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NITRATE AND NITRATE REDUCTASE IN PAPAW FRUIT

By ANNICE C. LLOYD, B.Sc., A.A.I.F.S.T.

SUMMARY

Because high nitrate (NO_3^-) levels in Queensland-grown papaws (*Carica papaya L.*) cause severe detinning in canned fruit products, some factors affecting the level of nitrate in this fruit have been examined.

The presence of nitrate reductase has been demonstrated in various parts of the fruit using a tissue piece assay method. The highest activity in the fruit was found in the exocarp (skin), while much lower activities were found in mesocarp (flesh), endocarp (cavity lining) and seeds.

Nitrate levels and nitrate reductase activity in these different tissues of the fruit were found to vary with the stage of development of the fruit. As fruit ripened, mesocarp nitrate levels fell, but mesocarp enzyme levels remained relatively low. Exocarp nitrate reductase activity reached a maximum when fruit were beginning to colour externally, and then decreased rapidly as fruit became fully coloured.

Nitrate reductase activity in green papaw exocarp fragments was greatly increased by exposure to light. The possibilities of producing low-nitrate papaw suitable for canned products are discussed.

I. INTRODUCTION

For several years, the detinning effect of nitrate in canned foods has been widely recognized. Tomato products, carrots, green beans, sweet potatoes, beetroot and celery have all been implicated in this type of corrosion problem. In Queensland, the corrosion problem has arisen mainly in connexion with canned papaw (*Carica papaya* L.) products (Seale 1968). Although the nitrate concentrations accumulating in papaws (up to 300 p.p.m. NO_3^-) are lower than those reported for many vegetable crops, the tin corrosion is accelerated because of the acid nature of the product (Strodtz and Henry 1954). At present in Queensland, all consignments of papaw to be used in canned products must be checked for nitrate levels. Fruit with less than 30 p.p.m. nitrate is used in conventionally packed canned tropical fruit salad (plain cans) but fruit with higher nitrate levels must be processed in internally lacquered cans. This is not fully satisfactory, because apart from the added container cost, fruit salad in lacquered cans darkens slowly due to oxidation, causing some reduction in the satisfactory shelf life of the product (Seale 1968). The accumulation of sufficient nitrate in papaw fruit to cause severe detinning in canned products is of importance with regard to both the food processing problem and the known toxicity of nitrates.

The toxicity to animals of high nitrate concentrations in forage has been well established (Wright and Davison 1964). Many leafy vegetables are known to accumulate large quantities of nitrate, and although toxicity to adult humans from this source has not been established, cases of infant poisoning due to high-nitrate water supplies are known (Burden 1961). Little is known about the accumulation

of nitrate in fruits. In normally metabolizing plants there are relatively small quantities of nitrate because nitrate nitrogen is rapidly reduced to other forms following uptake by the plant. However, nitrate metabolism in higher plants is known to be susceptible to a wide range of environmental and other factors and under certain conditions plants can accumulate relatively large quantities of nitrate in their tissues without showing any apparent toxic effects.

Preliminary investigations into the papaw nitrate problem revealed that molybdenum deficiency was not the cause, but there was a definite correlation between fruit nitrate and the amount of nitrogenous fertilizer applied (Seale 1968). Kruger and Ranson (1968) recommended that to produce low-nitrate papaws the amount of nitrogenous fertilizer should be reduced and application restricted to the earlier part of the rapid growth period. Urea foliar sprays could be applied during the winter to relieve any nitrogen deficiency which may affect developing flowers and the subsequent crop. However, these horticultural practices merely serve to minimize the nitrate accumulation problem, as there are cases where no fertilizer has been applied and papaws still accumulate high concentrations of nitrate.

A key enzyme in nitrate metabolism in higher plants is nitrate reductase, which has been demonstrated in a wide range of vegetable and grain crops (Beevers, Flesher and Hageman 1964), although there are relatively few reports of its occurrence in fruit tissue. Bar-Akiva and Sagiv (1967) have investigated nitrate reductase in citrus, while Grasmanis and Nicholas (1967) and Klepper and Hageman (1969) have reported on nitrate reductase in various parts of the apple tree.

As part of a programme to find a practical solution to the problem of nitrate accumulation in papaws, the presence and distribution of nitrate and nitrate reductase in the fruit and some of the factors which affect these have been investigated.

II. MATERIALS AND METHODS

Tissues examined and sampling procedures.—All fruit were obtained from field plots at Redlands Horticultural Research Station of the Queensland Department of Primary Industries, which is situated in south-eastern Queensland.

Nitrate reductase activity and nitrate concentrations were studied in four different parts of the papaw fruit: exocarp (skin), mesocarp (flesh), endocarp (inner lining of cavity, including seed stalks) and seeds. A limited number of enzyme and nitrate determinations were performed on leaf samples for comparison.

Fruit and leaf samples were normally harvested in the morning and assayed the same day. Exocarp was pared from fruit to a depth of approximately 1 mm and endorcarp was removed from the fruit cavity with a sharp knife. When exocarp or endocarp from one fruit was required in quantity, all of that tissue was removed, cut into approximately 5 mm square fragments then thoroughly mixed before 0·5 g subsamples were taken for enzyme assays. When an enzyme or nitrate determination for each tissue of one fruit was required, samples were taken from a slice of tissue approximately 2·5 cm thick cut transversely through the median section of the fruit, to minimize variability within the fruit. (M. D. Littmann (personal communication 1971) has shown variability in nitrate concentrations between the proximal and distal ends of a fruit.)

For leaf determinations, four medium-sized leaves were taken from each tree, cut into 5 mm square fragments and thoroughly mixed before samples were removed for assay.

Estimation of fruit maturity.—Fruit maturity was estimated on the basis of colour of each of the four tissues (exocarp, mesocarp, endocarp and seeds) and on seed size (expressed as number of seeds/g), the latter particularly for fruit which had not started to colour. The initiation of ripening in the papaw follows a well-defined sequence of visual changes as the colour progressively develops from the seeds to the exocarp (Wardlaw and Leonard 1935). Six broad maturity levels were defined (Table 1), ranging from very immature green fruit (usually 200–400 g) to ripe fruit (usually 1,000–1,500 g).

TABLE 1

STAGES OF PAPAW MATURATION AS DETERMINED BY COLOUR AND SEED SIZE

Exocarp	Endocarp and Mesocarp	Seeds	Maturity
Green	Both white	White > 20/g	Very immature green
Green	Both white	White < 20/g	Immature green
Green	Both white	Light brown to dark	Mature green
Green	Endocarp coloured Mesocarp white	Dark	Ripening, beginning of internal colour
Green with some yellow	Both coloured	Dark	Ripening, beginning of external colour
Yellow	Full orange colour	Dark	Ripe

Determination of nitrate.—Nitrate was determined by a method based on that of Bremner (1965). A boiled, filtered aqueous extract of the fruit tissue was prepared and an aliquot steam-distilled, first with magnesium oxide to remove free ammonia and then with Devarda's alloy to reduce the nitrate to ammonia. Both distillates were collected in a boric acid indicator solution and titrated with 0·1N HCl from a micro-burette. Results are expressed as p.p.m. nitrate (NO_3^-).

