

STUDIES ON THE GERMINATION CHARACTERISTICS
OF CITRUS RETICULATA ^(BLANCO) ✓ CV EMPRESS MANDARIN ^(BLANCO)
AND AN ANALYSIS OF THE EARLY SEEDLING
GROWTH OF THREE CITRUS ROOTSTOCK CULTIVARS

by

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DECLARATION

I hereby declare that the work reported on in this thesis was conducted by myself except for the assistance acknowledged on page 120. The work is original and has not been submitted to any other university.

Signed: 
L.A. VON BROEMBSEN

Why not a Title.

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1

INTRODUCTION

Early in 1973 the South African Co-operative Citrus Exchange Ltd. in association with the Department of Agricultural and Technical Services embarked on the first phase of the South African Citrus Improvement Programme (Von Broembsen & da Graca, 1976). Broadly the aim of the Programme is to improve the standard of citrus trees being sold to commercial growers. This is expected to contribute meaningfully towards the future of the citrus industry being structured on a stable foundation.

Capital in Reference? Inconsistent

The Programme deals with all aspects of citrus nursery tree production from the regulation of approved sources of propagation material to the many problems which attend the production of healthy seedlings and budlings in the nursery. The latter has become of special importance to nurserymen owing to a recent industry-wide swing to a new range of rootstock cultivars. Some of these cultivars are considered to be better adapted to the soil and climatic conditions of certain production areas than the traditionally used rough lemon rootstock. One such cultivar is *Citrus reticulata* (Blanco) cv. Empress mandarin.

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reference!

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Bredel

Empress mandarin is a highly polyembryonic South African seedling selection of the Australian Emperor mandarin (Hodgson, 1967). At first it was recognized solely as a promising loose-skinned fresh fruit, resulting in its extensive exploitation in the cool citrus production areas of the Transvaal, e.g. Rustenburg. However, its potential as a rootstock was noticed by Marloth in 1954 who later advised that it should be listed as a recommended rootstock (Marloth & Basson, 1961). Thereafter several growers introduced the new rootstock as is

Not a ref. list.

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 evidenced by scattered plantings of trees of Empress mandarin present today in many parts of the Transvaal. However, it is likely that its exploitation would have been far more widespread were it not for the difficulties it ^{seemingly} presented to nurserymen. Not only did the seed prove difficult and slow to germinate, but Empress mandarin seedling growth was always slower than the vigorous rough lemon to which nurserymen were accustomed (Oberholzer, 1969).
 Not according to your results

Since the work of Marloth & Basson (1961) it has become evident that while Empress mandarin may not serve as a better replant stock in old citrus soils it is decidedly more tolerant to *Phytophthora*-induced gummosis than rough lemon (Esselen, 1976). A close relative of Empress mandarin, the King mandarin, has also shown these characteristics (Klotz, Bitters, De Wolfe & Garber, 1967).
 Not in Reference list.
 Thus Empress mandarin appears a useful alternative to rough lemon in situations where vigorous scion growth is desired but where soil, climatic and cultural conditions favour the development of gummosis.
 Ref?

Several explanations for the customary poor germination of Empress mandarin seeds have been offered by nurserymen (Esselen, 1976). It has been claimed for example, that seeds extracted from immature fruit germinate faster and more uniformly than seeds from mature fruit. It has also been suggested that Empress mandarin seed responds well to being soaked in its own juice for several days before planting. Although these two theories were based on observation alone they seemed worthy of further investigation, as both implied the possible involvement of endogenous growth regulating substances in the control of the seed's germinability. It became obvious therefore that any study into the germination requirements and characteristics of Empress mandarin seed should be accompanied by an investigation into the changes of gibberellic acid

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Why?

concentrations taking place during seed maturation.

During the study it became apparent that high germination rates of Empress mandarin seed could be achieved quite easily. Thus attention became focussed upon another Empress mandarin problem, that of its slow and uneven seedling growth. The work reported on here therefore encompasses a detailed study of the germination requirements of Empress mandarin seed and an analysis of its seedling growth characteristics in comparison with those of two other important citrus rootstock cultivars, viz. *Citrus limon* Burm. f. cv. rough lemon and *Poncirus trifoliata* (L.) Raf. What about *C. jambhiri*

Not in Introduction!



CHAPTER I

SOME GERMINATION CHARACTERISTICS OF CITRUS
RETICULATA (BLANCO), CV EMPRESS MANDARIN
SEED

1.1. INTRODUCTION

It is known that many factors, both applied or inherent, exert an influence on the germinability of citrus seeds in general. (?) However, the specific sensitivity of Empress mandarin to such factors has been suggested by the erratic nature of its seed germination, a phenomenon which has troubled many citrus nurserymen in the past and threatened its future use as a rootstock cultivar. (?) In view of Empress mandarin's increasing popularity as a citrus rootstock, it was decided that an attempt should be made to discover some of the factors most responsible for the unsatisfactory germination tendencies of its seed and thereby to cast more light on the general germination characteristics of Empress mandarin seeds.

As with most angiosperms, the initial developmental stages of a citrus seed consist of the formation of the mature ovule with its central mass of nutritive cells (nucellus), the embryo sac within the nucellus, and two integuments surrounding the nucellus (Frost & Soost, 1968). After fertilization of the egg cell in the embryo sac, there follows a period of 2 to 8 weeks during which the nucellus greatly enlarges by cell division and the endosperm develops slowly. During this period the first signs of multiple embryo development become apparent in the nucellus of polyembryonic cultivars. At germination these multiple nucellar embryos give rise to some nucellar seedlings which, unlike the single seedling derived from the gametic embryo, all have genetic constitutions identical to the mother tree. (Ref.)

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Germination has been defined as the resumption in growth of the embryo resulting in the rupture of the seed coats and the emergence of the young plant (Leopold, 1964). Citrus seed of all commercially used cultivars except possibly *Poncirus trifoliata* do not have to undergo any specific dormancy-breaking treatment, although specific germination requirements have to be met before seed quiescence is overcome (Amen, 1963; Platt & Opitz, 1973).

At the start of citrus seed germination the hypogynous cotyledons absorb water and swell, while the tip of the radicle elongates and emerges through the micropylar end of the testa (Schneider, 1968). The metabolic reactions which precede and accompany these morphological changes are triggered and guided by the presence of specific enzymes whose synthesis is controlled by a delicate hormonal balance (Miller, 1956; Skinner, Talbert & Shive, 1958; Amen, 1968). Various pre-germination seed treatments act upon these promotor-inhibitor levels and by so doing exert an influence over germination characteristics of the seed. For example, the possibility that either the juice, pulp or seed coats of certain citrus cultivars contain germination inhibitors has been demonstrated (Elze, 1953; Mungomery, Agnew & Prodonoff, 1966; Sondheimer, Tzon & Galston, 1968).

Other pre-germination treatments which have been found to influence citrus seed germination include the removal of seed coats (Schneider, 1968; Mungomery *et al.*, 1966; Said, 1970), the control of micro-organisms on seed surfaces (Childs & Hrncair, 1948; Klotz, de Wolf & Wong, 1957; Ryan, 1958; Ryan & Stein, 1958; Klotz, de Wolf, Roistacher, Nauer & Carpenter, 1960; Roistacher & Nauer, 1961; Bridges & Youtsey, 1966; Nauer, Klotz, de Wolfe & Goodale, 1968), the conditions under which the seeds are stored (Barton, 1943; Childs & Hrncairn,

✓ 1948; ✓ Fu, 1951; ✓ Ryan & Frolich, 1961; ✓ Chacko & Singh, 1968; ✓
 Button, Bornman & Hackland, 1971; ✓ Anon., 1973), temperature (Camp,
 Mowry & Loucks, 1933; ✓ Chapot, 1955; ✓ Horanic & Gardner, 1958;
 Yelenosky, 1967) and the presence of foreign or unnatural substances
 in the germination medium (Patt, 1953; ✓ Singh, Shanker & Gosh, 1965;
 Gerdemann, 1973; Milne, 1973).

Original?
 Since little research work has so far been carried out on the response of Empress mandarin seed to such factors it was decided that a series of experiments should be conducted in an attempt to provide the foundation upon which to base further studies into its behaviour under specific conditions.

1.2. GENERAL METHODS AND MATERIALS

1.2.1. Selection of plant material

Why?
 All seeds used in these trials were extracted from fruit harvested on the estate of H.L. Hall & Sons, Nelspruit, from several healthy uniform seedling Empress mandarin trees in a cultivar block. In some cases the fruit was harvested several weeks or even months before the experiments were conducted and it therefore became standard procedure for a seed viability test to be conducted on each batch of seeds prior to the experiment being carried out.

Why this unnecessary long delay?

1.2.1.1. Determination of seed viability. Two methods were used to test seed viability. One simply entailed the germination response of a sample of seeds to conditions favourable for germination. The second involved the use of 2,3,5 triphenyl tetrazolium chloride (T T C) as a stain indicator. This chemical, being an indicator of active

respiratory processes, turns from colourless to pink in the presence of living tissue (Porter, Durrell & Romm, 1947; Roistacher & Nauer, 1961; Elias & Singh, 1967; Chacko & Singh, 1968; Nauer *et al.*, 1968).

Statistical

A trial comparing these two methods was conducted on freshly-extracted and well-rinsed Empress mandarin and *Poncirus trifoliata* seeds. Four hundred seeds of each cultivar were divided into 2 groups of 200 seeds. One group of 200 seeds of each cultivar was divided into 10 lots of 20 seeds. Each batch of 20 seeds was cut longitudinally and placed with cut surfaces facing down in 7cc of 1 per cent. TTC in each of 10 Petri dishes and left for 4 hours in the dark. The other groups of 200 seeds were subjected to a standard viability test by being treated with 8 hydroxyquinolene sulphate, placed 20 to a Petri dish and incubated on moist filter paper at 28°C. Germination percentages were assessed 20 days later.

Results, which are given in tabular form in Appendix Table 1 indicate that while the TTC method was far more rapid and accordingly more useful it gave statistically significant values 8-10 per cent. higher than the reliable but timeconsuming germination method over the range of 70-80 per cent. This was consistent with the findings of Chacko & Singh (1968), who determined that the TTC method gave ratings of about 11 per cent. higher than the actual germination percentages obtained.

In spite of this, and in view of its rapidity, it was decided that the TTC method would provide a sufficiently accurate picture of seed viability for the series of trials to follow. Since only batches of seed with an average viability less than about 70 per cent. would be unsatisfactory for general trial purposes it was decided that any batch

of seed which gave over 80 per cent. viability by the TTC method could be used with safety. The TTC test was accordingly conducted on a sample of 100 seeds before each trial was initiated.

1.2.2. Germination media

1.2.2.1. Soil and organic media. Previous workers have shown that unless vast differences in soil type exist, germination is not much affected (Perlburger & Reichert, 1938; Barton, 1943; Childs & Hrncaire, 1948; Elze, 1949; Patt, 1949; Burns & Coggins, 1969). Because normal germination occurs in any moisture retentive, well-aerated and well-drained medium it was decided to use a sterilized mixture of 1:1:1 sand, peat moss and vermiculite for trials where germination would be measured by the emergence of the epicotyl through the soil surface. At planting, care was taken that all seeds were oriented the same way, in spite of the findings of Marlangeon (1973), that the planting position of seeds is of little practical importance to seedling growth. Following the recommendations of Perlburger & Reichert (1938), seeds were planted to a depth of 20 to 25mm below the surface in flats 225 x 155mm in area and 60mm deep. Twenty seeds were planted per flat. After planting, soil moisture was retained by light daily applications of tap water. Care was taken not to waterlog the soil since Perlburger & Reichert (1938), and Kaufman (1969) had shown that although seeds germinate most rapidly when water is freely available, germination can be impaired where excessive water applications interfere with oxygen availability. *perlite?* *Analyzed.*

1.2.2.2. Artificial media. Petri dishes containing a single layer of moist filter paper were used. Seeds were rinsed and sterilized in 10 per cent hypochlorite solution and laid ten to a Petri dish.

where was this
Satisfactory moisture levels were maintained by the regular application of small amounts (5ml) of tap water to the dishes whenever this was necessary. Germination was considered complete when emerging radicles had reached a length of approximately 5mm. At this point emergence of the plumule had sometimes just commenced. It was not considered necessary to wait until the complete emergence of all seedling parts in order to measure germination; the degree of vitality of the seed was evident by the time taken for the radicle to reach a 5mm length.

1.3. EXPERIMENTS, RESULTS AND DISCUSSIONS

How time!

By a series of simple germination experiments, an attempt was made to determine which factors are primarily responsible for the erratic germination tendencies of Empress mandarin seed and thereby to provide a guideline for the more successful culture of this cultivar. An attempt was made to follow some of the factors thought to have a bearing on the germinability of the seed from its early development, through the various means of storing it and finally to whatever pre-germination treatments may enhance seed germination.

1.3.1. The effect of pre-extraction treatments on the germination of Empress mandarin seed

EXPERIMENT 1 The influence of fruit harvest date

Introduction Claims by some nurserymen that Empress mandarin seeds germinate faster and more uniformly when extracted from immature fruit, coupled with the suspicion that high gibberellic acid levels in immature seeds may prompt faster germination, were primary motives for this

Reference?

experiment. The findings of Elze (1949) that germination in citrus can be impaired by factors present in the fruit juice laid further emphasis on the possibility that the longer the seeds are under the influence of the juice the lower the germinability is likely to be.

Procedure Commencing at the beginning of February 1974, approximately 4 months after fruit set, representative samples of 20 Empress mandarin fruits were harvested at approximately two weekly intervals. Seeds were immediately extracted, sterilised in a 10 per cent hypochlorite solution and planted in each of 10 Petri dishes as described under General Methods. Germination counts of each batch were conducted four weeks later. At every second harvest approximately 10g seed was set aside in the freezer for gibberellic acid determinations. The fruit reached edible maturity in mid-June but fruit samples were taken until mid-July.

Results and Discussion Results are tabulated in Table 1.1 and show that germination rates of immature seed extracted during February and early March were low, increasing to a maximum by mid-May. This coincided with colour break and the onset of fruit maturity.

Poor germination of the immature seeds was manifest in two ways:

(a) by the failure of the radicle to emerge from seeds which appeared normal apart from their immaturity, and (b) as a result of rotting caused by fungal and bacterial invasion. After mid-May, small but insignificant changes in germination percentage occurred with a significant drop to 81 per cent. being measured in seeds harvested in mid-July at which time fruit drop had commenced. This reduction was due mainly to the onset of waste and not apparently due to changes in the inherent germinability.

Why not sterile conditions?

TABLE 1.1 Changes in the germination percentage of seed extracted from Empress mandarin fruit harvested progressively during the 1974 season (angular transformed data)

Harvest Dates	18/2	1/3	15/3	29/3	15/4	12/5	24/5	8/6	22/6	17/7
Fruit Appearance	Immature				Colour Development			Full Maturity		Senescence
Treatment Means	44	46	55	61	84	95	94	92	91	81
P = 0,05	a	a	ab	b	cd	d	d	cd	cd	c
P = 0,01	a	a	b	b	c	d	d	d	d	c

Duncan's Multiple Range Test

S.S.D. P = 0,05 : 11,27 CV = 20,01%
P = 0,01 : 14,95

Means in the row not followed by the same letter are significantly different at P = 0,01 and P = 0,05.

Conclusion

From these results it became clear that fruit should not be harvested before the onset of colour break (at Nelspruit in April) if good Empress mandarin seed germination is desired, but that the seeds are mature enough to germinate well 9 weeks before the fruit reaches edible maturity in mid-June. Results also showed that factors encouraging the interference of microorganisms could play a role in reducing seed germinability and that at certain times of fruit maturity, the seed was more predisposed to waste susceptibility. It also focussed attention on the probable importance of microorganism control in the period between the harvest of the fruit and the planting of the seed. This aspect is further investigated in Experiment 5.

EXPERIMENT 2 Comparisons between the effect of storing seed in or out of fruit under different temperature conditions and chemical treatments

Grandidea
Introduction Because as suggested by the results of Experiment 1 the freshly extracted seeds of Empress mandarin are prone to fungal invasion, the storage of such seed should be preceded by treatments aimed at keeping waste development to a minimum. In practice circumstances sometimes arise which make it difficult for nurserymen to execute such treatments, and for convenience the storage of seed within the fruit offers a practical alternative providing seed viability is not impaired. This experiment aimed at comparing the viability of seed stored in the fruit under certain conditions with that of extracted seed.

was
Procedure Express mandarin fruit harvested on 10th July 1975 was split into two groups. Seed was extracted from the one group and the fruit left intact in the second. About 200 extracted seeds were *How* briefly washed, surface sterilised in 10 per cent hypochlorite and after being surface dried, were placed in a plastic bag and well shaken up with 1g "benomyl" (methyl 1-(butyl-carbamoyl)-2-benzimidazole carbamate), a compound shown in pilot trials to control a wide range of seed fungi. Another similar batch of 200 seeds was washed, but received no chemical treatment before being sealed in a plastic bag. A portion of the intact fruit was dipped in an agitated solution of benomyl at *How big* 5 000 ppm (normal packhouse treatment is 1 500 ppm), "Dowicide" (sodium orthophenyl phenate) at 2 per cent. (normally applied at 0.15 to 2 per cent.), 2,4-D at 500 ppm, and 50 per cent water soluble "Citrashine" wax (Pelser, 1975). These fruit together with an equal number of untreated fruit and the two sealed plastic bags containing extracted seeds were placed in a cold room at a constant temperature of 5°C. An identical

replicate of fruit and seeds was placed in a room at ambient ($\pm 23^{\circ}\text{C}$) temperature. After storage for 79 days samples of 200 seeds were randomly selected from those kept in plastic bags under the 2 different storage temperatures. These together with batches of 200 seeds extracted from the treated and untreated fruits stored at the two temperatures were planted 20 to a Petri dish and germinated as described above.

Results and Discussion In Table 1.2 results are presented of the mean germination percentages obtained from seeds subjected to the various pregermination treatments.

