportion of soluble pectic compounds decreased. Perhaps ultimately all the pectic substances decompose into non-pectic substances.

DISCUSSION AND CONCLUSIONS

In fleshy fruits, generally some of the processes taking place during ripening are the increase of sugar content (Biale, 3) and conversion of protopectin to pectin (Pentmayr, et al., 15, Kertesz, 12, Appleman and Conrad, 2). It is, however, of vital importance to note that the conversion of protopectin to pectin has been considered as the most important biochemical change in the ripening of nearly all fleshy fruits. Hence, the emphasis of this investigation is on this phase. Hanson (9), Yang, et al., (20) and McCready and McComb (14), subscribe to this idea and confirmed that this conversion process is brought about by the action of some enzymes which are associated with ripening.

The findings of their investigations indicate that interruption of the important process of pectin conversion (and/or other processes associated with ripening) could lead to the failure of the fruits to undergo ripening and cause the delay of senescence. Biale (3), substantiated this statement in his review by advancing the theory that any treatment or condition which delays the onset of the climacteric rise as associated with ripening would also delay ripening and senescence. In papaya, the climacteric rise is at its peak at the time of the full ripeness and then decreases gradually (Jones and Kubota, 10). Lumps may occur as a local failure of climacteric rise to take place as a result of some interruption of the normal process in ripening of

the fleshy tissue. This possibility may be indicated by the fact that all of 12 tested chemicals at all levels enhanced the formation of lumps. It is intriguing to note, however, that for some reason, the control (distilled water) induced lumps. These lumps, however, were significantly lower in weight than those with higher concentrations of some of the tested chemicals.

In a special experiment, fruits which were injected with water alone produced lumps. The size of these lumps increased with an increase in the volume of water used. Distilled water in rates 0.4, 0.6, and 0.8 ml gave lumps averaging 6.37, 8.74, and 12.07 grams, respectively. A 0.4 ml volume was used in tests of the chemicals throughout the main series of experiments. Possibly the change in pH of the tissue due to the distilled water application had a bearing on the interruption of the enzymic activity thus causing the formation of lumps. This may have caused the failure in the transformation of protopectin into water soluble pectin. This assumption corroborates that of Wang, et al. (20) who mentioned that the polygalacturonase which he believed responsible for the degradation of pectin in brining cherries, was inactivated under acid conditions. This is substantiated further by the microscopic study of the pectin in the middle lamella which showed the failure of pectin transformation on the induced lumps as shown in Fig. 16 and Fig. 17.

The response chemicals varied. Six chemicals gave significantly

larger lumps than their controls. These were indoleacetic acid, gibberellic acid, coumarin, maleic hydrazide, triiodobenzoic acid and dinitrophenol. The following chemicals did not give significant results: 2, 4 dichlorophenoxyacetic acid, kinetin, chloramphenicol, copper sulfate, ethionine, and mannitol. Many of the latter had heavy lumps but so did the water check. Once water is injected, it is no longer pure. It becomes mixed with the contents of injured cells. There are undoubtedly "wound hormones" produced. One cannot rule out the mechanical damage to cells from hydrostatic pressure as the solution is forced into the tissue by hypodermic syringe.

The research of Glasziou, et al., (6) on the effects of auxin on membrane permeability and pectic substances in the bean endocarp may be pertinent. They disclosed that a greater amount of water-soluble pectin and a lower amount of protopectin or water-insoluble pectin was found in water treated endocarps, whereas high protopectin and low water-soluble pectin was observed in auxin (IAA) treated endocarps. They attributed the higher ratio of water soluble pectin in the water treated endocarps to indicate the activation of an auxin controlled pectin methylesterase (PME) or polygalacturonase (PG). It is likely that in these experiments with papaya, the significance of IAA over the control in increase of weights of lumps may be similar to the findings of Glasziou, et al., (6). Contrary to the finding of Glasziou on the inactivation of PME by IAA treatment, Yoda (21) found that

auxin (IAA) activates PME thus causes an increase in the water uptake of pea stem. In consonance with Yeda's findings, Bryan and NewComb (5), assumed that pectin methylesterase and polygalacturonase were controlled and activated by the auxin treatment.

Presuming that the protopectin without undergoing degradation would hold the cells rigidly together, it might be possible that IAA induced lumps were the result of local inhibition of protopectin conversion into water-soluble pectin associated to ripening. However, the inhibition due to direct or indirect enzymic inactivation could not be established in these experiments.