Measurement of nitrate reductase activity.—Attempts were made to measure nitrate reductase activity in crude extracts of papaw prepared and assayed using various modifications of the basic extraction and assay procedures of Beevers, Flesher and Hageman (1964). However, no nitrate reductase activity could be demonstrated in any papaw extracts, probably due to the destruction of the enzyme by the high proteolytic activity of papain present in the tissues.

In view of this, nitrate reductase activity was measured as reported elsewhere (Mulder, Boxma and Van Veen 1959; Bar-Akiva and Sternbaum 1965; Randall 1969) by the nitrite accumulating when tissue fragments were incubated in buffered nitrate. The routine assay procedure used was based on that of Randall (1969). Finely cut tissue (0·5 g) was infiltrated with nitrate by suspending samples in 5 ml of 0·1M KNO_3 buffered at pH 7·00 with phosphate (·005M), evacuating samples simultaneously in a microbiological anaerobic jar, and then readmitting the air. Assays were incubated aerobically in the dark for 2 hr. Reactions were stopped and the nitrite formed was determined colorimetrically by adding 2·5 ml of 1% sulphanilamide (w/v) in 2·5N HCl, followed immediately by 2·5 ml of 0·02% N-(-1-naphthyl) ethylenediamine dihydrochloride. Solutions were then filtered and the optical density at 540 mμ measured on a Unicam SP600 spectrophotometer. Nitrite formed (n moles/g fresh weight of tissue/2 hr) was calculated from a standard nitrite curve.

All assays were done in triplicate, unless otherwise stated. Boiled controls were frequently included to confirm that nitrite measured was being formed enzymically.

Light source.—An artificial light source consisting of two sets of lights was used in this investigation. Each set comprised three fluorescent tubes (two Grolux and one daylight each 40W) and two incandescent bulbs (100W).

III. RESULTS

(a) Examination of Tissue Piece Assay Method

Necessity for dark incubation.—Randall (1969) specified that, to measure nitrate reductase activity in wheat leaf fragments, it is necessary to incubate assays in the dark to allow nitrite to accumulate. A similar result has been obtained with photosynthetic papaw tissue (leaves and green exocarp). Nitrite formed by green exocarp fragments incubated for 0, 1, 2, 3 and 4 hr in the light and in the dark respectively is shown in Figure 1. Nitrite accumulation increased significantly with time ($P < \cdot 01$) and was significantly greater in the dark than in the light ($P < \cdot 01$). This implies that nitrite reductase of green parts of the papaw plant is dependent on a photoreducible cofactor such as ferredoxin as has been suggested for other plant tissue (Beevers and Hageman 1969). On the basis of these results all enzyme assays were incubated under dark conditions.

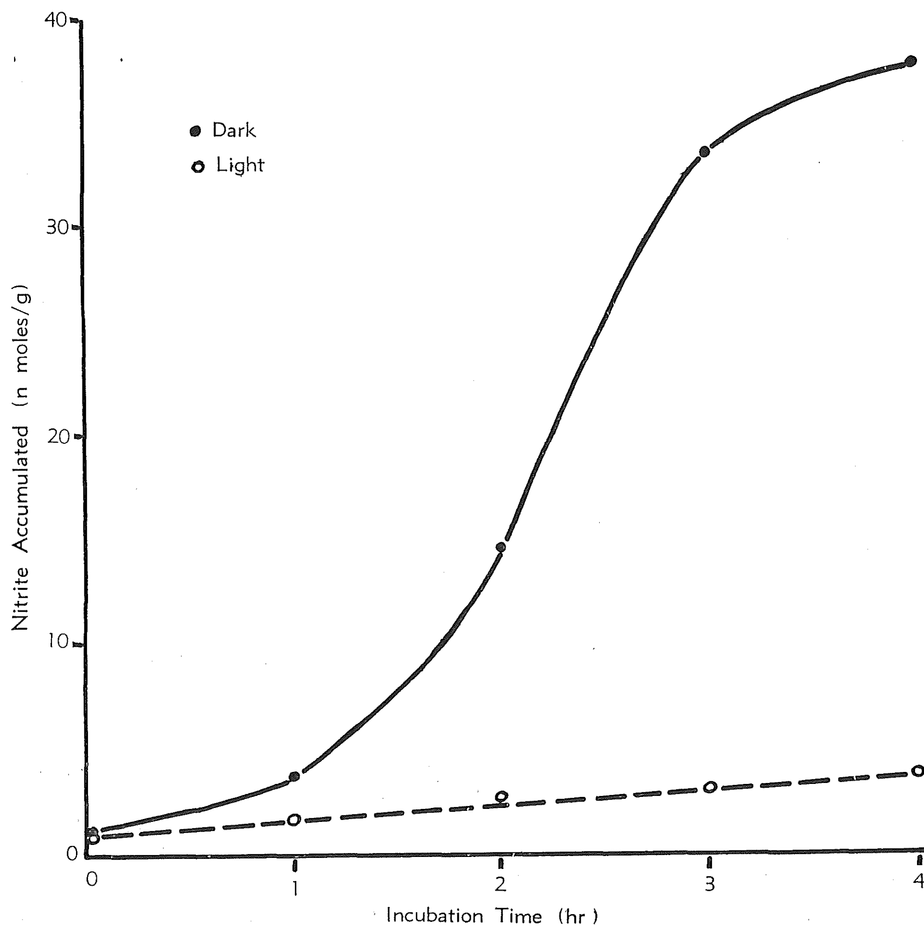

Fig. 1.—Effect of light on nitrite accumulation by green papaw exocarp. (Averages of 2 replications.)

Comparison of some assay technique variations.—Several modifications have been reported for the basic method of measuring nitrate reductase in plant tissue by incubating tissue fragments in nitrate solutions and then measuring the nitrite formed. Mulder, Boxma and Van Veen (1959) and Bar-Akiva and Sternbaum (1965) vacuum infiltrated nitrate with added malate or succinate into tissue fragments and then incubated the tissue fragments anaerobically in Thunberg tubes. Randall (1969) did not use malate or succinate, but vacuum infiltrated tissues with nitrate and then incubated under nitrogen, the latter giving a 15–20% increase in enzyme activity over incubation in air. The routine assay used (treatment B, Table 2) was based on Randall's method except that assays were incubated aerobically.