TABLE 1.2 Mean germination percentages of Empress mandarin seed stored for 79 days both in the fruit and after extraction. Extracted seeds and whole fruit were subjected to two temperature storage regimes and two chemical treatments

Chemical treatment	State of seed during storage	Storage Temperatures		Mean
		5°C	Ambient ($\pm 23^{\circ}\text{C}$)	
Treated	extracted	97,0	49,5	73,3
	not extracted	97,5	74,0	85,6
	Mean	97,2	61,8	79,5b
Untreated	extracted	58,0	28,0	43,0
	not extracted	87,0	29,0	58,0
	Mean	72,5	28,5	50,5e
Mean of treated and untreated	extracted	77,5	38,7	58,1d
	not extracted	92,2	51,5	71,6c
	Mean	84,6a	45,1f	64,9

L.S.D.s for main effects and treatment interactions $P = 0,05 : 2,05$
 $P = 0,01 : 2,72$

Significant differences ($P = 0,01$) between the means due to main

Not properly statistically analyzed.

treatment effects are indicated by the letters alongside the relevant means. The alphabetic order of the letters corresponds to the order of greatness of the means.

What about other temperatures?

- (a) Main effects: Significant differences ($P = 0,01$) were found to exist between all treatments means. Of all treatments, the low storage temperature of 5°C was most critical in maintaining a high level of seed germinability during the storage period. A significantly better ($P = 0,01$) germination percentage was obtained from seed stored in the fruit than after its extraction from the fruit. Untreated seed, or seed stored in untreated fruit gave a low mean germination percentage (50,5) but not as low as that of seed stored at ambient temperature (45,1).
- (b) Interactions: Treated seeds stored at 5°C germinated as well as the seeds of treated fruit stored at 5°C . However, where fruit or extracted seeds were not treated prior to storage at 5°C the germination percentage of the extracted seeds was significantly lower ($P = 0,01$) than that of the seeds which had been stored in the fruit.

Where seeds and fruit were treated and then stored at ambient temperatures the germination of seeds stored in the fruit was significantly higher ($P = 0,01$) than that of extracted seeds. No significant differences were found, however, between the germination percentage of untreated seed and the seed from untreated fruit which had been stored at ambient temperature.

Extracted seed stored at 5°C gave significantly lower ($P = 0,01$) mean germination percentages than seed stored in the fruit at the

same temperature. Similarly seed from fruit stored at ambient temperature germinated significantly better ($P = 0,01$) than extracted seed stored at that temperature.

Discussion or Conclusion.

From a practical viewpoint a cultivar with fruit as waste prone as Empress mandarin should not be stored for extended periods at ambient temperatures even if chemical treatments are applied to curb the development of fruit decay. The storage of seed in the fruit should only be undertaken where adequate cold storage facilities are available in which case good germination rates could be attained even when the fruit is not chemically treated. Excellent Empress mandarin seed storage results can be achieved by chemically treating extracted seed and maintaining the seed at a 5°C storage temperature. Even under these conditions, however, the extended viability of the seed would depend on other factors such as the loss of moisture from the seed during the storage period.

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looked at or tested or reference?*

1.3.2 Some factors affecting the viability of extracted seed during storage

EXPERIMENT 3 The effect of seed desiccation

Introduction During the storage of seed, nurserymen sometimes allow the seed to dry out. While this has the effect of retarding waste development, it has been shown to greatly reduce seed viability in a wide range of citrus cultivars (Barton, 1943; Childs & Hrncair, 1948; Fu, 1951; Button, Bornman & Hackland, 1971). To date no such tests have been conducted on Empress mandarin seed.

Why, was there any reason to believe it will be different surely it is logical.

Procedure Two independent trials were conducted. In the first,

Why?

freshly extracted seed was washed and separated into 5 batches of 100 seeds. The first batch was planted out directly into Petri dishes. Lots 2 to 5 were planted out after being allowed to dry out in the shade at about 19°C for 3, 6, 12 and 24 days respectively. Seeds were germinated at 27°C . In a second trial 100 fresh Empress mandarin seeds were allowed to dry out for 53 hours at 27°C before being planted while the control consisting of 100 fresh seeds, was planted out immediately. Both control and treated seeds were germinated at 30°C .

Temp. story not clear!

Results and discussion In the first trial, where seeds were allowed to dry out at ambient temperature over the 24 day period a steady reduction in viability was observed (Fig. 1.1a). After ^{three} 3 days, ^{drying} only 63 per cent. of the seed was still viable but after 24 days, ^{drying} this figure had dropped to zero. These results are similar to those of Kumar & Majmudar (1973) who found that the germination percentage of lime seeds kept in open storage for 0, 7, 14 and 21 days declined as follows: 58, 48, 26 to 0 per cent. respectively.

Results of the second trial indicated that where seeds are desiccated at 27°C damage occurs more rapidly than where the seeds dry out at a lower temperature (19°C). After drying out for only 53 hours at 27°C virtually all germination was inhibited for 27 days, and thereafter only 56 per cent. of the seeds succeeded in germinating (Fig. 1.1b). This shows that the viability of the seed during storage is very much a function of the temperature at which seeds desiccate, or the rate at which moisture is lost from the seed. This confirms the findings of Childs & Hrncair (1948) but contrasts with those of Fu (1951) who considers seed moisture content and not the rate of drying out to be the critical factor.

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Dis

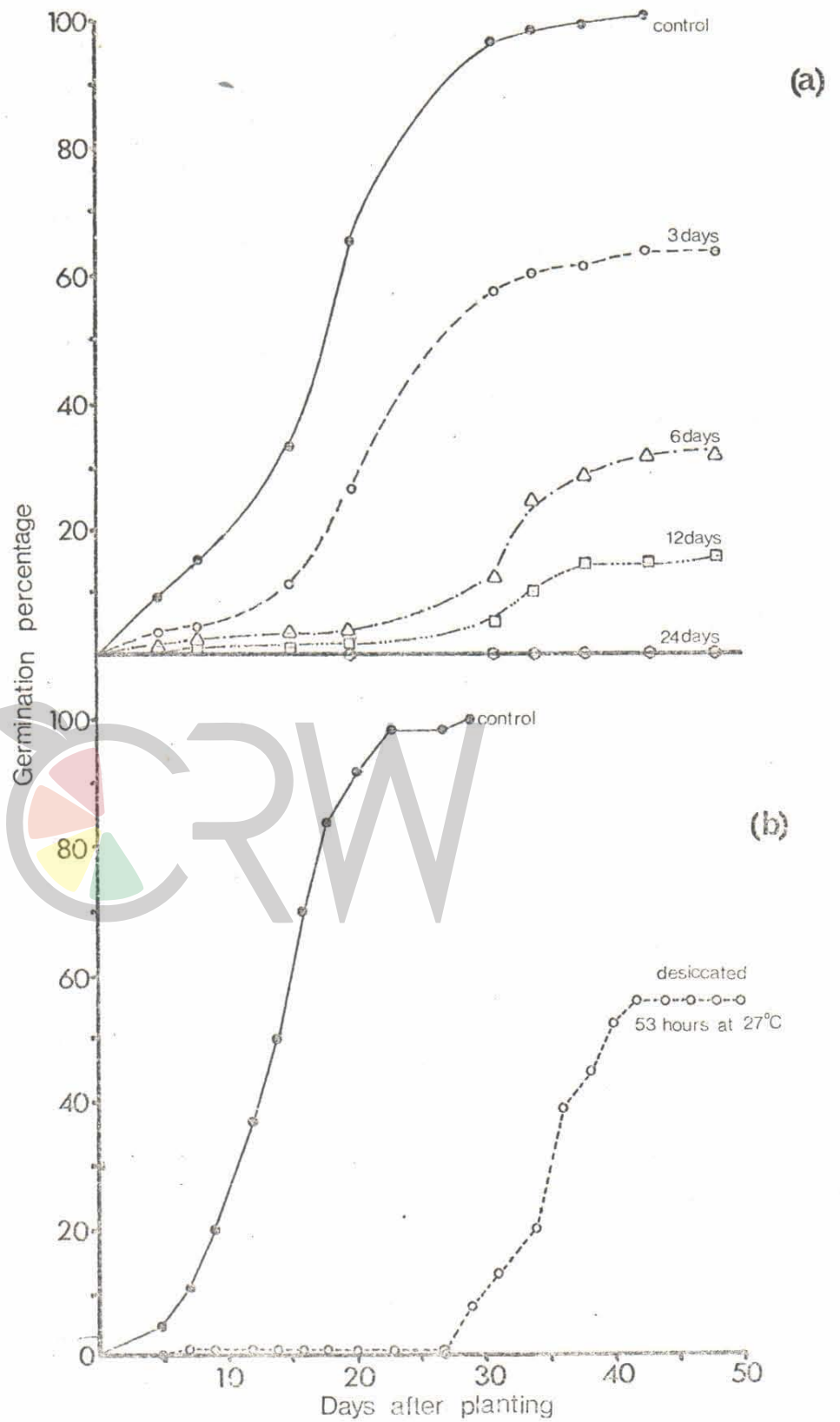


FIG. 1.1 The effect of Empress mandarin seed desiccation for various times before planting on the rate of its germination

Was this proved.

Not in Reference (1960)

No attempt was made to measure the changes in seed moisture content during storage and relate this to its germinability. Nevertheless, the experiment succeeded in showing that unlike the seeds of many other plant species (Hartmann & Kester, 1968), the seed viability of Empress mandarin is best retained by the prevention of its desiccation during storage. This can be achieved by storing the moist seed in sealed containers. Under such conditions however, a wide variety of micro-organisms flourish and these may greatly reduce seed viability as already shown.

Nothing to do with the present exp.

Further work aimed at investigating the effect of rapid and slow desiccation of Empress mandarin seed under low temperature storage conditions should be undertaken. This may reveal that providing storage temperatures remain low, Empress mandarin seed could undergo considerable desiccation before seed viability becomes impaired. In practice, however, the simplest method of retaining Empress mandarin seed viability during storage is required, viz., the storage in sealed containers immediately after extraction from the fruit.

Speculation

EXPERIMENT 4 The effect of thorough seed rinsing

Says who?

Introduction The presence of the sticky nutritive mucilage around extracted citrus seed constitutes a threat to the continued viability of the seed owing at least in part to its apparent attractiveness to a wide range of micro-organisms. It has been the experience of the author that this jelly-like substance is harder to remove from Empress mandarin seed than from Troyer citrange, *Poncirus trifoliata* or rough lemon seeds. It seemed possible therefore, that this factor may be important in somehow reducing the viability of seeds planted without first having this mucilage removed.

When harvested.

Very low.

Procedure Empress mandarin seed was divided into 4 batches of 100 seeds immediately after extraction from mature fruits. Preliminary work had shown that the mucilage could be effectively removed by a 4 minute rinsing in tap water under normal pressure (150 kPa). Accordingly this method was used in the experiment.

Treatments: Batch 1 : Unwashed untreated control
Batch 2 : rinsed for 4 minutes but no further treatment
Batch 3 : rinsed for 4 minutes and soaked in agitated 5 000 ppm benomyl for 5 minutes, then surface dried
Batch 4 : rinsed for 4 minutes then sprinkled lightly with "Trigosan"

Albimim?

After treatment seeds were planted 10 to a Petri dish each treatment being replicated 10 times. Seeds were incubated at 30°C and germination was assessed by inspection 28 days after planting.

Results and Discussion Details concerning the significantly low rate of germination (52 per cent.) of the untreated control and the significantly depressed germination of seeds treated with Trigosan (76 per cent.) in comparison to those either well-rinsed or rinsed and treated with benomyl (94 and 92 per cent. respectively) are presented in Table 1.3. The poor germination of control seeds could be attributed to the presence of seed-invading fungi in the Petri dishes. Trigosan-treated seeds showed very little fungal infection.

Surely he could see it this was the case.

Of importance in this experiment was the finding that seeds subjected to thorough rinsing germinated much better than unrinsed seeds and that the treatment of seeds with benomyl is not necessary providing, as was the case in this experiment, seeds are planted directly after extraction and rinsing and are not stored for any length of time. Use of "Trigosan"

TABLE 1.3 The effect of seed rinsing and chemical treatments upon the loss of Empress mandarin seed viability during storage

Treatment	(percentage germination)			
	4 Min. Rinse	Rinse + Benomyl	Rinse + Trigosan	Control
Means	94 a	92 a	76 b	52 c

Duncan's Multiple Range Test

S.S.D. $P = 0,05 : 5,2$ C.V. = 6,88%

S.S.D. $P = 0,01 : 9,0$

Means in the row not followed by the same letter are significantly different at $P = 0,01$.

(see Experiment 5) as a pre-germination treatment depressed germination significantly.

The high germination percentage of rinsed seed apparently resulted from the removal of the fungi-attracting mucilage. However it is conceivable that the water rinsing could also have served to remove germination-inhibiting substances from the seed coat. This is further investigated in Experiment 7. Another aspect worthy of further investigation was the effect of various chemical fungicides upon the retention of seed viability during storage.

EXPERIMENT 5 The effect of chemical treatment

Introduction Extracted seed may deteriorate as a result of the destructive effects of a variety of airborne and seed coat fungi or other organisms which, if they do not cause loss of seed viability,

may be responsible for albinism in seedlings by producing metabolites which inhibit chlorophyll synthesis in germinating seedlings (Tager & Cameron, 1957; Durbin, 1959; Ryan, Greenblatt & Delaminy, 1961). Various fungicides have been tested to eliminate such fungi including the inorganic salts of heavy metals, heavy metal carbamate fungicides and certain non-metallic organic compounds (Perlburger & Reichert, 1938; Childs & Hrncair, 1948; Ryan & Stein, 1958; Said & Ryan, 1961; Ryan & Frolich, 1961; Moore, 1961). Arising from such research the chemicals most widely used in California are 50 per cent. tetramethylthiuram disulphide (Arasan) and 8-hydroxyquinolene sulphate (Platt & Opitz, 1973).

Eschsch? *mark proved Albinism!!*

In South Africa little attention has been paid in the past to the screening of fungicides for seed storage purposes. A heavy metal compound, phenyl mercuric acetate ("Trigosan") has however, come into common use and therefore the need to compare this with other tested and untested chemicals was felt justified. This was especially so in view of results obtained in the previous experiment and two pilot trials where the germination percentage of "Trigosan"-treated Empress mandarin seed was found to be lower than that of Empress mandarin seed treated with other chemicals. Because the workers previously cited *who?* had established the most suitable concentrations to use for adequate control no attention was paid to further investigations of this kind.

???

Procedure Eight hundred freshly extracted and well-rinsed Empress mandarin seeds were split into four batches of 200 seeds. One batch was dipped for two minutes in 5 000 ppm agitated benomyl, the second soaked in a 10 per cent. solution of 8-hydroxyquinolene sulphate for 8 minutes, the third batch shaken with 3g "Trigosan" powder and the fourth left as an untreated control. "Arasan" was not available for

Why not!

inclusion in the trial. After treatment, the seeds in each batch were allowed to surface dry and were stored in sealed clear polyethylene bags at 5°C. After 35 days 100 seeds of each batch were placed in Petri dishes at 30°C for 38 days, after which germination counts were conducted. The balance were germinated in the same way after 89 days storage.

Results and Discussion

The use of suitable chemical treatments to control decay in stored Empress mandarin seed proved essential. After 35 days storage the germinability of untreated seeds had dropped significantly to 74 per cent. and after 89 days to 53 per cent.

TABLE 1.4 Effect of three chemical treatments on the germination percentage of Empress mandarin seeds after 35 and 89 days of storage at 5°C (angular transformed data)

Length of Storage (days)	Mean Germination Percentages				Mean
	Chemical Treatments				
	HQ	Benomyl	Trigosan	Control	
35	94,5	94,0	82,0	74,0	86,1 a
89	90,0	89,0	45,2	53,1	69,33b
Means	92,3a	91,5a	63,6b	63,5b	x What

L.S.D.'s for treatment means: $P = 0,05 = 7,10$ C.V. = 9,0%
 $P = 0,01 = 9,44$

Means in any vertical or horizontal column not followed by the same letter are significantly different at $P = 0,01$.

There was little to choose between benomyl and 8-hydroxyquinolene sulphate after either storage period whereas, the germination of Trigosan-treated seeds was significantly depressed after 89 days storage.

Save a penny for a

Reduction in the viability of control seeds was mainly due to the development of waste in these seeds whereas non-viable "Trigosan"-treated seeds were seldom infected. This suggests that the depressive effect of phenyl mercuric acetate ("Trigosan") on Empress mandarin seed viability is caused by some chemical interaction with the seed's metabolic processes, possibly inhibition of enzyme activity. *How?*

Extracted seeds will retain best viability over long storage periods if treated with either benomyl or 8-hydroxyquinolene sulphate.

"Trigosan" is less effective and should be avoided. When available, Arasan should be tested against "benomyl" and 8-hydroxyquinolene sulphate. *Weakness. Should have done it!*

EXPERIMENT 6 The effect of storing seeds in their own juice *How.*

Wash activation

Introduction The importance of storing Empress mandarin seed in an environment which discourages desiccation of the seed has been dealt with. The improved germination of seeds free of all fruit mucilage has also been demonstrated. In the latter case, it was concluded that the mucilage indirectly depressed germination by attracting seed-invading fungi. The possibility also exists however that the mucilage contains a germination inhibitor and that germination is facilitated by removal of the inhibitor. This theory conflicts with the belief of some nurserymen that the storage of Empress mandarin seed in the juice of the fruit may in fact improve germination. The need for an experiment to resolve this question was considered justified.

The practical usefulness to citrus nurserymen of holding seed for short periods in the pulp and juice of the fruit from which the seeds had been extracted, provided further motivation for the experiment. A trial was

thus designed to test the effect on seed germination of cool and ambient temperature storage of Empress mandarin seed in its own juice.

Procedure On 14th July, 1975 seeds were extracted immediately after harvest from about 100 sound Empress mandarin fruit. A portion of the juice from the extracted fruit was retained. Sixty seeds were placed into each of eight 50ml beakers containing this juice. Four of these beakers were kept at 5°C, the remaining four at ambient temperatures ($\pm 23^{\circ}\text{C}$). A control treatment consisting of 60 washed seeds were planted immediately after extraction, 20 to a Petri dish and incubated at 30°C. At two day intervals, 60 seeds from a beaker which had been kept at ambient temperature and a beaker kept at 5°C were well rinsed, sterilized in hypochlorite and planted out 20 to a Petri dish and incubated as above. A comparison was thus made between the germination rates of seeds stored in their own fruit juice for 0, 2, 4, 6 and 8 days at ambient temperature and at 5°C.

Results and Discussion The statistical analysis conducted on the germination percentages obtained is presented in Table 1.5.