The formation of lumps by the maleic hydrazide treatments could be related to the local delay of climacteric rise due to the effect of the treatment. Smock, et al. (18) found that adding maleic hydrazide to the ripening hormone at 270 ppm nullified the ripening effect of naphthalene acetic acid. The climacteric rise was delayed six or more days and the fruits were still at the firm stage. It is also possible that the same effect upon the delay of climacteric rise as with maleic hydrazide could have happened on the other inhibitors such as coumarin and triiodobenzoic acid to cause the induction of lumps significantly heavier than the control. Smock, et al. failed to indicate why maleic hydrazide delayed the climacteric rise.

The specificity of gibberellin in stimulating cell division, cell elongation or both in plants, seems to be very difficult to reconcile with the significant result of gibberellin treatment in the induction of lumps. It seems likely that

the induction of lumps with gibberellin could be attributed to its ability to delay the maturing of cells. Robbins (17) mentioned the action of gibberellin as a "youth hormone" based from his discovery that the arborescent form of ivy produced completely juvenile characteristics with gibberellin treatment. However, he failed to mention further the mechanism involved.

The results obtained from dinitrophenol in the induction of lumps are very difficult to correlate with its specificity as an uncoupling agent. Although the uncoupling effect of dinitrophenol was demonstrated by Biale and Young (4) on the mitochondria and its slight uncoupling effect on climacteric rise, yet, their findings need further elucidation to relate the activity of mitochondria to the metabolic changes in ripening fruit.

The increase in the weight of lumps as related to the length of time between treatment and harvest seems to be the result of the increase in the number of cells affected. Any inhibition of ripening caused by the tested substances upon the enzyme activity would result in increase in the weight of lumps as more cells are involved.

To recapitulate, inasmuch as all the injected substances including the water control produced lumps, it is likely that the cause of the lumps in papaya fruit may be due to a physiological interference with the normal processes of fruit ripening.

It is interesting that some of the substances tested did not show a significant increase in lump weight over the control but gave mean weights

higher than some of those which were significantly above the control at the same molar concentrations. It seems possible that translocation of substances by diffusion may have occurred in the said substances to augment the mean weight of the control relative to the treated. However, this has not beenestablished in these experiments.

It is possible that not all of the induced lumps were due to the direct effect on the inhibition of enzymic action. It could be that some substances caused the formation of lumps by interferring with the series of processes preceding the formation of the enzymes.

Cell division which might take place in the formation of a callus could also be a cause of the formation of lumps artificially, especially with the growth regulators (auxin, gliberellin, and kinetin). Such cell division might have failed to attain maturity at the time the other tissue had ripened. Microscopic examination of the lumps failed to show differences in the size of cells in the lumps compared to normally ripe tissues. No extensive study was made of this, however.

The theory that these lumps constitute unripened tissues was strengthened by observations on fruits that had been overlooked for five or six days. The lumps had become soft although the fruits were well past the "ripe" stage. By this time, the normal tissue was nearly liquified.

SUMMARY

1. A papaya quality problem involving the occurrence of hard portions or lumps in the flesh of ripe papaya of the Solo variety has been investigated.

2. The presence of lumps could only be determined after the fruit had ripened. It was postulated that the lumps were portions which, for reasons unknown, remained "unripe," possibly because of some interference with the breakdown of the pectic substances of the cell walls.

3. Methods of injection to produce lumps artificially were developed and described to test this hypothesis. Chemicals producing lumps significantly greater than the water control were indoleacetic acid, gibberellin, coumarin, maleic hydrazide, triiodobenzoic acid, and dinitrophenol.

4. Chemicals producing lumps not significantly greater than the water control were 2,4-dichlorophenoxyacetic acid, kinetin, chloramphenicol, copper sulfate, ethionine, and mannitol.

5. The size and weight of the induced lumps increased directly with the number of days from injection to harvest.

6. Histological studies revealed that cells of normal and lumpy tissue were essentially the same size.

7. Staining techniques revealed that pectic substances were concentrated in the middle lamella of the cell walls of the lumpy tissues but were dispersed in the cells of normally ripened tissues from the same fruit.

8. It is evident that lumps will result from the introduction of almost any foreign material into the papaya flesh.

9. It is probable that slight physiological changes within the tissues, change the activity of enzymes such as pectinesterase or inhibit the synthesis of their precursors thus delaying the ripening process.

10. The cause or causes of naturally occurring lumps in papaya fruit remains a matter of speculation.

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