TABLE 2

COMPARISON OF NITRATE REDUCTASE ASSAY METHODS—EXPERIMENTAL

Treatment	Phosphate–NO_3 Buffer (ml)	H_2O (ml)	0.1M Malate or Succinate (ml) (neut. with NaOH)	Vacuum Infiltration	Incubation*
A	4	1	0	No	Aerobic
B	4	1	0	Yes	Aerobic
C	4	1	0	Yes	Anaerobic
D	4	0	1	Yes	Aerobic
E	4	0	1	Yes	Anaerobic

* 1 hr for leaf tissue; 2 hr for fruit tissue.

To test if the assay method was responsible for some of the very low enzyme activities found in papaw tissue, the relative merits of the above techniques were examined using the experimental design outlined in Table 2. A microbiological anaerobic jar was used for infiltrating tissue and for anaerobic incubations. Analysis of these results (Table 3) showed that there were no major differences between treatments, i.e., vacuum infiltration, aerobic or anaerobic incubation with and without an added electron donor (malate or succinate) made no difference to nitrate reductase activity measured. Mesocarp tissue with negligible enzyme activity by the routine assay also failed to show any increase in activity when assayed by other methods. In view of these results, assay treatment B (Table 2) was used in all further work.

TABLE 3

COMPARISON OF NITRATE REDUCTASE ASSAY METHODS—RESULTS

Enzyme activity expressed as n moles NO_2/g tissue/1 hr for leaf tissue; per 2 hr for other tissues

Tissue	Assay Treatment (Table 1)				
	A	B	C	D	E
Leaves (1) ..	299	233	245	269	240
Leaves (2) ..	284	276	270	314	310
Leaves (3) ..	309	284	314	334	297
Leaves (4) ..	391	396	287	349	371
Leaves (5) ..	398	405	365	312	302
Leaves (6) ..	472	449	479	428	420
Exocarp (1) ..	31·4	37	36·8	37·8	33·8
Exocarp (2) ..	14·4	11·3	17	13	16·3
Mesocarp ..	·001	0	·002	·006	·006

Leaf samples 1–4 were infiltrated with malate, all others with succinate.

Leaf and fruit enzyme activities are the average of 6 and 3 determinations respectively, for each treatment.

(b) Presence and Distribution of Nitrate Reductase in Papaws

Nitrate reduction was demonstrated with papaw leaves, exocarp, mesocarp, endocarp and seeds. Since boiled tissue fragments showed no such nitrite accumulation, the reduction of nitrate is believed to be an enzymic process.

Enzyme activities in the various tissues are recorded in Table 4, which shows the results of assays on fruit of two broad maturity categories—green fruit (no colour development) and ripening fruit (internal and/or external colour development). The standard deviations included in the table demonstrate the wide range of activity found in each tissue. Most of the nitrate reductase activity in the fruit was located in exocarp tissue and was significantly higher ($P < \cdot 01$) in ripening than in green fruit. Endocarp nitrate reductase activity in ripening fruit was lower than that in green fruit ($P < \cdot 05$). The nitrate-reducing capacities of both mesocarp and seed tissue were very low at both stages of maturation. Leaf tissue had much higher nitrate reductase activity than any part of the fruit, the mean leaf activity being approximately eight times the mean exocarp activity.

TABLE 4

DISTRIBUTION OF NITRATE REDUCTASE ACTIVITY IN PAPAWS

Tissue	Nitrate Reductase Activity (n moles NO_2/g/2 hr)				
	Green Fruit (15 samples)		Ripening Fruit (9 samples)		Comparison of Maturities
	Mean	S.D.	Mean	S.D.	
Exocarp	16	26·2	95	70·5	Ripening > Green ($P < \cdot 01$)
Mesocarp	1·2	1·0	2·1	2·1	No significant difference
Endocarp	8·6	7·4	1·7	2·8	Green > Ripening ($P < \cdot 01$)
Seeds	0·8	1·0	0·6	0·9	No significant difference
	Exocarp > Mesocarp and Seeds ($P < \cdot 01$)		Exocarp > Mesocarp, Endocarp and seeds ($P < \cdot 01$)		
Leaves	734 (mean of 6 samples). S.D. = 139				

(c) Effect of Fruit Maturity on Nitrates and Nitrate Reductase Activity

Results in Table 4 indicate that nitrate reductase activity in different parts of the fruit was dependent on the stage of maturation.

To study the effect of maturity on nitrate and nitrate reductase, two trees of the same variety growing under identical field conditions were chosen and harvested approximately 10 weeks apart. The first tree (1) harvested in July supplied most of the data for the early stages of maturity, whereas the second (2) harvested in September supplied most of the data for the more advanced stages of maturity, because at this later stage in the season a much larger proportion of the crop was starting to show colour development. A selection of fruit was made from each harvest to cover the complete range of maturities on the tree at that time. Stage of maturation (as defined in Table 1), weight, volume, total weight of seeds and seed weight (as number of seeds per gram) were recorded for each fruit. Nitrate determinations and enzyme assays were performed on exocarp, mesocarp, endocarp and seeds for each fruit.

Figures 2–8 show the changes in nitrate and enzyme activity in various parts of the fruit with increasing maturity. Many smaller experiments, other than those for which results are given, have shown a similar pattern of enzyme and nitrate changes with maturity when a limited number of fruit covering all maturities have been harvested from a particular tree at one time.

Exocarp enzyme activity (Figure 2) changed significantly ($P < \cdot 05$) with increasing maturity once the fruit had started to colour internally. Enzyme activity reached a maximum in fruit which were starting to colour externally and then fell quickly as fruit became fully ripe.