TABLE 1.5 Mean germination percentages of Empress mandarin seed after storage from 0-8 days in its own juice. Means are calculated from 5 germination counts conducted during the period 11-19 days after planting

Storage temperatures	Storage time in juice (days)					Means
	0	2	4	6	8	
Ambient ($\pm 23^{\circ}\text{C}$)	81,5	64,0	51,8	50,4	57,1	60,7a
5°C	81,5	64,4	64,0	54,1	55,7	63,9a
Means	81,5a	64,2b	57,9c	52,3d	56,4cd	62,3

L.S.D.'s for storage time means $P = 0,05 : 4,6$ $P = 0,01 : 6,2$ C.V. = 8,75%

Means in the row not followed by the same letter are significantly different at $P = 0,05$.

Why? This seed
diff not same
as previous exp.

It is evident that each subsequent increase in storage time up until six days caused a further significant decrease in germination percentage. No decrease in germination percentage was recorded for seeds stored for eight days compared to seed stored for six days. No difference in germination percentage was found between seeds stored at 5°C and those stored at ambient temperature. *Why? if compared with exp. 2.*

Further details concerning the rates of germination are presented in Appendix Table 2. Seeds stored for two days in juice took five days longer to reach 100 per cent. germination than the control. Where seeds were stored in juice for eight days 10,4 per cent. lost their viability. Nurserymen would thus be ill-advised to store seeds in their own juice for longer than 6 days. *Not a proper conclusion after the exp. results!*

1.3.3 The influence of pre-germination seed treatments on Empress mandarin seed germination

EXPERIMENT 7 Effect of water leaching

Introduction The high germination percentage obtained in Experiment 4 for seeds well rinsed (4 minutes) before planting led to speculation that germination may be further improved by extension of the water rinsing period so as to constitute an actual water leaching of the seed. It was argued that if any endogenous inhibitors present in the seed coat were responsible for reducing germination rates, these may be removed by such treatment.

Why not different times only 2.
Procedure Extracted seeds were divided into two groups, one being leached for 24 hours in running tap water; the second being washed in running water for 2 minutes (control). It was decided that leaching

Why not 4

for periods under 24 hours would only be tested if results obtained by the 24 hour period proved promising.

In July 1974 seeds were planted 30 to a flat, 5 such flats constituting a water leach treatment. Germination was checked approximately every two days after the first epicotyl broke the surface 19 days after planting.

Results and Discussion

Very!
The slightly faster rate of germination achieved by seed leached for 24 hours is shown by Fig. 1.2a. Of importance is the fact that after 57 days no difference existed between the total number of seeds germinated under the two treatments and therefore the effect of extended water leaching upon germination was confined to the rate of germination only. It is possible that the germination of leached seeds was accelerated by the 24 hour period during which imbibition was able to proceed. However, citrus seed may not always depend upon imbibition as the initial phase of germination because viable seeds usually contain high moisture contents. *bad sentence*

Of course!
The first epicotyl emerged 19 days after planting and between the 36th and 44th day after planting 16 per cent. more 24-hour leached seeds had germinated than control seeds. While this may sound substantial, and while statistical analysis shows that highly significant differences existed between the mean germination obtained by the two treatments (Appendix Table 3) the fact that water leaching in no way improved the final germination percentage limits its use as a commercial practice. Results of this experiment suggest that, in the light of the time taken for the leaching and the requirement of additional facilities in order to carry it out, it is not worthy of further consideration for improving Empress mandarin seed germination.

USEFUL EXP.

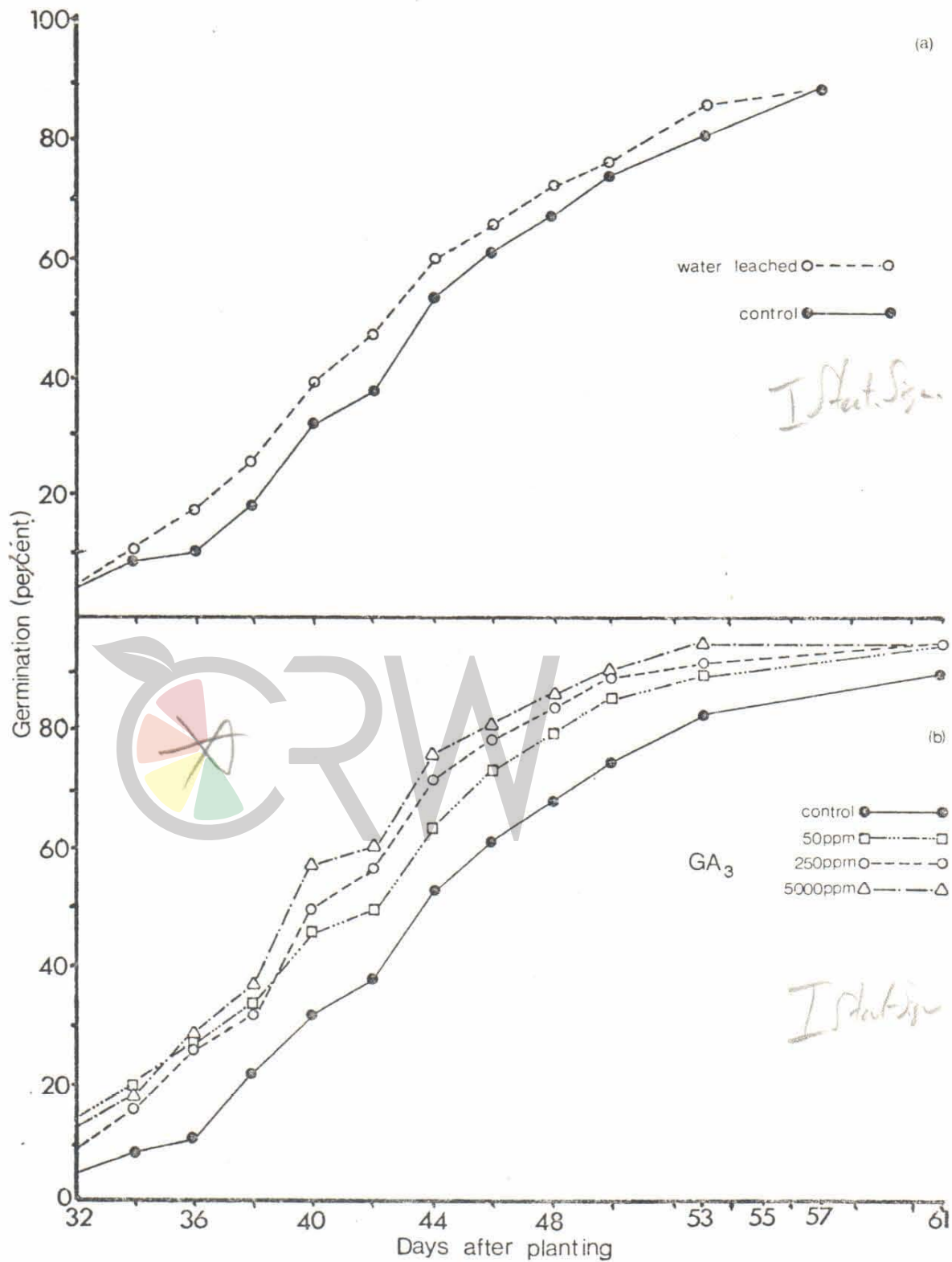


FIG. 1.2 Effect of a 24 hour water leach and GA seed soak treatments on the germination rate of Empress mandarin seeds

* Should not be here but after p28

Not in Ref List

EXPERIMENT 8 The influence of gibberellic acid seed soaks

Introduction Both Burns & Coggins (1968) and Achituv & Mendel (1973) found that the soaking of citrus seeds in gibberellic acid increased the number of germinating seeds and the rate of germination in several rootstock cultivars. The influence of such treatments on Empress mandarin was considered important in view of the possibility that if low concentrations of free gibberellins exist in mature seed this may be responsible for retarded germination rates or even contribute to reduced seed viability.

Procedure Varying concentrations of GA_3 were used as 24 hour Empress mandarin seed soak treatments.

The trial was laid out according to randomised blocks design with GA_3 being applied at 0, 50, 250, 1 000 and 5 000 ppm. Each treatment consisted of 30 seeds planted per germination tray, replicated 5 times.

Results and Discussion The response of Empress mandarin seeds to GA_3 seed soaks is shown in Fig. 1.2b, where it can be seen that seeds treated with all levels of GA_3 from 50 to 5 000 ppm germinated faster than the untreated control. The 1 000 ppm curve has been excluded to improve the clarity of the figure but coincides closely with the other GA_3 curves.

It was evident that at certain stages many more GA_3 seeds had germinated than untreated seeds. For example, 40 days after planting only 32 per cent. of the control seeds had germinated compared to 57 per cent. of those treated with 5 000 ppm GA_3 . Results of the statistical analysis of the experiment which are presented in Appendix Table 4 show that as

from 38 days after planting, all GA_3 treated seed germinated significantly faster (at $P = 0,01$) than the control. The 1 000 ppm treatment induced significantly slower germination than the 5 000 ppm treatment (at $P = 0,05$) although this was not the case for either the 50 ppm or 250 ppm treatments.

At the conclusion of the experiment 61 days after planting (results shown in Fig. 1.2b) only 3 per cent. more GA_3 treated seeds had germinated than control seeds. This shows that while the GA_3 soaks hastened germination and therefore may reduce the time between seed planting and budding, they had little effect on the number of seeds which actually germinated. Since more difficulty is experienced by nurserymen in getting Empress mandarin seed to germinate than obtaining a more rapid germination, GA_3 soaks seem of little practical value. The stimulatory effect of GA_3 upon germination rate may, however, prove to be of use in the cool production areas where germination is normally slow, and may in time lead to a greater interest in Empress mandarin being shown by nurserymen in these areas.

*Unjustified conclusion
(stratification?)*

Seeds treated with GA_3 were observed to produce taller seedlings with longer internodes than untreated controls. This may prove an important means of inducing seedlings to reach budding height more rapidly but did not form part of the present study.

*Contrary to usual
effect of GA_3 ?*

EXPERIMENT 9 The influence of seed coat removal

Introduction Citrus embryos are enclosed by two seed coats. The tegumen, or inner coat is a thin delicate membrane composed of remnants of the endosperm, nucellus and the true inner wall called the inner integument. Its characteristic brown-red to purple colour is derived

?

from several layers of cells in the chalaza adjacent to the nucellus. The outer seed coat is composed primarily of the outer epidermis of the ovular wall, and during the final stages of seed maturation the epidermal cells form lignified secondary walls which are often wrinkled (Schneider, 1968).

The seed coat may retard germination by harbouring growth inhibitors (discussed earlier), by physically obstructing the emerging seedling during germination or by preventing the movement of water or oxygen to the seed (Zabala & Guardiola, 1974). To determine these effects on Empress mandarin seed an experiment was conducted in which the germination rate of seeds with both seed coats removed was compared to that of intact seeds. This trial was run in conjunction with a further investigation into the influence of gibberellic acid soaks upon germination rate.

Why? *To early according to Table 1.1*
Procedure Seeds extracted from immature fruit harvested on 7th April, 1975 were well rinsed and sterilised. Both seed coats were removed from 80 seeds but left intact in a second batch of 80 seeds. Both batches of excised and normal seeds were subdivided into 4 groups of 20 seeds and treated as follows:

- | | | |
|---------------|-----------------|---------------------------|
| Treatment (a) | 24 hour soak in | 50 ppm GA ₃ |
| (b) | " " " " | 500 ppm GA ₃ |
| (c) | " " " " | 5 000 ppm GA ₃ |
| (d) | " " " " | sterile water |

Seeds were planted 10 to a Petri dish and germinated at 30°C. The experiment was terminated after 36 days.

In order to test the presence or absence of seed coats upon the rate of water uptake, a further small trial was conducted. Outer seed coats were removed from 50 seeds and left intact on 50 seeds all of which had been allowed to dry out in the shade at 21°C for 53 hours after extraction. After ^{the masses were determined} being weighed, both batches of 50 seeds were soaked in water for 21 hours, surface dried, then ^{their masses were determined} weighed again. Why?

Mass determined.

Results and Discussion The high degree of polyembryony in Empress mandarin was immediately evident when seeds were germinated without seed coats. In many instances as the seeds absorbed water they fell apart into their several separate embryos, which made germination counts difficult to conduct accurately. In some cases where polyembryony was excessive (4-5), germination was sometimes delayed perhaps because none of the embryos were large enough to sustain immediate radicle development. In spite of this, results as presented in Table 1.6a showed that the germination rate of excised embryos was very much more rapid than that of intact seeds. In most cases all excised seeds had already germinated before normal seeds even commenced germinating.

As found in Experiment 8, seed soaks in certain concentrations of GA₃ speeded up germination. Best results for intact seeds were obtained at 500 ppm GA₃ while excised embryos responded well to both 500 and 5 000 ppm. Excised seeds reacted more noticeably to the GA treatment than did normal seeds. This suggested that the seed coat may contain germination inhibitors such as ABA or restrict uptake by the seed of water (in this case dissolved GA) or oxygen. Not very conclusive. Water lit.

As shown by Table 1.6b seeds with their outer seed coats removed increased in ^{mass} weight by 71 per cent. during the 21 hour imbibition period whereas the figure for intact seeds was 33 per cent. This suggests

TABLE 1.6(a) Influence of seed coat removal and GA₃ seed soaks upon germination rate of Empress mandarin seed

What happened to the other 10

Treatments	Reps.	(No. out of 10 germinated)									
		Days after planting									
		5	7	13	15	17	21	27	32	36	
Excised embryos											
Control	1	2	3	7	7	8	?	?			
	2	2	3	8	9	9					
50 ppm	1	4	5	5	6	6	?	?			
	2	3	5	7	7	7					
500 ppm	1	6	7	8	8	9					
	2	6	7	10	10	10					
5 000 ppm	1	8	9	10	10	10					
	2	5	5	10	10	10					
Normal embryos											
Control	1	-	-	1	-	-	-	-	3	6	
	2	-	-	1	-	-	-	4	5	8	
50 ppm	1	-	-	-	-	1	-	2	9	9	
	2	-	-	-	-	-	2	3	6	7	
500 ppm	1	-	-	-	-	-	3	9	-	-	
	2	-	1	-	-	-	2	10	-	-	
5 000 ppm	1	-	-	2	3	-	5	9	10	-	
	2	-	-	1	-	-	4	10	10	-	

TABLE 1.6(b) Changes in mass of 50 seeds with and without seed coats after a 21 hour water soak

	(g)	
	Seed coats removed	Seed coats intact
Mass of dry seed(g)	4,30	4,90
Mass of moist seed	7,35	6,55
Percentage mass increase	71	34

9.1 that seed coats may retard germination by restricting water uptake by the seed. This together with the restraining effect of the seed coat upon emergence of the radicle seems, in the light of water leach experiments previously discussed, to primarily account for the slower germination rates of intact seeds. However, it does not discount the possibility that the water soak leaches out germination inhibitors nor that seed coats may be responsible for restricting the uptake of oxygen during early stages of germination.

2 The removal of seed coats is too timeconsuming to be of practical use, but some scarification treatment aimed at rupturing the outer testa may prove worthwhile. Why didn't he test it.

1.3.4 The influence of conditions during germination upon Empress mandarin seed germination

Many factors are known to influence the rate and percentage germination of viable citrus seeds. These include the type of germination medium, planting depth, moisture regimes and, probably of greatest importance, temperature. Since the influence of the former factors are of general application to most cultivars used as rootstocks for citrus, the results of previous work done in this field (cited in the introduction to this chapter) probably hold equally well for Empress mandarin. However, the influence of temperature, which vitally influences the rate of all metabolic reactions and to which different cultivars vary in their response, was a factor thought worthy of further investigation.

EXPERIMENT 10 The influence of temperature

Introduction Little has been written about ideal temperatures for

citrus seed germination though it is generally accepted that germination will not occur below 13°C (Webber, 1948a), while the optimum lies somewhere between 27°C and 32°C (Platt & Opitz, 1973). It is important to know the response of seed germination to different temperatures because if planted too early before the soil is warm enough seeds may rot, whereas seed planted too late may not respond well to the high soil temperatures.

Two separate trials were conducted to determine the effect of temperature on Empress mandarin seed germination. The first constituted part of a larger experiment, which also concerns the influence of temperature on seedling growth and is more fully discussed in Chapter III. The second was a straightforward germination trial.

Procedure (a) On 31st July, 1973, 50 seeds were planted in each of 9 flats. Three flats were placed in each of three separate constant temperature glasshouses set at 20°C , 25°C and 30°C .^{35?} Seeds were kept moist with water of the same temperature as that of the chamber in which they had been planted. *What about a high temp 35?*

(b) Five Petri dishes each containing 20 seeds were placed in constant temperature incubators set at 15° , 18° , 21° , 24° , 30° , and 36°C .

In both trials germination was recorded every second day.

Results and Discussion Where seeds were planted in flats, epicotyl emergence at 30°C was very much more rapid than at either 25°C or 20°C . For example, 26 days after planting over 70 per cent. of the seeds planted in the 30°C chamber had produced emergent epicotyls, while the

corresponding figures in the 25°C and 30°C chambers were 35 and 30 per cent. respectively. A feature of the first trial was that no significant differences in the rate of germination of Empress mandarin seeds at 20°C and 25°C occurred (see Fig. 1.3a and Appendix Table 5a).

In the second trial where a far wider range of temperatures were tested it was evident from Fig. 1.3b and Appendix Table 5b that germination rates during the first 12 days were greatest at 36°C but that final germination percentages were lower for temperatures above 30°C. The reason for this was primarily an increased incidence of fungal invasion in spite of the seeds having been treated with 8-hydroxy-quinolene sulphate prior to planting out. After 4 days, 10 per cent. of the seeds at 36°C had germinated compared to only 5 per cent. of those at 30°C. It may be speculated therefore that while the optimum temperature of Empress mandarin seed germination is probably not lower than 36°C, it is likely that a number of seeds, especially those predisposed to develop waste (due to such factors as length of and conditions during storage, maturity of the seed, presence of disease organisms within the seed coat, sterility of the germination medium, etc.) will do so at these high germination temperatures, thereby reducing the overall number of seeds which finally germinate.

At 15°C germination percentage was low and germination was extremely slow. However, germination increased appreciably as temperatures rose through 18°C, 21°C and 24°C. Under circumstances where rapid germination rates are important in producing seedlings of budding height by the onset of winter, it is evident from Fig. 1.3b that over 3 weeks can be saved if germination temperatures can be held at 30°C or 24°C rather than at 21°C or lower. This provides motivation for germination in soil given bottom heat or under a plastic canopy.

Only one?