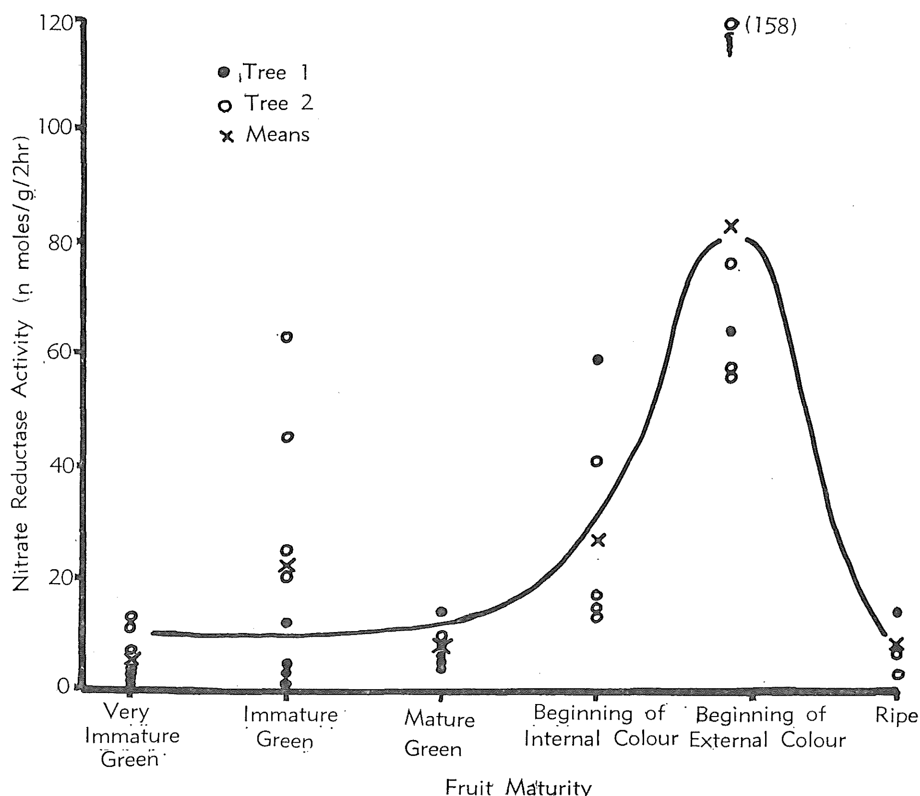

Fig. 2.—Effect of maturity on exocarp nitrate reductase activity (n moles NO_2/g/2 hr). (Each point represents the mean of triplicate assay on a single fruit.)

Data obtained from enzyme assays on the exocarp of a single fruit (Figure 3) also showed enzyme activity being dependent on the stage of colour development of the tissue. The exocarp of a fruit which had coloured unevenly was divided into a number of distinct colour regions ranging from green to orange. The nitrite accumulated by replicated samples each of 7 mm x 9 mm discs of exocarp (approximately 0·5 g) from each of these coloured areas was determined. Nitrate reductase activity was clearly dependent on degree of colour in the area sampled. This also served to demonstrate the necessity for obtaining a completely randomized sample when an average exocarp enzyme activity for a fruit was required.

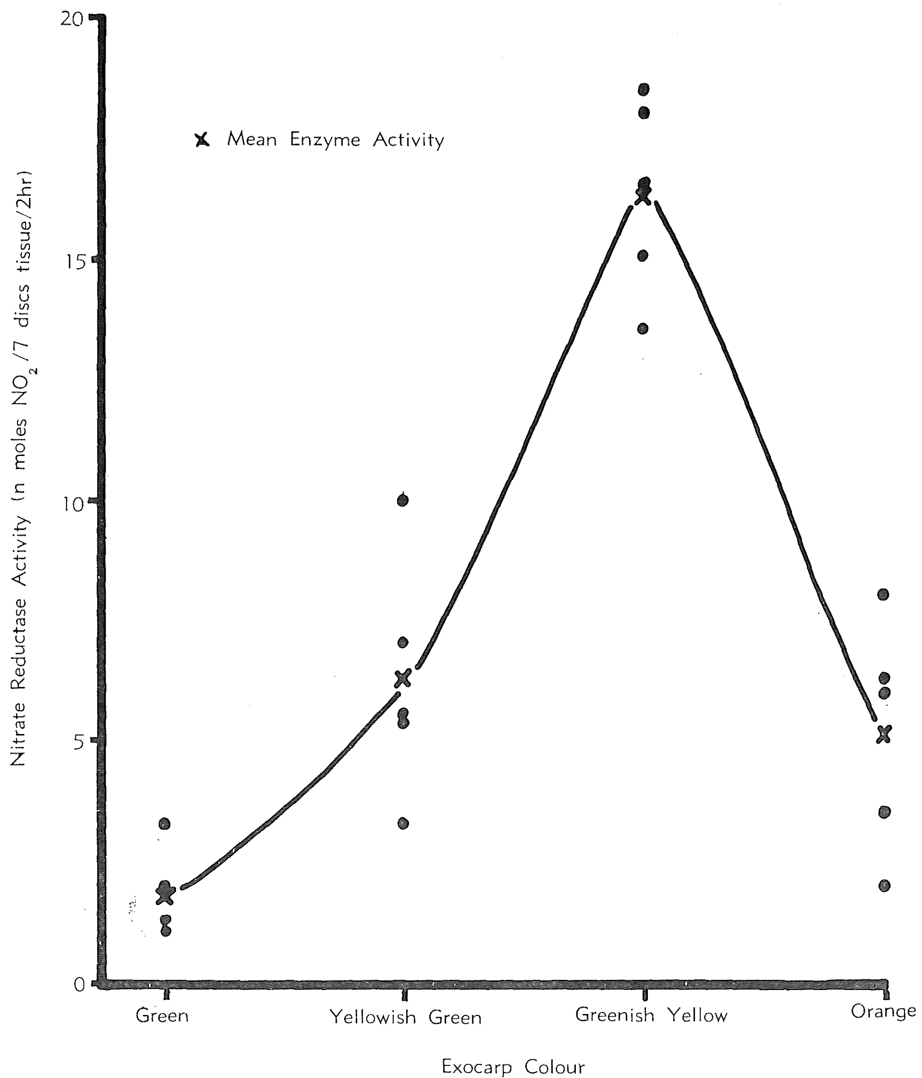

Fig. 2.—Effect of maturity on exocarp nitrate reductase activity (n moles NO_2/g/2 hr). (Each point represents the mean of triplicate assay on a single fruit.)

Endocarp nitrate reductase (Figure 4) was highest ($P < \cdot 05$) in immature green fruit, whereas seed enzyme activity was highest ($P < \cdot 05$) in ripe fruit. Maturity had no significant effect on mesocarp nitrate reductase activity. Endocarp, mesocarp and seed nitrate-reducing capacities at all stages of maturity were very much lower than that of the exocarp.