Why not
have
sandy

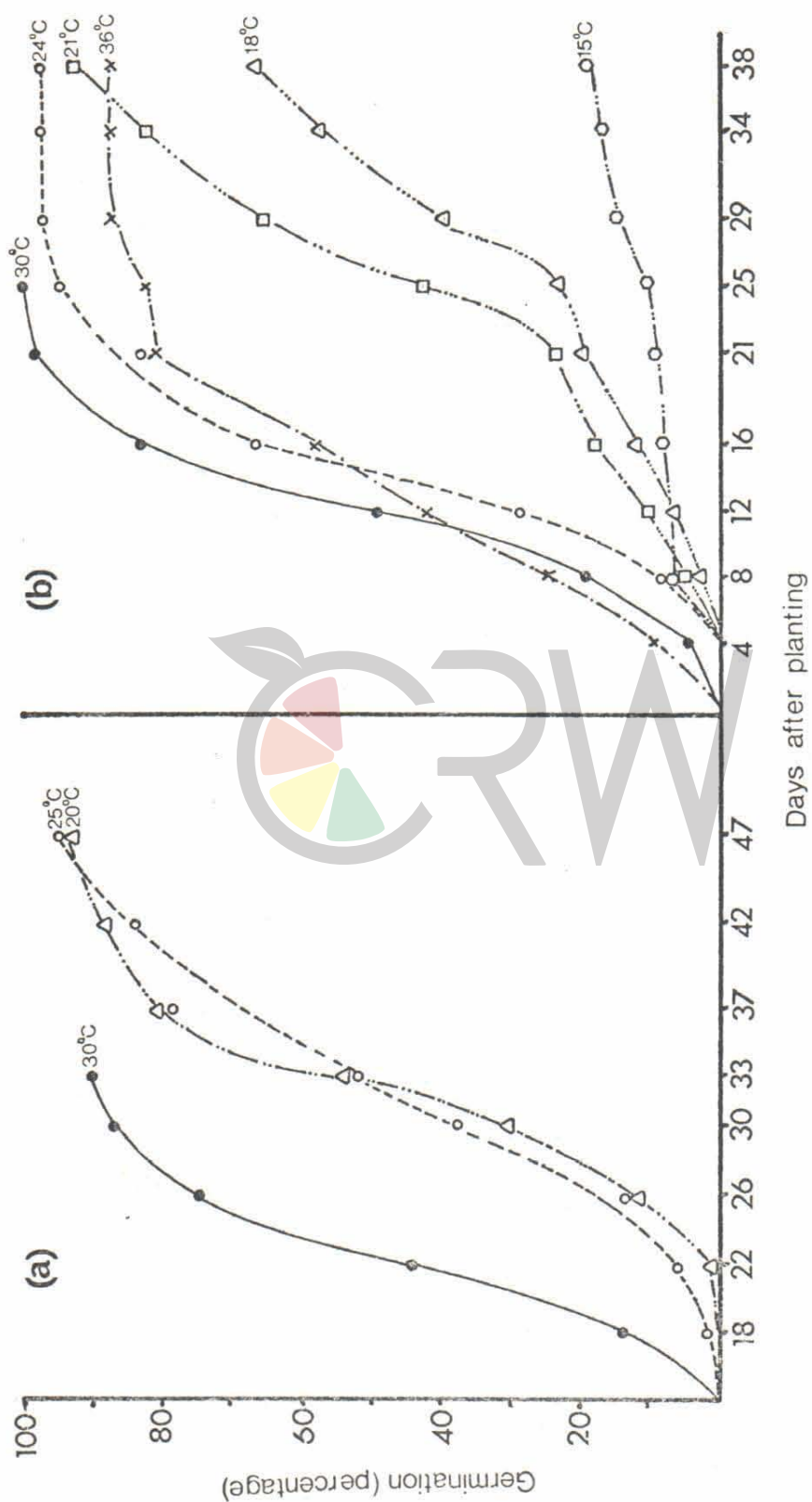


FIG. 1.3 Effect of temperature on the rate of Empress mandarin seed germination

The former is expensive and has little influence on rates of photosynthesis of the developing seedling. ✓ The use of plastic canopies as described by Hall and Besemer (1972), however, is a practical way of increasing temperatures in seed bed soils to boost germination rates and also to maintain rapid seedling growth. This was highlighted by the results of a small trial in which Empress mandarin seed was planted both under canopy and alongside in an exposed position of the same seed bed at Ukulinga Research Station, Pietermaritzburg in mid-September, 1973. Although temperature differences were not recorded, 87 per cent. of the seed under canopy had germinated 32 days after planting whereas only 43 per cent. of the exposed seed had germinated by then.

Not with side monitoring

Why not a proper exp to prove this point!

1.4 GENERAL DISCUSSION + CONCLUSIONS

I doubt it.

It was possible, as a result of the trials described in this chapter, to obtain a clear picture of the factors most responsible for the erratic germination tendencies of Empress mandarin seed. A surprising discovery, quite early on in this work, was that the poor reputation Empress mandarin has developed among many nurserymen is not well founded and reflects more the lack of attention paid by nurserymen to basic seed extraction, storage and pre-germination treatments than to any hereditary peculiarity on the part of the seed. The lack of attention by some nurserymen to these factors may be partially the result of their long association with rootstock cultivars such as rough lemon which are apparently less sensitive in their response to injudicious pre-germination handling.

Why not all exp. compared with Rough Lemon

Experiments revealed that Empress mandarin seed can be stored successfully for long periods in the fruit or in sealed plastic bags at 5°C

What of Eschmuss work.

providing seed is not permitted to dry out and appropriate waste control measures are taken. In this regard the use of benomyl at ^{5000 ppm} 5 000 ppm or 10 per cent. 8-hydroxyquinolene sulphate is recommended ahead of the more commonly used phenyl mercuric acetate ("Trigosan").

Rutbush.

Storage of seed in the juice of the fruit from which it was extracted also offers a practical means of overcoming short-term (under 6 day) storage problems. The destructive effect of the waste-promoting organisms on seeds in storage was found to be as detrimental to the viability of seeds planted out to germinate as to that of seeds held in storage. This focussed attention on the importance of using adequate sterilizing treatments prior to planting out, even in cases where seeds are freshly extracted from fruit just harvested. It was confirmed that such sterilising treatment should always be preceded by a thorough rinsing of the seed so as to remove the fungi-attracting mucilage from its surface. Thereafter the proven hot water treatment to eliminate *Phytophthora* species (Klotz, de Wolfe & Wong, 1958), followed by an 8 minute soak in 10 per cent. 8-hydroxyquinolene sulphate should provide good protection against waste-promoting organisms.

Observation
No indication
of result of
exp

What of Barclay now?

Such as

As with the other citrus rootstock cultivars, Empress mandarin is also very sensitive to drying out. However, whereas Troyer citrange and species of *Poncirus* may retain their seed moisture for longer owing to the nature of their seed coats and their suspected adaptation to periods of post-extraction stratification, Empress mandarin is rapidly affected by desiccation. This points to the importance of moisture retentive soils being used in seedbeds or alternatively to the use of mulches or sun protection devices to prevent the rapid drying-out of seedbed soils. Care should be taken not to plant Empress mandarin seeds too shallowly and a minimum depth of 15mm is advocated.

Explain how

No exp. evidence for this

While being sensitive to drying out, Empress mandarin seed also showed itself to have an optimum germination temperature of $\pm 30^{\circ}\text{C}$. To create conditions under which both high humidity and high temperatures can best be satisfied, the use of plastic canopies to enclose seedbeds is suggested. Here, moisture is trapped beneath the canopy so permitting soil moisture levels to remain high and even. At the same time a build-up of heat is able to occur to within the optimum range (Hall & Besemer, 1972). On the strength of this supposition, plastic tunnels about 750mm in height have already come into successful use by certain nurserymen in the cooler production areas (Rustenburg, E. Cape), though there is a great need for further research work to be conducted in this specific field.

? The modification of environment by plastic tunnels or other means should enable nurserymen to produce dense batches of strong seedlings at a time of year that is normally unfavourable. The action of the environment upon seed germination probably takes place through changes in specific enzyme systems which are regulated by growth regulating substances (Amen, 1968). There seemed scope for a study to be made of the role these regulators play and the extent to which their specific levels are linked to seed germinability at different times of the year and at different stages of seed maturity. This is reported on in Chapter II.

these

CHAPTER II

VARIATIONS IN ENDOGENOUS GIBBERELLIN-LIKE
SUBSTANCES IN DEVELOPING EMPRESS MANDARIN SEEDS

2.1. INTRODUCTION

Gibberellins are an essential part of the hormonal system regulating plant growth and have been found in the seeds of many higher plants (Stowe & Yamaki, 1957; Phinney & West, 1960; Adler, Medwick, Johl & Manzelli, 1961; Weaver, 1961; Crane, ~~3, 6~~, 1964; Paleg, 1965).

Changes in the levels of gibberellin-like substances during the ontogeny of seeds are also well documented (Skene & Carr, 1961; Corcoran & Phinney, 1962; Hashimoto & Rappaport, 1963; Ogawa, 1963a; Ogawa, 1963b; Baldev, Lang & Agatep, 1965; Hashimoto & Rappaport, 1966). Gibberellins are able to terminate rest in the seeds of many species (Ikuma & Thimann, 1963; Lang, 1965; Weaver, 1972) and it has been proposed by Wareing & Saunders (1968) that this is achieved by two distinct modes of action, viz. as a reductant for releasing latent hydrolytic enzymes and as an initiator of enzyme synthesis presumably via RNA control. Van Overbeek (1966), suggests that gibberellins perform the dual role of providing soluble food for embryo development and weakening integument restrictions.

Gibberellins have been isolated from various tissues in a wide range of citrus species (Sumiki & Kawarada, 1961; Khalifah, 1965; Monselise & Goldschmidt, 1967; De Lange, 1969) and the exogenous application of gibberellic acid to citrus fruit for a variety of reasons has become a popular practice in many countries (Lewis, Coggins, Labanauskas &

Dugger, 1967; Coggins, 1969; Coggins, Platt & Opitz, 1969; Goldschmidt & Eilati, 1970; Turner, 1972; Gilfillan, 1976).

In view of the above, and the influence pre-germination gibberellin seed soaks were found to have towards hastening germination of certain citrus seed cultivars (Burns & Coggins, 1969; Button, Bornman & Hackland, 1971; Achituv & Mendel, 1973; Eshuys, 1975), it was decided that an investigation into the gibberellin-like substances present in Empress mandarin seed should be conducted. More specifically, quantitative estimates of GA content at different stages of seed maturation were considered likely to shed light onto some of its supposedly erratic germination characteristics. By so doing, it was hoped that some link would be found between the germinability of the seed and its content of GA-like substances at that time.

Attempts were made to extract GA-like substances by standard techniques and to estimate the amounts present by photofluorometric and bioassay methods.

Photofluorimetry has been effectively used by previous workers to make quantitative determinations of gibberellins in plant tissue (Elson, Jones, MacMillan & Suter, 1964; Gordon & Pankratz, 1968; Wiltbank & Krezdorn, 1969; de Lange, 1974) although difficulty is generally experienced in obtaining an extract pure enough to be free of interfering substances (Coombe, 1975).

Intact plants, plant parts and seeds have been used in bioassays of gibberellins and gibberellin-like substances. Some bioassays are based on the ability of gibberellins to overcome light-imposed growth reduction in stems. These include the rice seedling (Murakami, 1957),

cucumber hypocotyl (Brian & Hemming, 1961), dwarf pea (McComb & Carr, 1958) and lettuce hypocotyl (Frankland & Wareing, 1960). In certain single-gene dwarf mutants the plant is unable to biosynthesize endogenous gibberellins. Such plants are good monitors of applied GA and include the dwarf maize (Neely & Phinney, 1957) and the dwarf bean (Brian, Hemming & Lowe, 1964). Two popular bioassays which are based on gibberellin-stimulated production of alpha-amylase in germinating seeds are the rice endosperm (Ogawa, 1963a) and the barley endosperm (Paleg, 1961; Paleg, Coombe & Buttrose, 1962). According to Coombe (1975), if low quantities of GA are present the Rumex leaf bioassay is best to use, but the barley endosperm is recommended above all if routine analysis is to be conducted. It is also a bioassay sensitive to a broad range of active gibberellins (Coombe, Cohen & Paleg, 1967). On account of this, it was decided to use the latter bioassay in these determinations. The dwarf pea bioassay was also used, in part because of the local availability of the recommended indicator cultivar selections.

2.2. GENERAL METHODS AND MATERIALS

2.2.1. Selection and preparation of plant material

The Empress mandarin fruit from which seeds were extracted, were harvested at monthly intervals commencing in February 1974 from three healthy uniform trees at H.L. Hall & Sons. Immediately after harvest, seeds were extracted from the fruit, washed thoroughly in running tap water and stored in a deep freeze at -25°C until being analysed.

where the GA not washed out

2.2.2. Extraction and Separation

For fluorometric analysis the extraction and separation methods perfected by Wiltbank (1967) were used with a few minor modifications. At each analysis a 5g seed sample was homogenised for 3 minutes in acetone and water and centrifuged at 10 000 rpm for one minute. The supernatant extract was decanted and the homogenate twice more agitated with 10ml acetone. Acetone in the combined extract was removed under vacuum. The aqueous extract was acidified to pH 2,5 with 25 per cent. sulphuric acid and extracted three times with 10ml portions of pH7 buffer. The pH of the cloudy lower aqueous solution was reduced to 2,5 and extracted three times with ethyl acetate and the supernatant acetate fraction evaporated to dryness.

The acetate sample residue was redissolved in 0,5ml ethyl acetate and streaked 2cm from the bottom of a Kieselguhr silica gel TLC plate. The plate was developed using the solvent system di-iso-propyl ether: acetic acid, 95:5. Gibberellin was detected by spraying with 90 per cent. ethanolic sulphuric acid, heating for 5 minutes at 100°C and viewing under longwave ultraviolet light (365 mμ).

The R_f of standard GA₃ determined in this solvent system was found to be 0,13. For determination on the photofluorometer, the chromatogram was cut into strips and the silica gel scraped from each strip into fritted glass funnels where they were eluted with 2:1 chloroform:methanol. The eluates were dried under vacuum in test tubes. Two mls H₂O was added to each tube and cooled for 5 minutes in an ice bath. Ten ml chilled H₂SO₄ was added to each tube which was then placed on ice for a further 10 minutes before being removed and allowed to remain overnight at room temperature prior to the determination of its

relative fluorescence intensity.

Different extraction methods were used for the two bioassays. For the barley endosperm bioassay the extraction methods described by Monselise & Goldschmidt (1967) were followed. Homogenised seed was extracted with 80 per cent. methanol and evaporated under vacuum. The water residue of crude extract was partitioned into three other fractions; a neutral fraction (fraction I), a mildly acidic fraction (fraction II) and an acidic fraction (fraction III). All fractions were concentrated *in vacuo*, streaked on Whatman 3MM filter paper and chromatographed in an ascending system using isopropanol:ammonia:water (80,0:0,1:19,9). An amount corresponding to 0,5g fresh material was applied to chromatographs. After development TLC strips were cut then divided into 3 equal portions and placed in incubation flasks containing endosperm segments.

On the recommendation of Coombe (1975) it was decided that an attempt should be made to determine gibberellins in the butanol fraction of seed extract. Accordingly the method described by Hashimoto & Rappaport (1966) was used for GA separation and determination using the dwarf pea bioassay. The seed sample was macerated in methanol and the water phase adjusted to pH 7,5 with NaHCO_3 and extracted 3 times with equal volumes of ethyl acetate to give the neutral ethyl acetate fraction. The remaining water phase was adjusted to pH 3,0 with dilute H_3PO_4 and shaken 3 times with ethyl acetate to give the acidic ethyl acetate fraction. The remaining water phase was further extracted with n-butanol giving acidic butanol fraction. The acidic butanol and acidic ethyl acetate fractions were streaked on Whatman No. 3 MM paper and chromatographed using iso-propyl alcohol-ammonium-hydroxide-water 10:1:1 as the developing solvent.

2.2.3. Sensitivity of spectrophotofluorometer to GA

All determinations of relative fluorescence intensity (RFI) were made using the F.O.C.I. Mark II spectrophotofluorometer. RFI of standard GA solutions was determined in order to establish the sensitivity of the instrument to gibberellic acid in the absence of interfering substances. A standard GA solution was prepared by dissolving 2mg GA in 2ml ethyl acetate. From this standard, an aqueous solution containing 120 µg GA per ml was prepared. Dilutions were made of the aqueous solution to obtain final concentrations from 0,06ppm to 6ppm. One ml of the aqueous solution was placed in a test tube and cooled for 5 minutes in an ice bath. For the control, ~~one~~ ml of distilled water was placed in the test tube. Five ml of cold 85 per cent. sulphuric acid was then added to each test tube and the contents thoroughly mixed. The test tubes remained in the ice bath for 10 minutes after mixing. After standing at room temperature for one hour, RFI of each GA concentration was determined on the spectrophotofluorometer (Fig. 2.1).