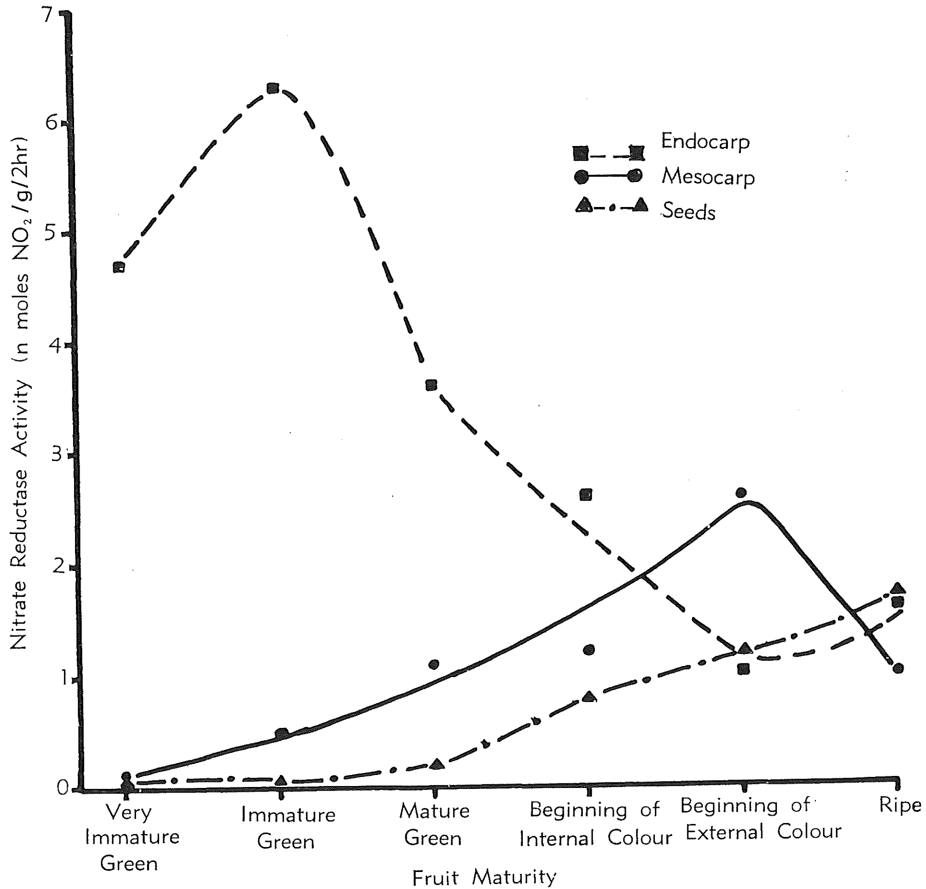

Fig. 4.—Effect of maturity on mesocarp, endocarp and seed nitrate reductase activity. (Each point represents the mean enzyme activity for all fruit at that particular maturity.)

Nitrate concentrations in the exocarp (Figure 5) showed no significant changes with fruit maturity, although the mean value suggested an increase in exocarp nitrate in mature green fruit. Mesocarp nitrate levels (Figure 6), however, were significantly dependent on fruit maturity ($P < \cdot 05$), first decreasing in from very immature to immature green fruit, then increasing to mature green fruit, then decreasing again as colour development proceeded.

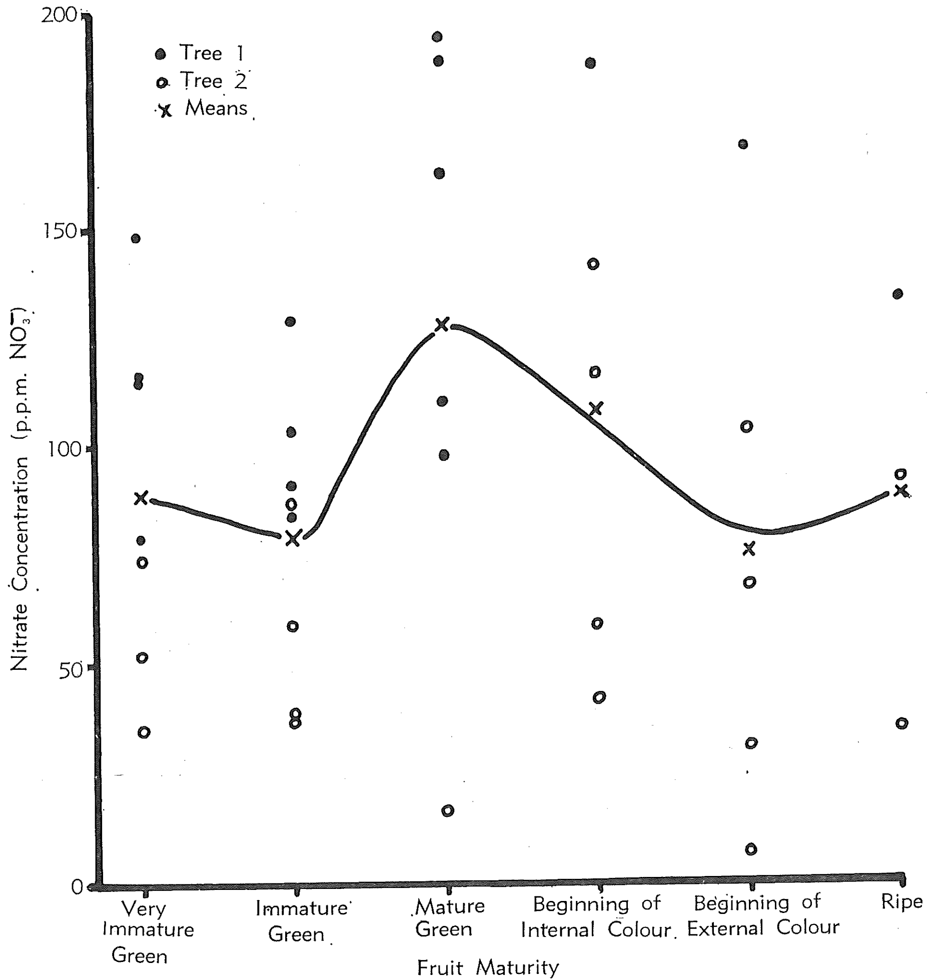

Fig. 5.—Effect of maturity on exocarp nitrate levels. (Each point represents the nitrate concentrate in exocarp of a single fruit.)