2.2.4. Bioassay techniques

2.2.4.1. Barley endosperm bioassay. The discovery by Paleg (1960) of the effects of GA upon amylase activity and the release of reducing sugars from respiring barley endosperms was followed by an investigation into the parameters of the response system by Coombe *et al.* (1967). It was shown that variations in varieties, techniques, additives, conditions and even gibberellins confirmed the stability, sensitivity and adaptability of the hormone-induced response thereby emphasizing its utility as a GA bioassay. In 1967, Chrispeels & Varner showed that GA does not merely trigger amylase synthesis but is

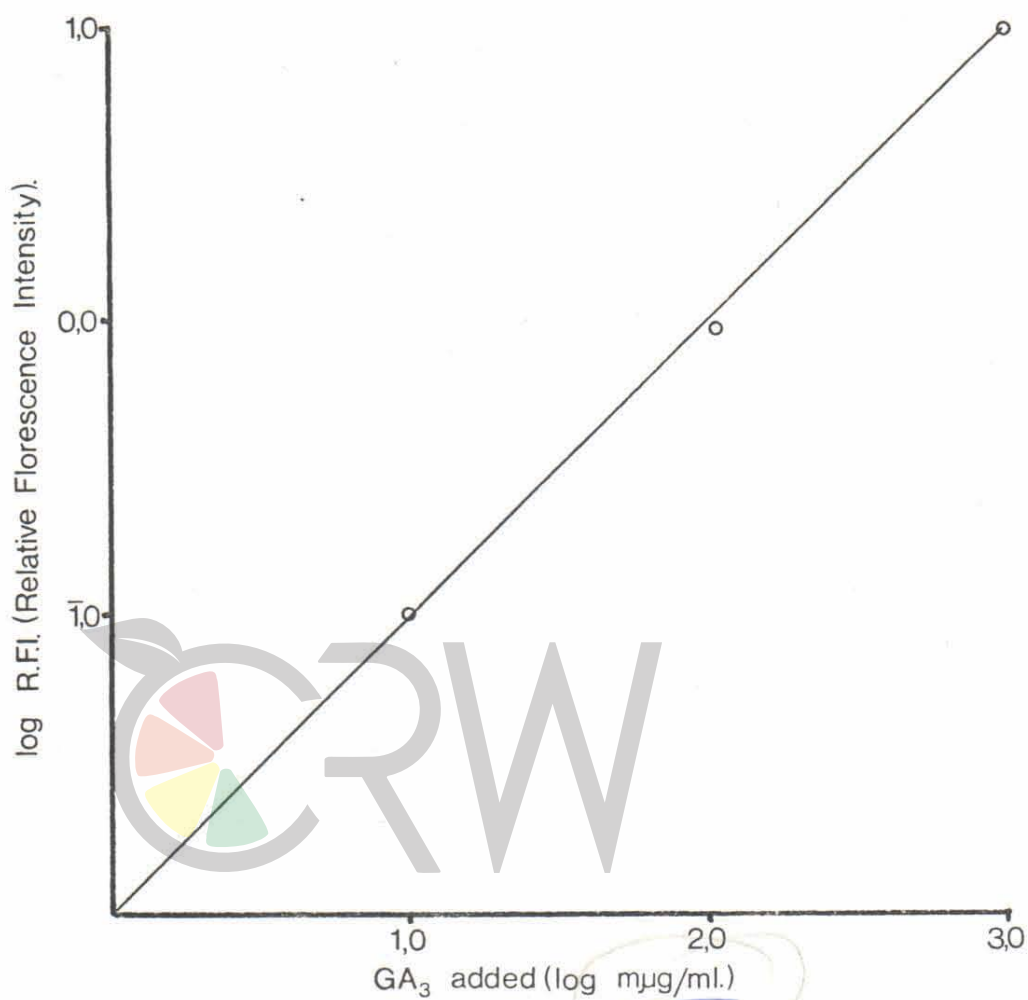


FIG. 2.1 Changes in RFI through increases in GA₃ concentrations (standard curve)

What is this

continuously required during the period of enzyme formation. More recently, McNeil, Albersheim, Taiz & Jones (1975) discovered that the walls of aleurone cells which are composed of 2 major polysaccharides, arabinoxylan (85 per cent.) and cellulose (8 per cent.), are degraded when the isolated tissue is treated with GA. Since the wall may be a barrier to the release of enzymes this may suggest the mechanism by which GA application initiates enzyme activity and endosperm starch hydrolysis.

Use in these investigations was made of a local huskless barley cultivar, Kaalgars. The bioassay methods of Coombe *et al.* (1967) were followed with a few small variations. To minimize contamination by micro-organisms which can greatly increase the level of reducing sugars found after incubation, certain precautions were followed. Barley seeds were soaked for 2 hours in 5 per cent. hypochlorite and then rinsed in running tap water. It was decided to determine whether shorter rinsing times than the 20 hours recommended would not render the test more sensitive by reducing the opportunity for endogenous gibberellins to move from the embryo to the endosperm. Accordingly various rinsing periods were evaluated in conjunction with tests made to find optimum incubation periods.

Rubish.
Sterilized and washed seeds were cut in two about 4mm from the distal end thus separating the embryo from ^{most of} the endosperm. Three endosperm segments were placed in each 12ml incubation flask together with 2ml deionised water and the paper chromatography strips.

After incubation total reducing sugars were measured by the degree of colour development of test solutions after their treatment with Somogyi (1952) and Nelson (1944) reagents according to the procedures

Not a ref. but (1964)

prescribed by Pateg (1960) and slightly modified by ^D de Lange (1969).
 Colour intensity was determined at 750 ^{nm} on a spectrophotometer.

Sugar determinations were also made of standard GA solutions (instead of seed extracts) taken through the entire procedure and of standard glucose solutions (see Figs. 2.7a & b). These values enabled the concentration of extracted GA to be established.

2.2.4.2. Dwarf pea bioassay. For this bioassay developed and used by Brian & Hemming (1955) and McComb & Carr (1958), use was made of a local dwarf pea cultivar, Onward which had already proved successful in work done by ^D de Lange (1969). The seed was soaked for 24 hours in running tap water and sown in trays 24 X 17 X 6cm containing a mix of equal parts commercial peat and vermiculite. Germination was allowed to proceed for 4 days in total darkness. After 7 days all abnormal seedlings were removed to provide a uniform stand. At 9 days, 20 μ l of the test solution containing 0.1% Tween 20 surfactant was applied to the base of the leaf subtending the third node. At this time each plant had about 3 nodes. Response was measured by the distance between the 3rd and 6th nodes 6 days later.

2.3. EXPERIMENTS, RESULTS AND DISCUSSION

2.3.1. Photofluorometric Determinations

EXPERIMENT 11 Effect of thorough glassware cleansing on the amount of impurities in the final extract

Introduction Initially the high degree of variation in RFI values of seed extracts and the apparent lack of correlation between GA Rf's

determined under UV light and those determined on the photofluorometer indicated impurities in the extract which could have been derived from inadequate extraction technique or from contamination of glassware by fluorescing substances. Accordingly an investigation was made into the effect of glassware impurities upon results.

Procedure (a) One blank was taken through the entire extraction procedure and chromatographed. The chromatogram was divided into 9 equal strips each of which was eluted. A second blank was made up of chloroform:methanol eluates of 9 clean strips of silica gel scraped from an unstreaked chromatogram the same widths as those above. Readings on the instrument were based on a blank consisting of 2ml deionised water and 10ml 85 per cent. sulphuric acid.

(b) A sample of immature Empress mandarin seed was divided in two groups. Half the seeds were both extracted and eluted in glassware washed in the normal way with soap. The other half was purified and eluted in glassware thoroughly washed with hexane and methanol.

Results and discussion (a) As indicated by Fig. 2.2, TLC strips of the blank which underwent the entire extraction procedure (Fig. 2.2a) gave higher fluorescence than TLC strips of the blank derived directly from the chromatogram (Fig. 2.2b). It seems likely therefore that when taken through the entire extraction procedure, fluorescing substances present in glassware washed in the normal way do contaminate the extract to some extent.

(b) In Figs. 2.2c & d the influence of glassware washing upon RFI is further illustrated. There is

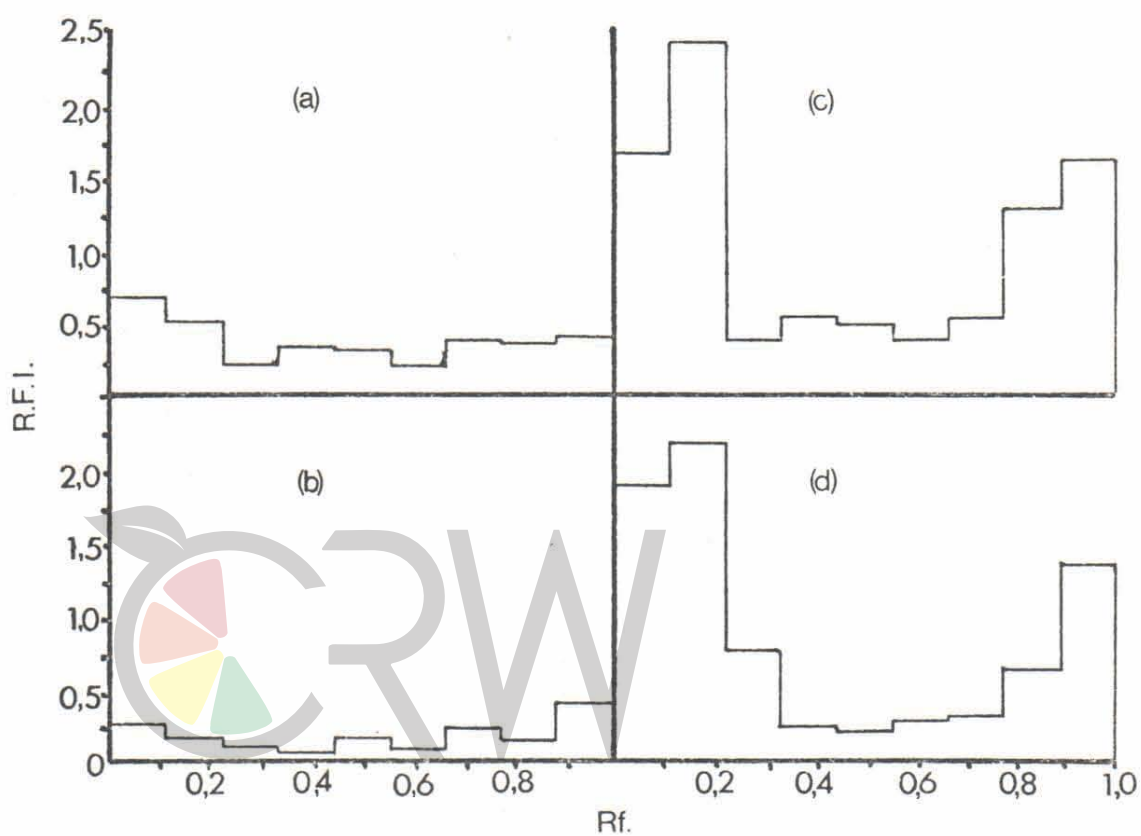


FIG. 2.2 The effect of a thorough washing of glassware with hexane and methanol (b & d) upon the RFI of blanks (a & b) and extracts (c & d)

fluorescence in almost all the segments derived from extraction in hexane/methanol-cleaned glassware.

As a consequence of results obtained in this experiment care was taken in all future determinations to use only glassware well-washed as recommended. Furthermore, because it was found difficult to completely eliminate impurities, it was decided that for future determinations the instrument would be zeroed on an eluted blank which had undergone the whole extraction procedure. In this way, RFI values registered on the photofluorometer would be unaffected by those impurities which even thorough cleansing was unable to eliminate.

EXPERIMENT 12 Determination of gibberellin-like compounds in
immature Empress mandarin seed by photofluorometry

Does sound as if it was still not good enough

Introduction Having reduced the effect of glassware contamination as much as possible attempts were made to determine the RFI levels of GA₃-like compounds in immature Empress mandarin seed. Results of this experiment would determine the reliability of the technique for tracing changes in the levels of GA-like substances during seed growth.

Procedure Immature Empress mandarin seed was extracted from fruit harvested in February 1974. After purification the GA-containing residue was redissolved in 1ml ethyl acetate. This was divided into two equal portions and amounts corresponding to 2.5g seed streaked on each of 2 silica gel TLC plates which were developed to 12cm in di-isopropyl ether, acetic acid 95:5 and cut into ¹²twelve strips of equal width commencing below the origin so that the first strip constituted a blank. Each strip was eluted and then the RFI's of the eluates determined.

Results and Discussion In Fig. 2.3a the histogram of averaged RFI values of the 24 strips is presented. An RFI value of 1,57 which according to the standard curve represents a concentration of $33 \text{ m } \mu\text{g GA mL}^{-1}$ was given in the same Rf zone (0,13) as the standard GA_3 when observed under UV light. This compares interestingly with the range of 60-160 $\text{m } \mu\text{g GA g}^{-1}$ fresh mass Wiltbank (1969) obtained from the ovaries and young fruits of Navel oranges, and suggests the possibility that a portion of the GA present in young developing fruit up to about 35mm diameter is located in the immature seeds. This may well apply to Empress mandarin as well.

Difficult to explain is the fluorescent activity of substances located near the TLC front. The indications were that this fluorescence was caused by a compound or compounds much like GA in respect of their chemical structure but not necessarily so in terms of bioactivity. To confirm the fluorescent activity of compounds in this zone, its excitation spectrum was conducted against a standard GA_3 solution of $6 \text{ m } \mu\text{g mL}^{-1}$. The result is presented in Fig. 2.3b. The wavelength of optimum excitation of both compounds was found to be practically identical (462 nm), this confirming some similarity in their molecular behaviour. Attempts to determine the bioactivity of these high Rf compounds were carried out by means of the dwarf pea bioassay on subsequent seed extracts, this being reported under Experiment 17.

A disturbing aspect of this GA determination was the high degree of impurities in the extract. Throughout the chromatogram, compounds were present which gave varying degrees of fluorescence and cast doubt upon the efficacy of the purification technique used. The high RFI values corresponding with the position of GA_3 on the TLC cannot be considered very accurate because of the interfering substances which

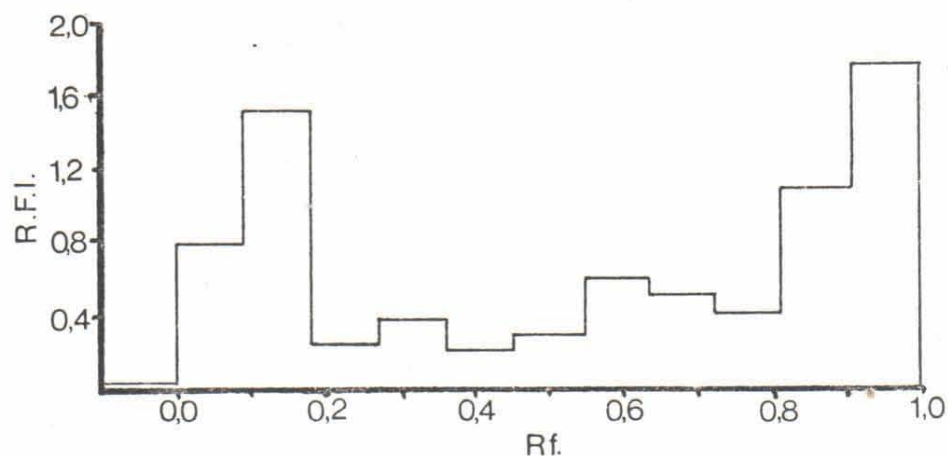


FIG. 2.3a RFI response of immature Empress mandarin seed extract after development in di-iso-propyl ether:acetic acid, 95:5 solvent on a Kieselguhr silica gel TLC plate

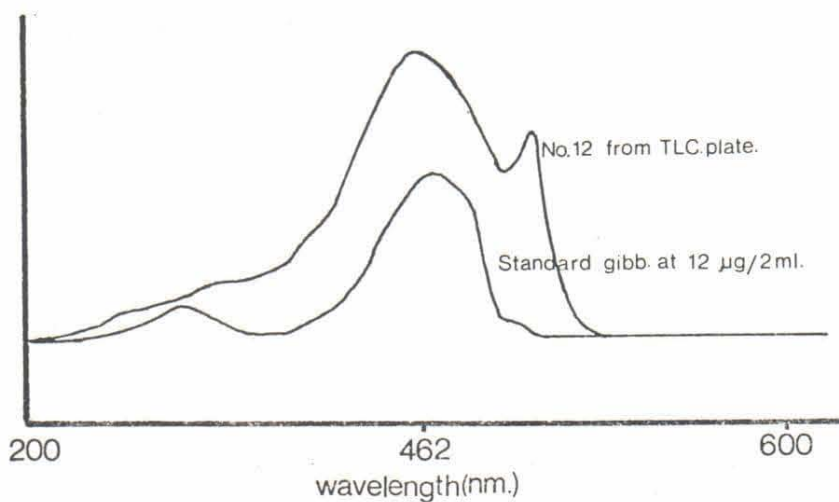


FIG. 2.3b Excitation spectrum of the compounds present in the frontal zone of the TLC plate in Fig. 2.3a in comparison to that of a standard GA_3 solution

were present.

*This leaching the wash not
of very high value*

The results obtained in the early experiments confirmed the conclusion of Coombe (1975) that photofluorometry is inadequate for qualitative GA determination and only useful quantitatively providing a high degree of purity is achieved in the extract. Citrus seed, which like the seeds of most species, is a concentrated storehouse of many organic compounds in various chemical states did not appear suited to determinations of GA by photofluorometry owing to difficulty in extracting pure GA. In view of the availability of other techniques for the quantitative analysis of GA it was decided that attention should rather be turned to bioassay methods.

2.3.2. Bioassays

EXPERIMENT 13 Effect of variations in rinsing and incubation times upon the level of GA-like compounds detected by the barley endosperm bioassay

Introduction After the sterilisation of barley seeds in 5 per cent. hypochlorite a period of seed rinsing follows. Rinsing time is fairly critical because if carried out for too long, endogenous gibberellins may prematurely be lost by moving from the embryo to endosperm. On the other hand soaking or leaching for too short a time can reduce the sensitivity of the test. Coombe *et al.* (1967) recommended a 20 hour rinsing period which if shortened could improve the efficiency of the bioassay.

Incubation periods are also critical. Until hydrolases are secreted some 8-10 hours after the addition of GA₃, an aleurone membrane

proliferation occurs by the action of two enzymes, phosphorylcholine-cytidyl and phorylcholine glyceride transferases, within minutes of GA₃ administration (Yomo & Varner, 1971; Ben-Tal & Varner, 1974). The process of sugar release effectively commences shortly after GA administration which poses a question whether shorter incubation periods than the hours recommended could not be just as effective.

Procedure After being sterilised for 2 hours in 5 per cent. hypochlorite the barley seeds were split into three groups which were subjected to 5, 10 and 20 hour rinsing periods respectively. Thereafter standard GA₃ solutions at 10^{-9} , 10^{-10} and a blank were administered to each group of seeds which were incubated in flasks for 10, 20 and 30 hours as shown in Table 2.1.