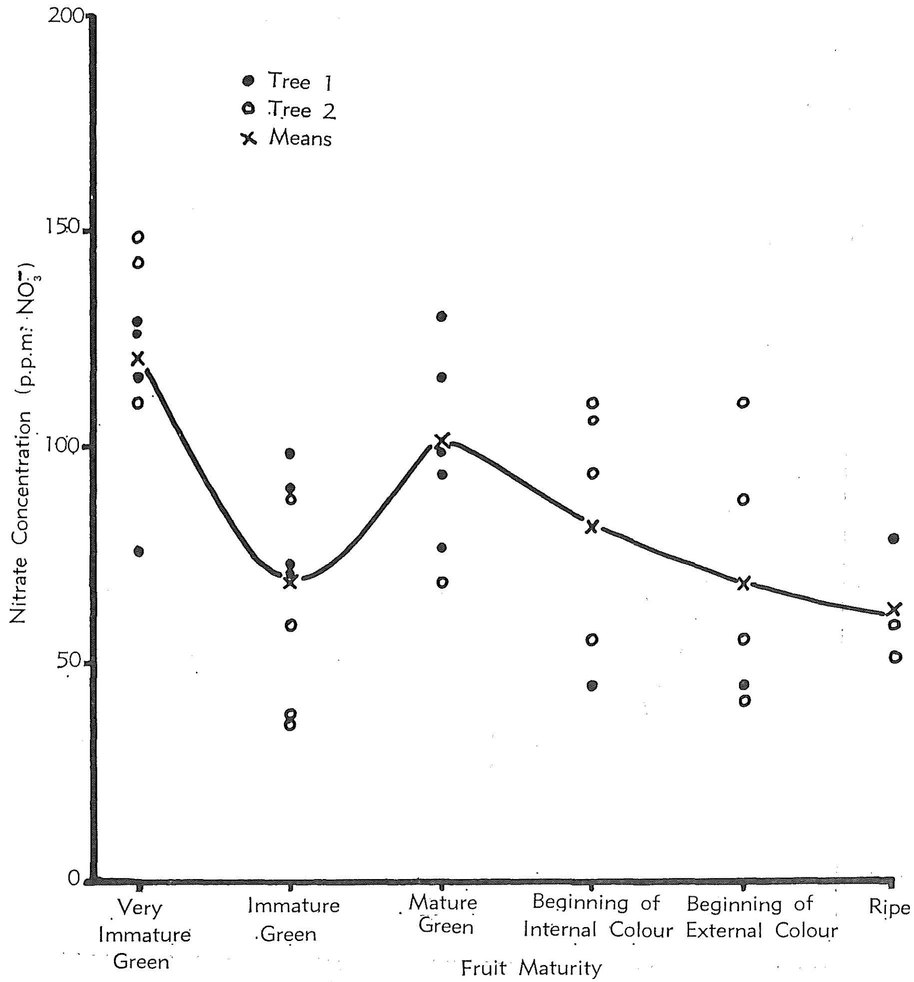

Fig. 6.—Effect of maturity on mesocarp nitrate levels. (Each point represents the nitrate concentration in mesocarp of a single fruit.)

Analysis of the results for the effect of maturity on endocarp nitrate (Figure 7) showed that during development of fruit from very immature green to the mature green stage, nitrate fell significantly ($P < \cdot 05$), and then increased again as fruit colour developed ($P < \cdot 05$), for tree 2 only, there being insufficient data for analysis on tree 1. Nitrate levels in seeds decreased significantly ($P < \cdot 01$) as fruit ripened (Figure 8). The data suggested a slight increase in seed nitrate during the final ripening stage but this difference was not significant.

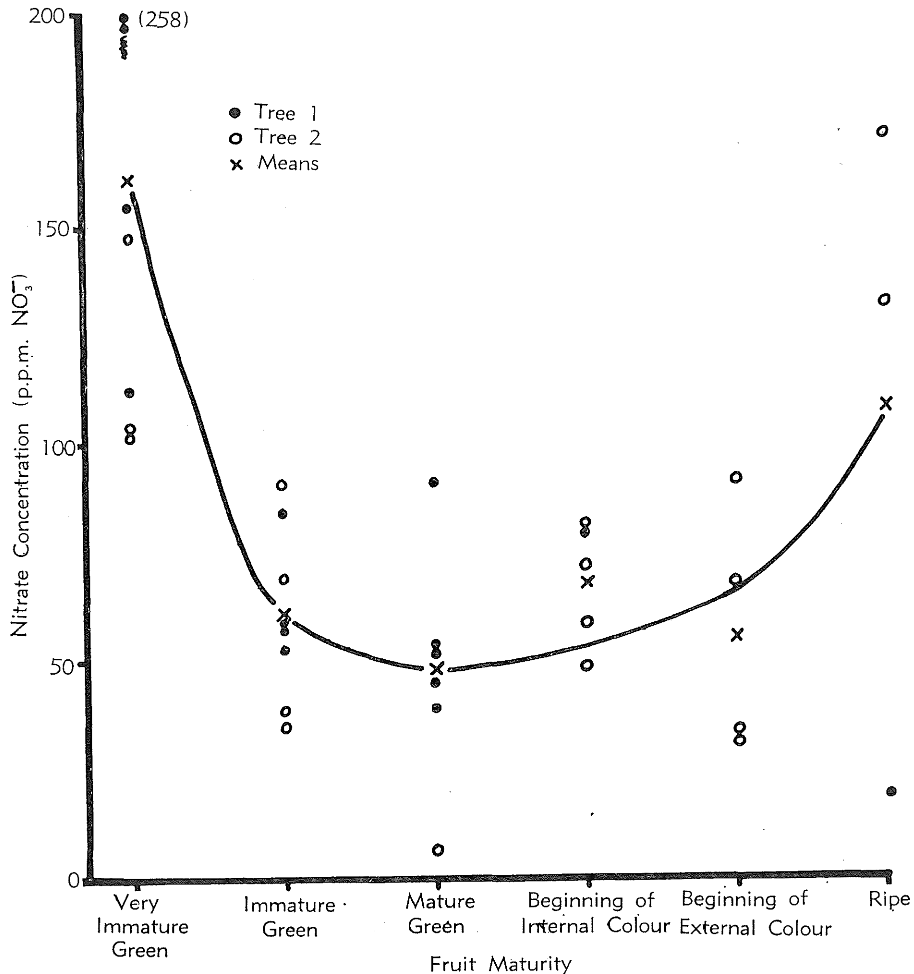

Fig. 7.—Effect of maturity on endocarp nitrate levels. (Each point represents the nitrate concentration in endocarp of a single fruit.)

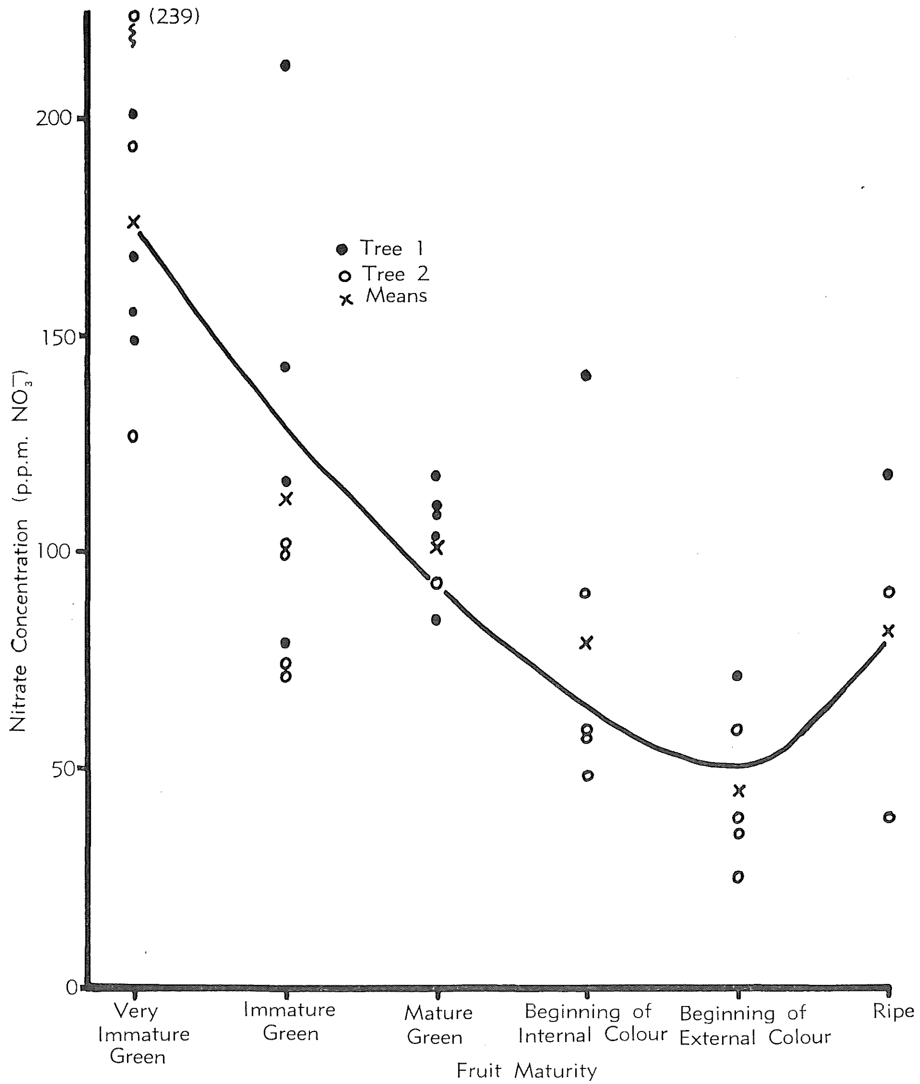

Fig. 8.—Effect of maturity on seed nitrate levels. (Each point represents the nitrate concentration in seeds of a single fruit.)

The spread of points at each stage of maturity in Figures 2–8 shows the large variations in nitrate and enzyme which occur in fruit of a particular maturity from trees of the same variety grown under similar conditions. The greatest variations were observed in the peak enzyme activities in fruit starting to colour externally, some fruit exhibiting exceptionally high activities.

The data above are shown on graphs with a constant difference horizontal scale of maturities, although the time for fruit development from very immature green to mature green is actually much greater than the time that elapses between the beginning of internal colour development and the development of full exernal colour.

Time for fruit development in Queensland papaws has been shown to vary from 175 to 275 days according to the month of flowering (Agnew 1948).

(d) Effect of Light on Nitrate Reductase Activity

The stimulating effect of light on nitrate reductase activity is well established (Hageman, Flesher and Gitter 1961) so it seemed probable that some form of artificial light treatment might increase enzyme activity in papaw fruit and so assist in reducing nitrate levels.

The effect of light induction with and without exogenous nitrate (0·1M) in phosphate buffer was studied using exocarp tissue (0·5 g samples) from mature green fruit. Following light induction, nitrate reductase activity was assayed in the dark, those samples which were in phosphate buffer being first transferred to phosphate-nitrate buffer. Results on exocarp of four individual fruit (Table 5) show that 2 hr light induction in the presence of nitrate resulted in significantly higher enzyme activity than light induction alone, for exocarp with both moderate and very low initial enzyme activity ($P < \cdot 01$). The proportional increase in enzyme activity in fruits 3 and 4 (very low initial activity) was significantly greater ($P < \cdot 01$) than that for fruits 1 and 2 (moderate initial activity). The effect on nitrite accumulation of incubating the routine assay in the light instead of in the dark was examined and it was shown, as before, that light greatly reduced the amount of nitrite accumulation ($P < \cdot 01$).

TABLE 5

EFFECT OF LIGHT INDUCTION ON NITRATE REDUCTASE ACTIVITY IN GREEN PAPAW EXOCARP

Treatment	Enzyme Activity n moles NO_2/g/2 hr (averages of triplicate assays)				
	Fruit 1	Fruit 2	Fruit 3	Fruit 4	Means
2 hr Dark + NO_3 (routine assay) ..	13·3	16	2·6	0	8·9
2 hr Light + NO_3	2·8	2·9	1·8	0	1·8
Induction: 2 hr Light − NO_3 Followed by 2 hr Dark + NO_3	49	47·6	14·3	10·1	29·6
Induction: 2 hr Light + NO_3 Followed by 2 hr Dark + NO_3	60	74	25·6	30·4	47·5

Necessary differences for significance in comparison of treatments—5% (5·2); 1% (6·9).

Figure 9 shows that increasing the time of light induction significantly increased enzyme activity both in the presence of nitrate ($P < 0 \cdot 01$) and in its absence ($P < \cdot 01$). The effect of longer light induction periods was significantly greater ($P < \cdot 01$) when exogenous nitrate was present. Longer induction periods with this particular light system would presumably result in a plateau value of enzyme activity.