TABLE 2.1 Experimental design to determine the influence of seed rinsing and incubation periods at 3 levels of GA₃ upon the release of reducing sugars from Empress mandarin seed

		Rinsing time (hours)		
		5	10	20
Time of incubation (hours)	10	0 10^{-10} 10^{-9}	0 10^{-10} 10^{-9}	0 10^{-10} 10^{-9}
	20	0 10^{-10} 10^{-9}	0 10^{-10} 10^{-9}	0 10^{-10} 10^{-9}
	30	0 10^{-10} 10^{-9}	0 10^{-10} 10^{-9}	0 10^{-10} 10^{-9}

Results and Discussion It is evident from Fig. 2.4a, b & c that the 20 hour incubation period produced 10 to 20 times more sugar than the 10 hour period. This leads to the speculation that depending on the rate and manner in which sugar is released during the 10-20 hour period, there may be a time, e.g. 16 hours, at which the test is most sensitive. This would be so only if the glucose release pattern of GA-containing and control solutions gave the type of curves given in Fig. 2.5. It is possible however that both curves would be influenced in the same way by incubation period in which case either the 10 or 20 hour periods would be equally accurate. Figs. 2.4a, b & c show that there is no advantage to be gained from incubating for longer than 20 hours since there is little increase in the concentration of reducing sugars beyond this time. The increase which is observed may be due to the contamination of flasks by sugar producing fungi. This is borne out by Fig. 2.4c where the greatest increase in sugar occurred in the control flask. It is also of interest that there was a lower reducing sugar level in flasks containing seeds washed for ¹⁰ten hours than those washed for ⁵five hours (Fig. 2.4a & b). An explanation is offered that the 10 hour rinse removed (by leaching) a greater amount of free sugars present in the endosperm than the 5 hour rinse. Thus, in the case of the 5 hour rinse the reducing sugars measured after incubation did not reflect only those sugars released as a result of the exogenous GA.

The increased levels of glucose measured after the 20 hour rinse is evidence that the second stage of germination (Hartmann & Kester, 1968) had commenced. However, De Lange (1969) had shown that to achieve greatest sensitivity rinsing should be extended for as long as possible. It was thus decided that for all future determinations the 20 hour period should be slightly shortened to 19 hours.

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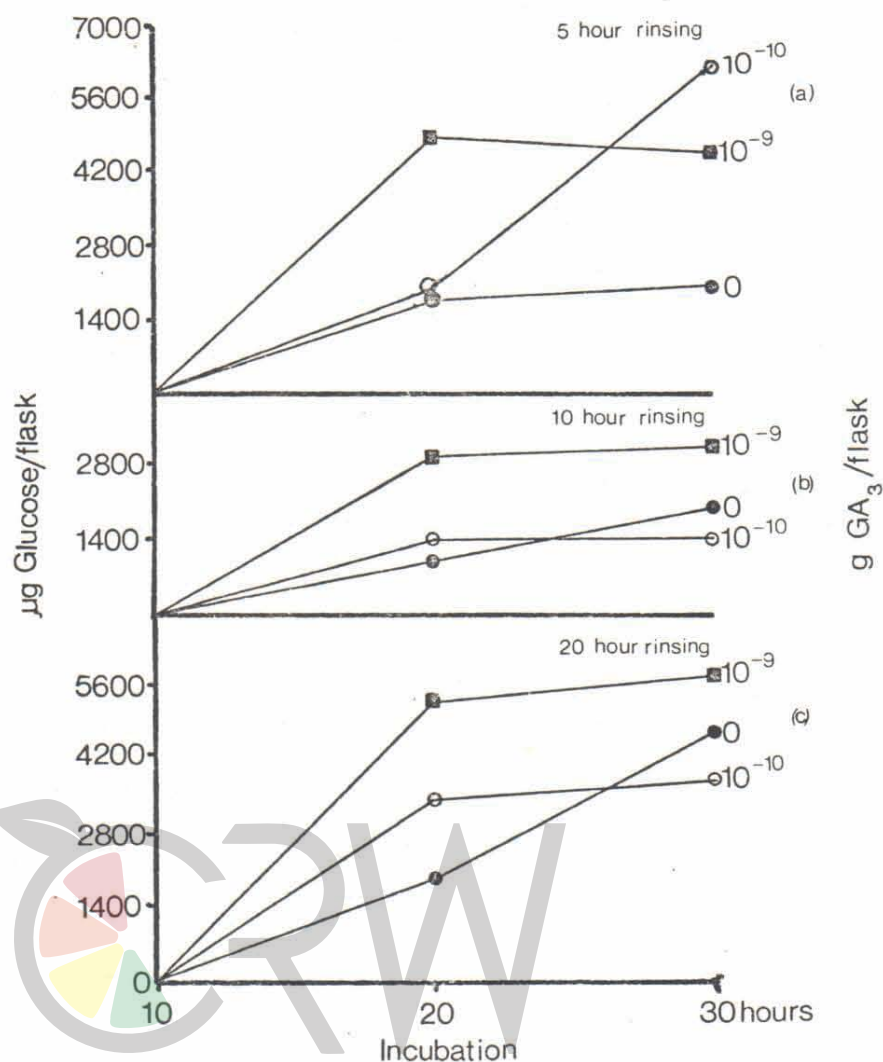


FIG. 2.4 Effect of various rinsing and incubation times on the amount of glucose produced by excised barley endosperms in the presence of 3 levels of GA_3

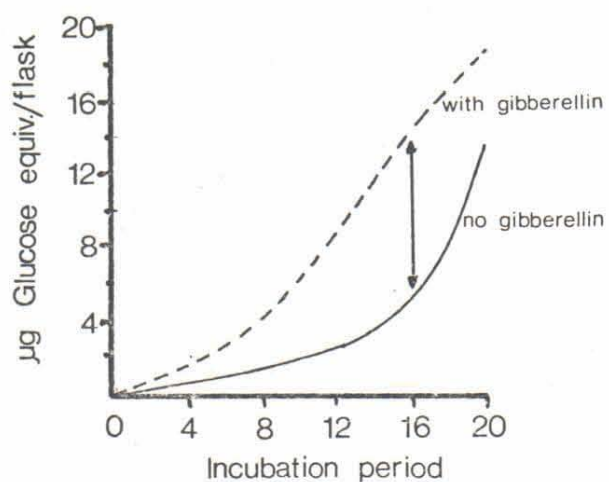


FIG. 2.5 Proposed glucose release patterns in flasks containing excised barley endosperms with and without GA added

EXPERIMENT 14 Determination of GA-like substances in 3 fractions
of Empress mandarin seeds by the barley
endosperm bioassay

Introduction According to Monselise & Goldschmidt (1967) GA extracted from young flushing lemon branchlets was found in greatest amounts in the acid etheric fraction. Since it was not known in which fraction gibberellin-like substances extracted from seed would appear and because it would be a laborious task to isolate and assay all fractions in all future determinations this trial was deemed necessary.

Procedure The extraction method of Monselise & Goldschmidt (1967) was conducted on 5g immature Empress mandarin seed. Each fraction was tested for GA-activity by the barley endosperm bioassay.

Results and Discussion It is apparent from Fig. 2.6 that although some activity was evident in fractions I & II, the greatest amount of sugar was measured in the Rf 0,7 to 0,8 range of the acid etheric fraction. The Rf range in which this activity was found corresponded to that of standard GA₃ developed in the same system as described in the General Method.

While this cannot be looked upon as a factor confirming that the acid etheric fraction contains GA₃, it does show that in comparison to the other fractions, the acid etheric fraction is most likely to yield GA-like compounds.

The average amount of GA-like compound found in the acid etheric fraction corresponded to about 6×10^{-11} g GA per flask (see standard

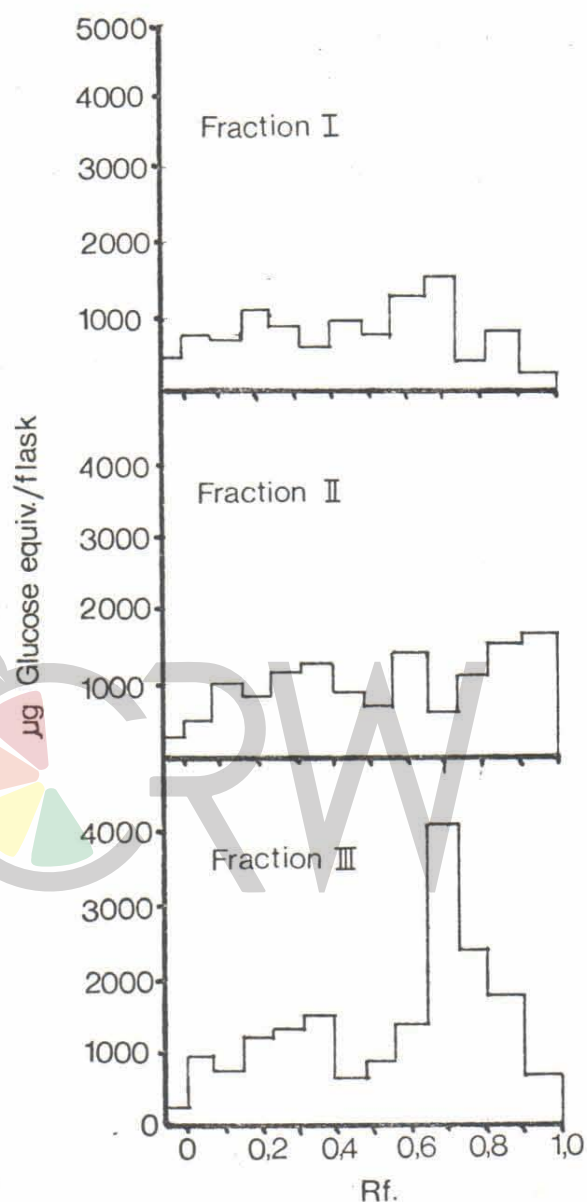


FIG. 2.6 Sugar release measured in flasks containing excised barley endosperms to which 3 fractions of extracted Empress mandarin seed were added

curves Fig. 2.7a & b) which is much lower than the 8×10^{-6} g per 1g fresh seed obtained by photofluorometry and suggests that the values obtained for the latter method were boosted by the presence of impurities in the extract.

EXPERIMENT 15 Changes in the amounts of GA-like substances present in the acid etheric fraction of developing Empress mandarin seeds by the barley endosperm bioassay

Introduction Having determined that the acid etheric fraction provided an index of gibberellin-like substances present in extracted seed, it was decided that this technique should be used for the routine analysis of gibberellin-like substances in the maturing seed. It was hoped that the changes in GA content of the seeds would shed some light on the germination characteristics of Empress mandarin seed.

Procedure Routine analysis as described earlier was conducted on 5g seed samples extracted from fruit harvested at monthly intervals. Only the acid etheric fraction was tested for bioactivity by the barley endosperm bioassay. Analysis was successfully completed on seed of fruit harvested in March or April, and values obtained in the previous experiment were used for the immature February-harvested fruit. At each analysis chromatogram strips were cut into 3 equal portions to serve as replicates.

Results and Discussion As shown by Fig. 2.8, a reduction in the concentration of GA-like substances occurred as the seed matured. Because the slope of the curve between the first two points could not be accurately determined, it is impossible to know how rapidly the GA

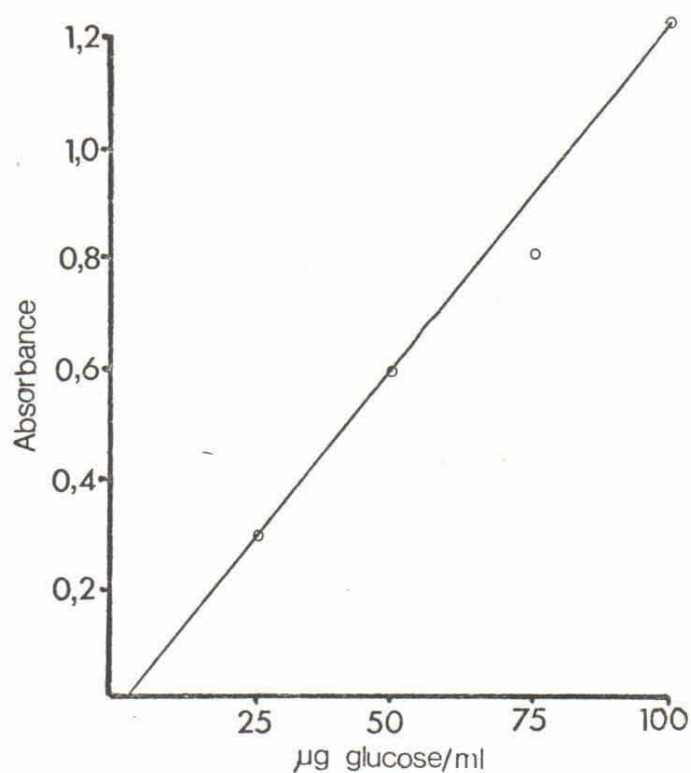


FIG. 2.7a Relationship between absorbance and glucose concentration measured by the arseno-molibdate colorimetric determination

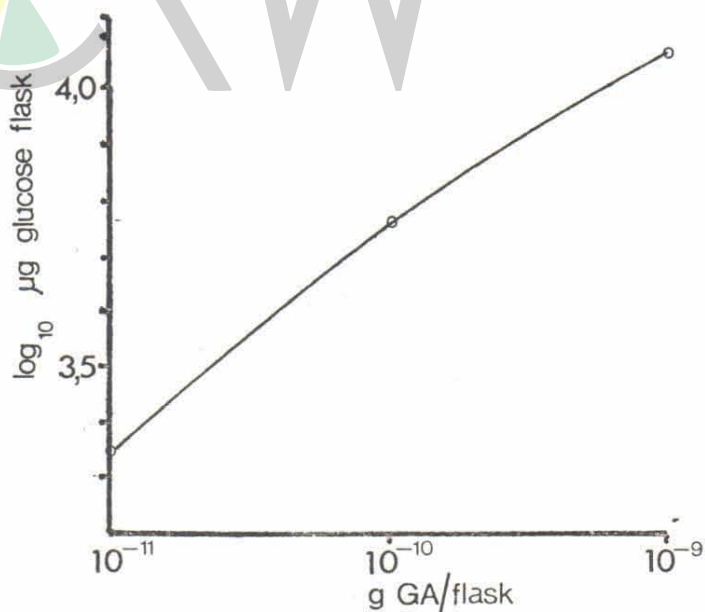


FIG. 2.7b The influence of various concentrations of GA_3 upon the production of reducing sugars by the barley endosperm tissue



FIG. 2.8 Changes in the content of GA-like substances in developing Empress mandarin seed determined by the barley endosperm bioassay

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concentration dropped in the maturing seed. However, the most significant aspect of this experiment was the relationship between the drop in GA-substances and the acquisition of seed viability as previously demonstrated (Experiment 1) during this period (March-April). A possible explanation is the conversion of free GA to bound GA as seeds approached maturity.

EXPERIMENT 16 Determination of the chemical fraction in which most free GA-like compounds extracted from immature Empress mandarin exist for analysis by the dwarf pea bioassay

Introduction In an attempt to reinforce the data obtained by the barley endosperm bioassay use was made of Hashimoto & Rappaport's (1966) extraction technique as recommended by Coombe (1975) and the dwarf pea bioassay. The first experiment was conducted in order to determine the fraction in which most free gibberellins were present.

Procedure Seeds of fruit harvested in February were extracted and separated as described in general methods. The eluates from R_f zones of the chromatograms produced from extracts of 5g seed were applied to each pea plant.

Results and Discussion At various zones on the TLC plate mild activity was registered in the acidic ethyl acetate fraction (Fig. 2.10). In the acidic butanol fraction elongation of pea plants corresponding to values exceeding 0,008 µg GA were registered (see standard curve, Fig. 2.9). According to Coombe (1975), this fraction contains the very polar free gibberellins and the acidic glucose ethers. By the extraction methods used for the barley endosperm bioassay these gibberellins may not have been measureable.

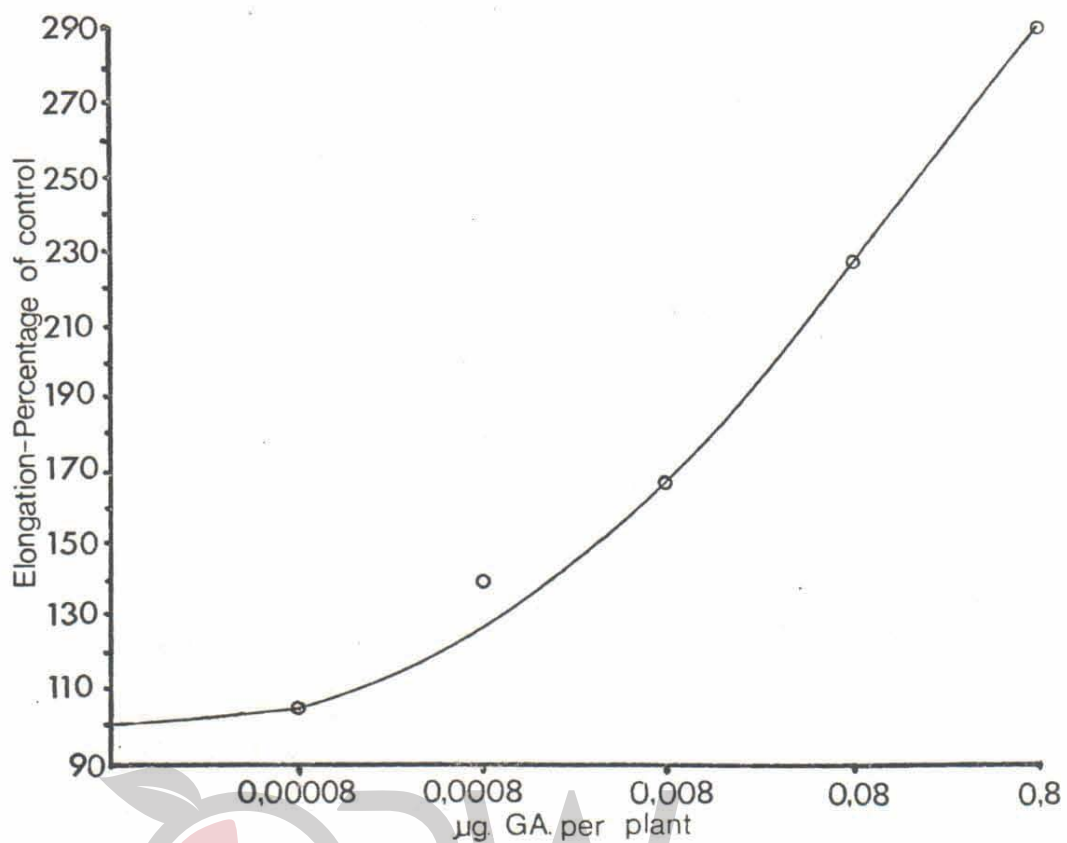


FIG. 2.9 The influence of GA_3 upon the stem elongation of dwarf pea seedlings

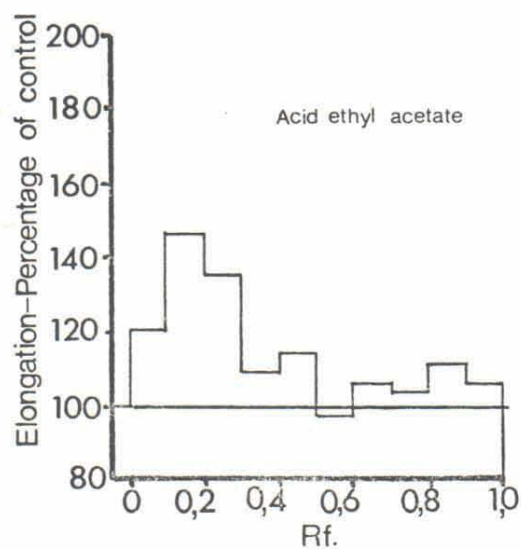


FIG. 2.10 The stimulation of dwarf pea stem elongation by compounds present in various TLC zones of the acid ethyl acetate fraction of immature Empress mandarin seeds

EXPERIMENT 17

Determination by the dwarf pea bioassay of the bioactivity of the TLC segment found to give an excitation spectrum similar to that of standard GA

Introduction In Experiment 2 it was found that a substance, GA-like in its fluorescence characteristics, but located at the frontal zone of the chromatograph gave the same excitation-spectrum as a GA₃. The dwarf pea bioassay was considered a reliable way of determining the biological activity, if any, of compounds in this zone.

Procedure The acidic ethyl acetate fraction of GA-like substances were extracted from mature Empress mandarin seed and chromatographed and eluted as already described under 2.2.2. The evaporated eluates of each chromatogram strip were redissolved in 1ml water containing 0,5 per cent. Tween 20. Twenty μ l of the extract so derived from each of the 10 strips were administered to each of ^{five} 5 pea plants for bioassay.

Results and Discussion The R_f zone which showed the same excitation spectrum as GA, did not possess biological activity as determined by the bioassay. While some biological activity is present in the R_f zone corresponding to that of the GA standard, there is no evidence of such activity at the TLC frontal zone. Thus the method used to isolate gibberellin does not appear to have delivered a sufficiently pure extract for fluorometric determination as the presence of interfering substances prevents an accurate quantitative estimate being made.

EXPERIMENT 18

To determine differences in the amount of GA-like compounds present in the acidic butanol fraction of immature and mature Empress mandarin seeds by the dwarf pea bioassay

Introduction

In Experiment 6 it was shown that gibberellin-like substances could be measured by the dwarf pea bioassay in the acid-butanol fraction according to the methods of Hashimoto & Rappaport (1966). It was thus thought possible that this method could be used to confirm the results showing the change in GA-like substances from immature to mature Empress mandarin seeds as already carried out by the barley endosperm bioassay.

Procedure

Mature seeds extracted from Empress mandarin fruit in mid-July were washed and surface dried. GA-like substances were determined in the acid-butanol fraction as described by Hashimoto & Rappaport (1966). GA activity was measured by the dwarf pea bioassay.

Results and Discussion

Stem elongation corresponded to a GA concentration of approximately 0,0008 μg per plant which is ten times less than the GA concentration of immature seed as illustrated in Fig. 2.8 (Experiment 6). This compares interestingly with the results obtained by the barley endosperm bioassay where levels of GA-like substances in immature and mature seeds were found to be considerably lower.

2.4. GENERAL DISCUSSION

Differences obtained in the results between the amount of GA-like compounds found present in immature and mature Empress mandarin seeds as

determined by the three separation and assay techniques were found to range from 8×10^{-6} to $1,8 \times 10^{-4} \mu\text{g GA g}^{-1}$ mass fresh seed. This could be attributed to differences in the content of GA-like substances in the various fractions extracted. It has been shown (Hashimoto & Rappaport, 1966) for example that the water phase, which is often discarded after the acid ethyl acetate fraction has been isolated, may contain a major portion of butanol soluble GA-like substances. Not all GA-like substances could possibly have been recovered from the seed by the techniques used because in seed, particularly when mature, gibberellins are often present in bound forms and the extraction procedures used in this investigation would not have measured these. Coombe (1975) suggests that alkaline hydrolysis followed by purification of free gibberellins could serve as a means of obtaining more meaningful results. Indeed McComb (1961), found that treating homogenates of mature pea seeds with ficin, a proteolytic enzyme, resulted in larger amounts of acidic ethyl acetate-soluble gibberellin-like substances than were present in extracts not treated with the enzyme.

From the findings of workers previously cited it seems acceptable that a reduction in the acidic ethyl-acetate soluble gibberellins could be expected as the seeds mature. Possibly these gibberellins are required for seed growth. The neutral substances, which were not tested owing to their very low activity in immature seed when assayed by the barley endosperm method, may possibly increase seed maturity. Future research in this direction should include analysis of the neutral fraction in mature seeds.

The factors which determine the age at which a developing seed reaches germinability for the first time, and which continue to act upon its rate of germination after seed maturation, are probably far more

complex than variances in the content of GA-like compounds in the seed. The drop in the amount of acidic ether-soluble GA-like substances in Empress mandarin seed between February and May does coincide with the period during which the seed reaches sufficient maturity to germinate, and may even play some role in it. The factors which prevent germination of seed in the fruit but which allow it to commence once the seed has been extracted, washed and planted in a suitable environment are most likely linked to shifts in the hormonal promotor-inhibitor balance. Here, most GA-like promotors are chemically bound and require some environmental trigger to be released. This may constitute the exchange of gases, the presence of light or the removal of an inhibiting chemical.

In any event, it is apparent from the investigations carried out that changes in the amounts of GA-like substances do occur during the ontogeny of Empress mandarin seed but that many factors apart from this probably contribute to its germinability. If future attempts are made to isolate and determine GA-like compounds in citrus seed, bioassay methods should be preferred to photofluorometric methods even if only quantitative data ^{are} required. Gas liquid chromatography may be the physical method most likely to succeed for routine measurement but according to Coombe (1975) the sensitivity is still expected to be a limitation. For a thorough investigation of changes in the content of GA-like substances in citrus seeds it is suggested that changes in the neutral, acid ethyl acetate and acid-butanol fractions be followed. Bound gibberellins and the interconversions from free to bound gibberellins will also constitute an important future study.

?
Why not
used

CHAPTER III

GROWTH ANALYSIS OF CITRUS SEEDLINGS

3.1. INTRODUCTION

Important citrus nursery operations such as transplanting and budding are most efficiently conducted on uniformly sized seedlings. For this reason, differences in seedling growth rate between the various common citrus rootstock cultivars can lead to significant increases in the cost of nursery tree production. In spite of this little work has been carried out to combine quantitative measures of the growth and photosynthetic characteristics of citrus and related species. Khairi & Hall (1976) used rates of carbon dioxide uptake to identify adaptive mechanisms and parent material for possible use in breeding programmes to improve the adaptation of citrus cultivars to unfavourable climates. However, growth analysis techniques have not yet been used for this purpose, nor used to any extent as a research tool for estimating the relative growth rates and photosynthetic capacities of citrus cultivars. The reasons for this probably lie in the difficulties of applying growth analysis methods to perennial and woody plants owing to their slow growth, their size and complexity, high degree of individual variation, and the large quantities required for increasing the precision of results.

Webber (1932) and later Singh & Ghosh (1965) described the variation in citrus seedling growth and its relation to rootstock selection. However, the first use of increases in the mass and leaf area of growing citrus seedlings as a measure of relative growth and photosynthetic efficiency was made by Monselise (1951). By then, such studies had

grading a size? etc.
large

Is this true

Further literature

All is cited by somebody else

Not in Ref. list.

70

been carried out for pure lines of field crops or annual horticultural plants by, *inter alia*, Gregory (1917); Briggs, Kidd & West (1920); Heath (1937) and Bolas, Melville & Selman (1938). Further growth analysis studies on citrus seedlings were conducted by Maggs & Alexander (1969) who over an 18 month period were able to show differences in absolute and relative growth rates and in the allometric growth of leaf, stem and thorn of three citrus rootstocks.

Investigations by the above workers have shown that the usefulness of growth analysis methods extends beyond establishing the criteria for defining seedling growth and the quantitative interrelations between plant parts. They also provide a means of comparing the assimilation rates of plants. To this end various models have been described for the daily potential photosynthesis of leaf canopies of certain plant species, using such parameters as incoming short-wave radiation, day length, light intensity, leaf area and mass gain (Vernon and Allison, 1963; de Wit, 1965; Harris, 1971; Patefield & Austin, 1971; Thornley, 1972).

In other cases photography and microscopy have been used as aids in accumulating growth analysis data (Powell & Vink, 1969).

The aim of the study described below was to investigate the influence of temperature upon the relative growth and net assimilation rates of three citrus rootstock seedlings, using as a guide the first of two analytical methods described by Radford (1967). It was also intended to use the growth analysis data to compare some quantitative interrelationships between plant parts.

Why not be framed in the review.

3.2. GENERAL METHODS AND MATERIALS

3.2.1. Raising of seedlings

Three citrus rootstock cultivars were selected on the basis of their commercial popularity and differential growth rates. *Citrus limon* Burm. f. cv rough lemon was compared with *Citrus reticulata* (Blanco) cv Empress mandarin and *Poncirus trifoliata* (L.) Raf. (Rubidoux), the latter hereafter referred to as trifoliolate. All seeds came from fruit harvested at H.L. Hall & Sons, Nelspruit on 4th April, 7th June and 28th June, 1973, respectively. Seeds were extracted from fruit of all three cultivars on 24th July, 1973 and stored moist in plastic bags at 5°C until used. *Treated with 8-hydroxyquinoline?*

Fifty seeds of each cultivar were planted into each of 9 flats containing an evenly proportioned steam sterilised sand, vermiculite, peat mix on 31st July, 1973. Three flats containing seeds of each cultivar were placed in each of three glasshouses held at constant day and night temperatures of 20, 25 and 30°C ^{35°C} $\pm 1^\circ\text{C}$. Seeds were kept moist and seedlings subsequently irrigated with water held at the same temperatures as the respective glasshouses.

On reaching a height of 25mm, 90 uniform seedlings of each cultivar were transplanted into 70 X 120mm black plastic sleeves on the dates given in Table 3.1. Genetic uniformity could not be guaranteed, but the high rate of seed polyembryony in the cultivars used served to reduce inherent seedling variability. The exterior of the sleeves were painted white to reduce temperature variation in the event of their exposure to sunlight. *Small!*

Why not selected for uniformity?

TABLE 3.1 Dates of seedling transplanting after seed germination in glasshouses at 20^o, 25^o and 30^oC constant temperature

Glasshouse temperature	Cultivar	Transplant Date
30 ^o C	Rough lemon	25-8-73
25 ^o C	Rough lemon	4-9-73
30 ^o C	Empress mandarin	5-9-73
30 ^o C	Trifoliate	6-9-73
25 ^o C	Empress mandarin	15-9-73
20 ^o C	Rough lemon	15-9-73
25 ^o C	Trifoliate	16-9-73
20 ^o C	Trifoliate	26-9-73
20 ^o C	Empress mandarin	27-9-73

Seedlings were transplanted into the same steam sterilised mix as that used for germination, but 750g of a 2:3:2 NPK fertiliser per cubic metre was included. Commencing on 18th September, half-strength Hoagland solution as prescribed by Bonner & Galston (1967) was applied to seedlings at two to three weekly intervals. On 17th October and 3rd December, 1973, each plant received about 120ml liquid fertiliser mix containing 0.51g NH₄NO₃; 0.31g KCl and 0.9g NH₄H₂PO₄ l⁻¹ in order to counter possible increases in soil pH owing to the Ca (NO₃)₂ applied in the Hoagland's solution (Nauer, Roistacher & Labanauskas, 1967). No nutrient deficiency symptoms were observed during subsequent growth.

Depending on their rate of growth and consequently on the size they attained, batches of seedlings were transplanted to larger plastic sleeves when necessary. *When was that?*

To overcome the effects of temperature gradients resulting from the regular exposure of certain seedlings to sunlight, the sleeves on the bench were rotated at regular intervals. *Such as? How often?*

Pesticide treatments were applied whenever necessary to ensure unimpaired leaf development during the growth period. *Such as?*

3.2.2. Sampling, measurements and definitions

Growth rates were measured by the serial harvest of 9 randomly selected seedlings of each cultivar/temperature combination at approximately three weekly intervals for the first 100 days after transplanting the first seedlings. One further harvest was conducted a further 114 days later.

When every treatment sampled on their respective days from planting? see p 72

At sampling the selected seedlings were removed from the sleeves and any adhering soil mix gently washed from the roots. All leaves were clipped off at the proximal end of the petioles. Roots and aerial plant parts were separated at a point on the stem corresponding to about 3mm below soil level. Seedling height was measured. Total

leaf area (A) was determined using a specially constructed apparatus

made by Paton Industries, S. Australia, and

working on the suction principle (Department of Pasture Science, *Reference?*

University of Natal).

How does this work.

The combined dry mass of stems and leaves (S_m) and roots (R_m) was determined after a 24 hour oven drying at 80°C (Monselise, 1951).

Total dry mass (M) was calculated from $S_m + R_m$.

Was this sufficient to ensure constant temp in an

The data so collected were used to determine absolute growth rates and shoot:root relationships. They were also used for the derivation of relative growth rates (RGR) and net assimilation rates (NAR) according to the equations developed by Blackman (1919), Fisher (1920), Williams (1946) and summarised by Radford (1967).

All cited by Radford?

In contrast to the absolute growth rates, RGR at an instant in time is the increase of plant material per unit of material per unit of time. Since it is not practical to make a continuous record of the changes of M and A , it is customary to measure these variates at a number of isolated times and then attempt to evaluate their average over the period of successive harvests. To determine the mean RGR ($\overline{RGR}$) the only necessary assumption is that M varies without discontinuity. Since data in this study fulfilled this requirement direct substitution into Fisher's equation was possible.

$$\overline{RGR} = \frac{\log_e M_2 - \log_e M_1}{(t_2 - t_1)} \dots\dots\dots (1),$$

where M_2 and M_1 = mean dry mass of 9 seedlings at t_2 and t_1 respectively.

The NAR is a measure of the plant's photosynthetic capacity at an instant in time and is defined as the increase in plant material ^{weight, volume} per unit of assimilatory material per unit of time. Mean NAR ($\overline{NAR}$) over a growth period can be calculated from the following formula providing the relationship between A and M is linear.

$$\overline{NAR} = \frac{M_2 - M_1}{A_2 - A_1} \cdot \frac{(\log_e A_2 - \log_e A_1)}{t_2 - t_1} \dots\dots\dots (2),$$

where A_2 and A_1 = mean leaf area, and M_2 and M_1 = mean dry mass of 9 seedlings at t_2 and t_1 respectively. At one time NAR based on leaf area was looked upon as a quantity constant throughout the plant kingdom and not influenced by widely differing environments. Monselise (1951), however, showed that different citrus species do not all have the same NAR.

3.3. RESULTS AND DISCUSSION

3.3.1. Absolute dry masses and leaf areas

Mean dry masses of plant parts and mean leaf areas are presented in Table 3.2. These data are the basis of subsequent calculations and are therefore given in some detail. The main points arising are the much faster growth of rough lemon, the marked effect of temperature on rate of growth and the increase in standard errors as the plants grew.

Differences in absolute growth rate between cultivar/temperature combinations are further illustrated by Figs. 3.1 & 3.2 and Plate 1. The greater rate of seedling growth and dry mass increase of seedlings at 30°C over those grown at either 20 or 25°C is clear. For rough lemon seedlings, the 5°C temperature difference from 20-25°C caused a greater seedling height response than the 5°C difference between 25 and 30°C. For Empress mandarin, however, a greater difference in seedling heights was measured between those grown at 25 and 30°C than between those grown at 20 and 25°C. Trifoliate seedlings at 25°C grew faster than those at 20°C by about as much as those at 30°C grew faster than those at 25°C. The seedling growth pattern for Empress mandarin suggests that their growth is more active in a temperature range above 25°C. The fact that mean monthly summer temperatures never reach this range in any of the citrus sub-areas except Messina and occasionally Malelane (Anon., 1977), provides one of the possible explanations for the reputation Empress mandarin has gained as a slow grower. Even at 30°C, however, the slow growth rate of Empress mandarin and trifoliate in comparison to that of rough lemon is well illustrated. In this regard it is interesting to note that after about 125 days at 20°C the difference in dry mass between rough lemon seedlings on one hand and

For a
single
seedling?