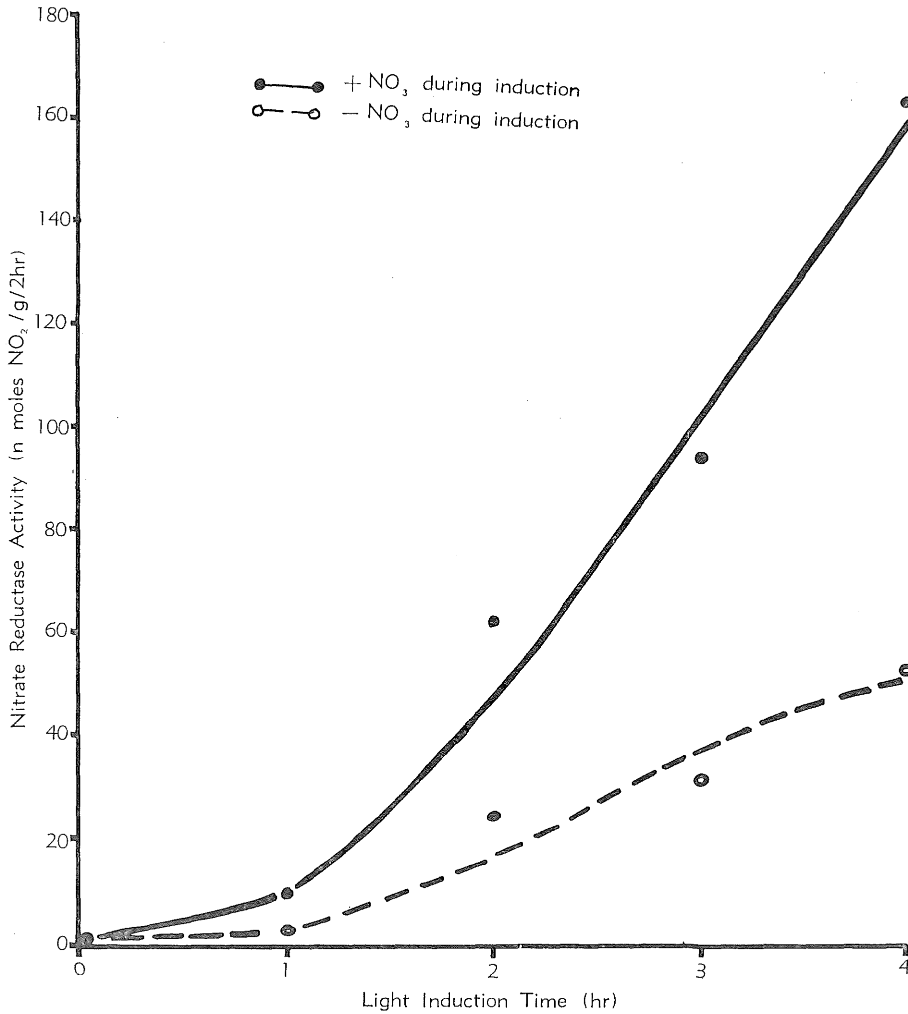

Fig. 9.—Effect of time of light induction on nitrate reductase activity in green papaw exocarp. (Averages of 2 replications.)

IV. DISCUSSION

The highest nitrate reductase activity in papaws occurs in the exocarp when the fruit begins to show external colour development. At this and subsequent stages in development, the mesocarp nitrate reductase activity is very low. This implies that nitrate may be translocated through the mesocarp to the exocarp for reduction. According to the data of Wardlaw and Leonard (1935) on the changes involved in papaw ripening, the onset of the respiratory climacteric coincides with the first development of external colour, which is the stage of maturity at which the exocarp nitrate reductase activity reaches a maximum. The gross biochemical and physiological changes occurring in the fruit at the onset of ripening may be responsible for an increased supply of electron donors which could result in increased nitrate reductase activity.

Nitrate metabolism in green tissues is intimately associated with photosynthesis and in non-green tissues appears to be related to respiratory metabolism, but the mechanism by which nitrate is reduced to ammonia in the latter is not well established (Beevers and Hageman 1969). It is well known, however, that nitrate reductase is inducible and that different levels of nitrate are required for optimum induction in different species, in some cases quite high concentrations of nitrate being required. The non-photosynthetic papaw tissues, endocarp and mesocarp, contain considerable amounts of nitrate, but exhibit very low nitrate reductase activity at all stages of maturation. Beevers and Hageman (1969) have postulated that failure to demonstrate the occurrence of appreciable nitrate reductase in tissues containing nitrate could be due to the nitrate being retained in vacuoles and not able to function as an inducer, or to the tissue having a lowered capacity for protein synthesis, or to a depletion of electron donors and ATP. However, when malate or succinate was infiltrated with nitrate into papaw tissue pieces, no significant increase in nitrate accumulation was detected (Table 3).

There is little published information on nitrate or nitrate reductase activity in fruit tissue. Wright and Davison (1964) claimed that fruits and seeds usually contain very little nitrate. Hoff and Wilcox (1970) found that nitrate accumulation in tomato fruit occurred to a much smaller extent than in leaves (1–5% of leaf concentrations). In contrast, nitrate concentrations in papaw leaves, under the conditions studied here, were found to be only slightly higher than those occurring in fruit tissue. The nitrate concentrations for the leaf samples in Table 4 were in the range 200–400 p.p.m., compared with exocarp levels approaching 200 p.p.m. (Figure 5), and endocarp levels reaching 258 p.p.m. (Figure 7). Green tomato fruit under various conditions accumulated approximately 20–200 p.p.m. NO_3^-, nitrate levels in green fruit tending to be slightly higher than in ripe fruit (Hoff and Wilcox 1970). These results are comparable with those obtained for papaw fruit. It was also reported that significant enzyme activity could not be detected in tomato fruit, but was observed in the stem end in high-nitrate fruit when the fruit was dissected and the individual parts analysed separately.

Bar-Akiva and Sagiv (1967) have reported that nitrate reductase of sour orange fruit flavedo and albedo fragments was 56 and 128 n/moles NO_2/g fresh tissue/3 hr respectively. These activities are comparable to the maximum activities found in papaw exocarp. The average nitrate reductase activities reported for sour orange leaf fragments (628 n moles/g/2 hr) is only slightly lower than the average value for papaw leaf fragments (734 n moles/g/2 hr).

Nitrate reduction in plant tissue is known to be stimulated by light (Hageman, Flesher and Gitler 1961), but the mode of operation of light is complex and not fully understood. At least for green papaw exocarp it has been shown that light will increase nitrate reductase activity considerably, and to an even greater extent if exogenous nitrate is present during the induction period. This implies that although the nitrate concentration in exocarp may be quite high, it has not reached an induction saturation point so far as the enzyme is concerned. The practical importance of the effect of light is that it may be able to be used as a post-harvest treatment on papaw fruit to accelerate the natural reduction in mesocarp nitrate which has been shown to occur as fruit ripen. Further work would be needed to determine at what stage of maturity fruit should be harvested to give both minimum nitrate levels and optimum texture in the ripe fruit for canning. It is doubtful, however, that a prolonged light treatment, with corresponding heating problems, would be a commercially economic proposition.

Nitrate reductase in plant tissue generally is so sensitive to changes in environment that it appears unlikely that control of any single factor can guarantee low-nitrate papaws. However, nitrate reductase in plants is known to be genetically controlled and Zieserl and Hageman (1962) have shown that corn inbreds and hybrids differed widely in their seasonal level of nitrate reductase, but by proper combination of inbred lines, followed by selection based on enzyme assays, a hybrid could be developed with a high, medium or low level of nitrate reductase activity as required. In the same way it is not unlikely that the nitrate problem in papaws could be overcome by breeding.

Much more information is needed on horticultural and physiological factors, such as uptake and translocation of nitrate by the plant and the fruit, before a complete picture of nitrate metabolism in papaw plants is available.

V. ACKNOWLEDGEMENTS

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The author is an officer of Horticulture Branch, Queensland Department of Primary Industries, stationed at Sandy Trout Food Preservation Research Laboratory, Brisbane.