TABLE 3.2. Mean dry masses, leaf areas, seedling heights and their SE values for seedlings of three citrus rootstock cultivars grown over a period of 5 months at constant temperatures of 20°, 25°, and 30°C

Cultivar	Sampling date	Total Dry Mass (g)					Root Dry Mass (g)					Seedling Height (g)					Total Leaf Area (cm ²)									
		20 ^o	SE	25 ^o	SE	30 ^o	SE	20 ^o	SE	25 ^o	SE	30 ^o	SE	20 ^o	SE	25 ^o	SE	30 ^o	SE							
R L*	4-9	-	-	-	-	0.09	0.019	-	-	-	-	-	-	-	-	-	-	-	-	-						
	19-9	0.07	0.012	0.09	0.024	0.12	0.026	0.01	0.003	0.03	0.010	0.05	0.014	3.7	0.48	4.3	0.77	4.8	0.83	4.2	0.6	6.8	2.09	9.9	1.48	
	0-10	0.09	0.022	0.13	0.024	0.20	0.042	0.04	0.014	0.06	0.010	0.11	0.022	4.4	0.77	4.6	0.62	5.1	0.51	5.8	2.12	9.6	3.45	10.0	2.36	
	31-10	0.20	0.043	0.24	0.069	0.32	0.082	0.09	0.017	0.10	0.020	0.14	0.039	5.1	0.93	5.3	0.74	7.1	1.43	11.0	3.31	18.0	4.64	21.4	3.78	
	21-11	0.31	0.095	0.48	0.096	0.59	0.100	0.13	0.046	0.17	0.040	0.20	0.048	6.3	0.96	8.0	0.70	10.2	1.37	18.9	3.96	37.9	7.95	59.7	11.06	
	16-1	1.37	0.399	3.72	0.43	4.97	0.403	0.41	0.447	1.17	0.173	1.64	0.182	15.4	3.28	28.1	4.94	39.6	8.66	93.4	16.27	213.2	26.70	269.7	57.20	
E M*	10-5	21.75	-	44.49	-	73.39	-	7.69	-	18.15	-	25.80	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	19-9	-	-	0.06	0.013	0.07	0.017	-	-	0.01	0.002	0.03	0.010	-	-	3.2	0.30	3.6	0.53	-	-	3.8	0.49	4.4	0.81	
	9-10	0.07	0.010	0.07	0.012	0.09	0.022	0.03	0.006	0.03	0.012	0.05	0.010	2.9	0.28	3.0	0.58	3.9	0.44	4.2	0.81	3.1	0.94	5.1	1.09	
	31-10	0.14	0.017	0.13	0.019	0.14	0.028	0.07	0.011	0.05	0.020	0.07	0.018	3.6	0.39	3.8	0.57	3.7	0.33	6.0	1.30	7.8	1.56	7.0	1.43	
	21-11	0.20	0.046	0.21	0.040	0.25	0.041	0.10	0.022	0.09	0.022	0.09	0.017	4.3	0.54	4.8	0.58	6.2	0.73	9.0	1.41	12.5	2.01	21.0	3.80	
	16-1	0.53	0.061	0.74	0.312	1.31	0.219	0.22	0.041	0.28	0.026	0.43	0.089	6.8	0.76	10.8	1.72	20.1	3.66	22.8	3.15	45.6	10.05	80.7	13.09	
P.t.*	10-5	4.25	-	6.89	-	19.90	-	1.59	-	2.75	-	7.33	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	19-9	-	-	0.13	0.024	0.11	0.033	-	-	0.03	0.01	0.04	0.010	-	-	6.4	0.66	7.5	1.67	-	-	3.8	0.774	4.2	1.64	
	9-10	0.16	0.033	0.18	0.039	0.16	0.043	0.06	0.012	0.09	0.026	0.06	0.020	6.1	0.44	6.3	0.73	8.2	1.38	4.6	1.0	2.9	0.656	3.8	1.23	
	31-10	0.27	0.040	0.25	0.047	0.21	0.034	0.26	0.040	0.99	0.024	0.08	0.014	6.3	0.95	8.9	0.94	9.6	1.09	5.1	0.63	6.9	1.64	5.2	1.45	
	22-11	0.36	0.065	0.38	0.065	0.32	0.058	0.16	0.056	0.15	0.036	0.12	0.022	7.7	1.05	11.1	1.70	11.6	1.91	6.9	1.55	9.5	1.58	10.0	1.68	
	18-1	0.75	0.079	0.91	0.241	1.21	0.237	0.42	0.052	0.39	0.120	0.33	0.088	13.2	3.26	16.8	1.41	28.3	3.16	11.6	3.38	16.1	2.85	42.4	7.32	
P.t.*	10-5	2.57	-	6.96	-	13.61	-	1.01	-	3.03	-	4.50	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	19-9	-	-	0.13	0.024	0.11	0.033	-	-	0.03	0.01	0.04	0.010	-	-	6.4	0.66	7.5	1.67	-	-	3.8	0.774	4.2	1.64	
	9-10	0.16	0.033	0.18	0.039	0.16	0.043	0.06	0.012	0.09	0.026	0.06	0.020	6.1	0.44	6.3	0.73	8.2	1.38	4.6	1.0	2.9	0.656	3.8	1.23	
	31-10	0.27	0.040	0.25	0.047	0.21	0.034	0.26	0.040	0.99	0.024	0.08	0.014	6.3	0.95	8.9	0.94	9.6	1.09	5.1	0.63	6.9	1.64	5.2	1.45	
	22-11	0.36	0.065	0.38	0.065	0.32	0.058	0.16	0.056	0.15	0.036	0.12	0.022	7.7	1.05	11.1	1.70	11.6	1.91	6.9	1.55	9.5	1.58	10.0	1.68	
	18-1	0.75	0.079	0.91	0.241	1.21	0.237	0.42	0.052	0.39	0.120	0.33	0.088	13.2	3.26	16.8	1.41	28.3	3.16	11.6	3.38	16.1	2.85	42.4	7.32	

*R L = rough lemon P.t. = Poncirus trifoliata E M = Empress mandarin

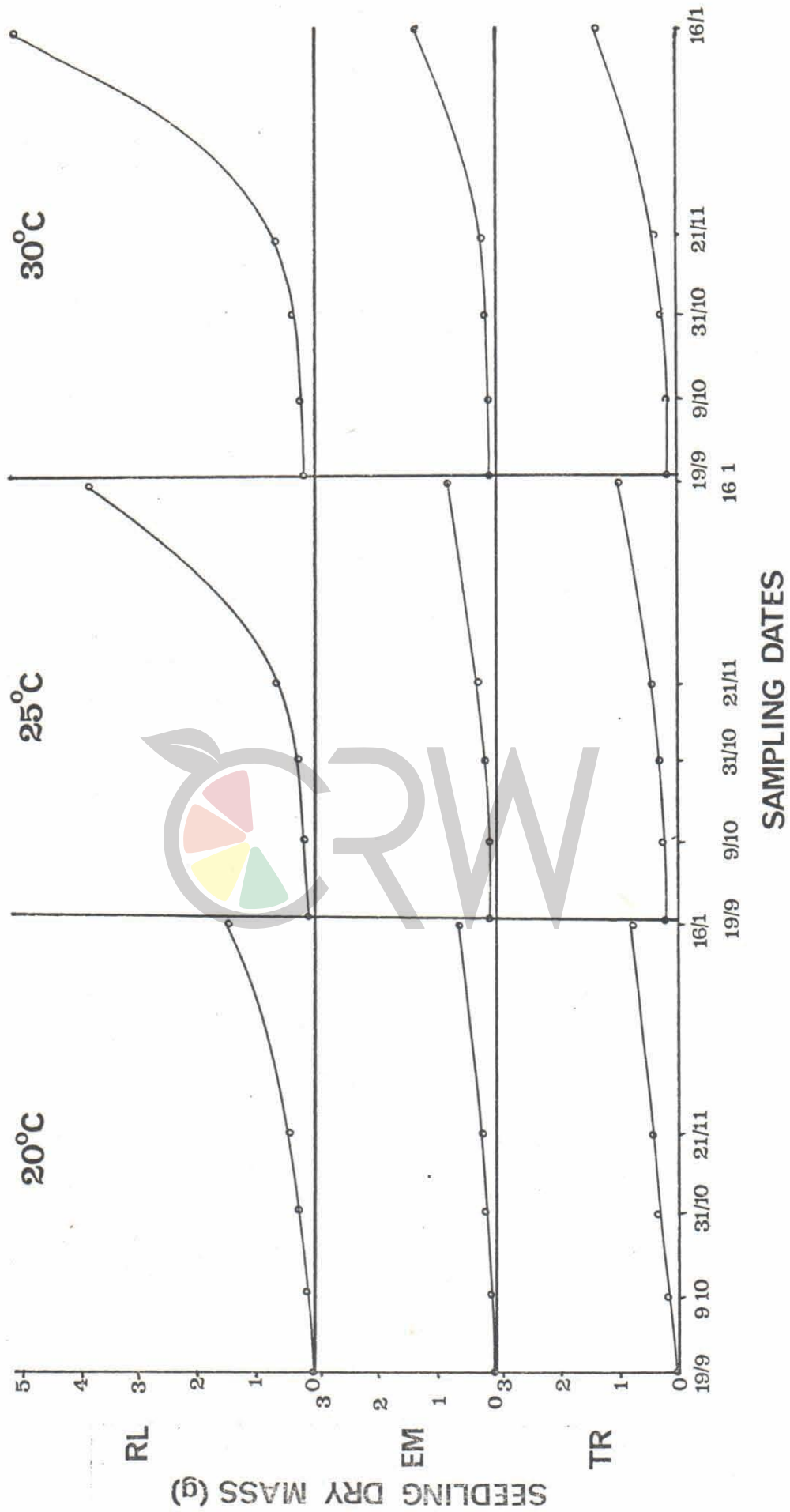


FIG. 3.1 Mean increases in total dry mass of 9 seedlings of 3 rootstock cultivars grown at constant temperatures of 20, 25 and 30°C

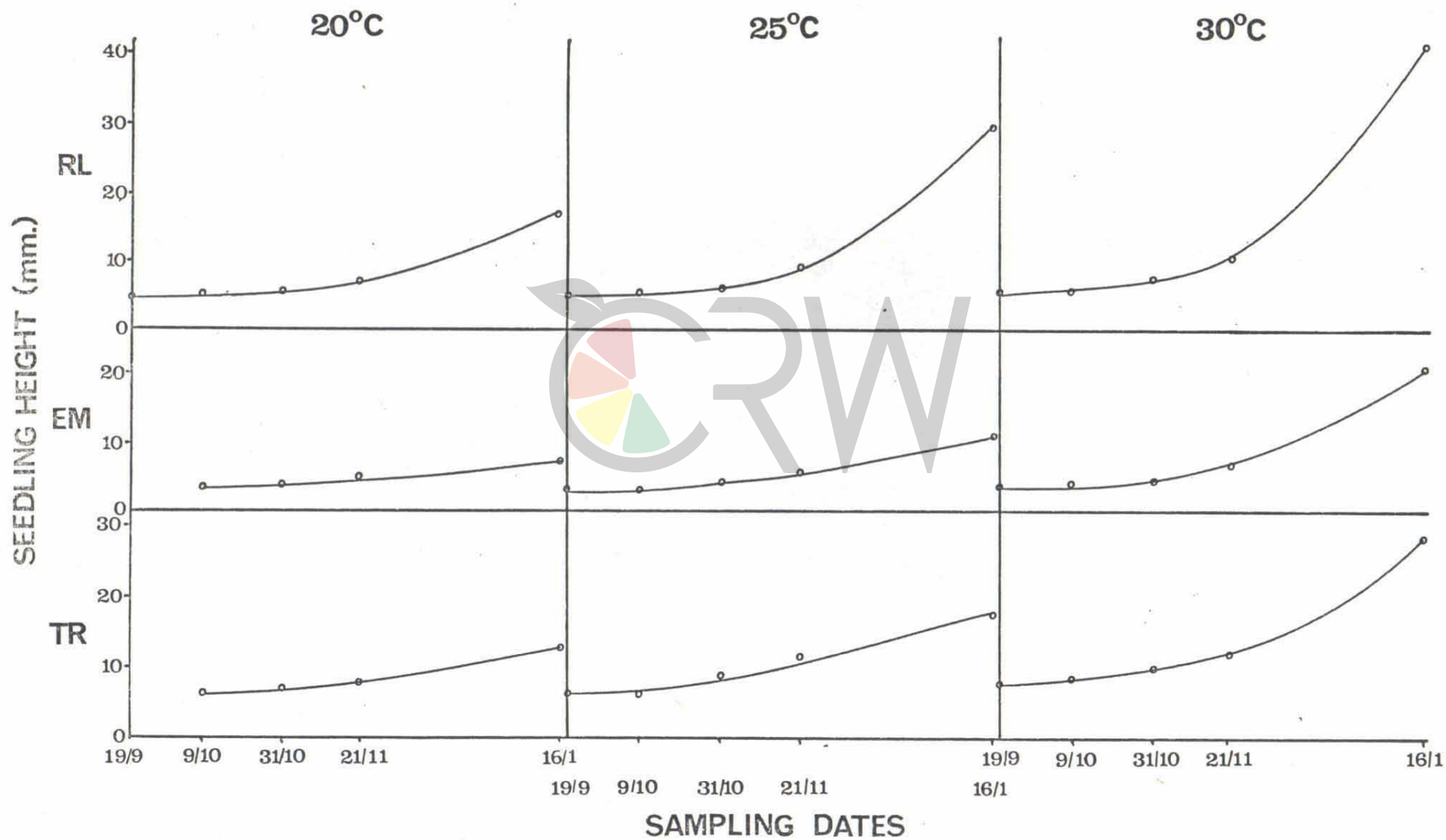


FIG. 3.2 Mean increases in height of 9 seedlings of 3 rootstock cultivars grown at constant temperatures of 20, 25 and 30°C



30°C

RL

TR

EM



25°C

RL

TR

EM



20°C

RL

TR

EM

PLATE 1

The comparative size, 148 days after seed planting of rough lemon (RL) trifoliate orange (TR) and Em-press mandarin (EM) seedlings grown in Separate constant temperature glasshouses at 20, 25 & 30°C. (The length of the ruler is 30cm)

Empress mandarin or trifoliolate on the other was less than the difference at either 25 or 30°C. This is illustrated in Table 3.3.

Table 3.3 Empress mandarin and trifoliolate seedling dry mass expressed as a percentage of the dry mass of rough lemon seedlings approximately 125 days after transplanting to glasshouses at 20, 25 and 30°C

Cultivar	Temperature		
	20°C	25°C	30°C
Rough Lemon	100	100	100
Empress mandarin	38,7	19,9	26,4
Trifoliolate	54,7	24,5	24,4

3.3.2. Shoot : root ratios

The effect of temperature upon changes in the proportion of roots to whole seedlings is shown in Fig. 3.3. The proportion of roots increased for seedlings of all cultivars grown at 25 and 30°C from 50 to 70 days after seed planting. A similar effect was noted for seedlings grown at 20°C though here the increase occurred 70 to 90 days after planting. Thereafter a steady reduction in the proportion of roots was noted for all cultivar temperature combinations except for trifoliolate seedlings at 20 and 25°C. Further details concerning the effect of temperature are presented in Table 3.4 in 3.3.2.1.

The pattern of root growth preceding shoot growth may represent the first growth cycle of these young seedlings and develop to form the periodic growth rhythms (flushes) associated with all citrus cultivars (Krishnamurthi & Kulasekharan, 1957). It is suggested that apart from hereditary influences, factors such as photoperiod (Simanton, 1971) and

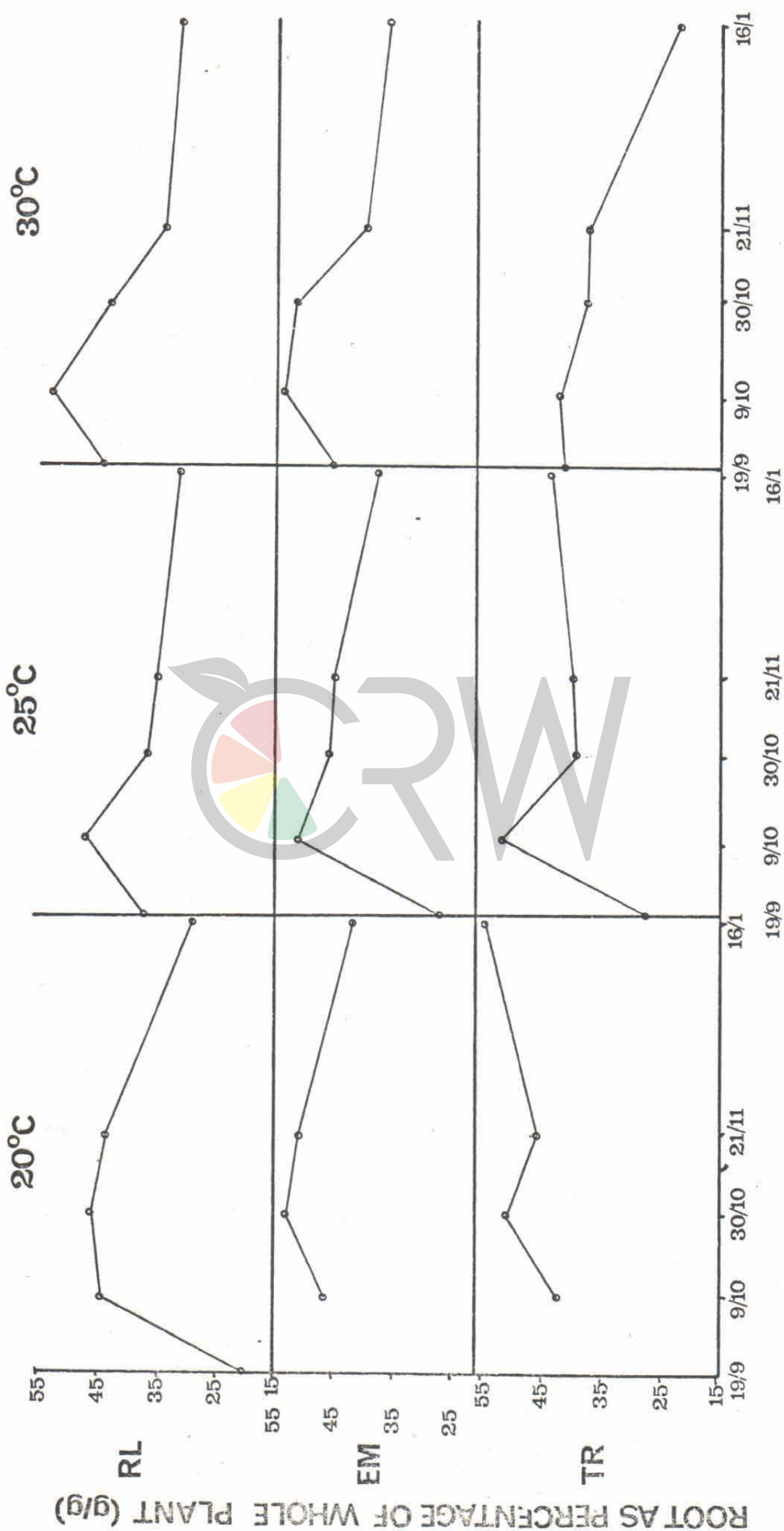


FIG. 3.3 Changes in the proportion of roots to whole seedlings (g/g) of 3 cultivars grown at constant temperatures of 20, 25 and 30°C for approximately 120 days

Root?

light intensity may account principally for their regulation (Marloth, 1950; Cooper, Peynado, Furr, Hilgeman, Cahoon & Boswell, 1963; Singh & Ghose, 1965).

3.3.2.1. The effect of temperature

From Table 3.4 it is evident that the proportion of roots to whole seedlings was higher in plants grown at 20 and 25°C than those grown at 30°C.

Table 3.4 The effect of temperature on the mean root percentage of three citrus rootstock cultivars calculated from dry mass data measured at 4 sampling dates (roots as a percentage of the whole plant g/g)

Temperature	Cultivar			Mean
	Rough lemon	E. mandarin	Trifoliate	
30°C	41,0	43,8	41,2	42,0
25°C	45,0	51,7	52,3	49,7
20°C	46,9	51,5	50,4	49,6
Mean	44,3	49,0	48,0	47,1

For all cultivars there were greater differences in the proportion of roots to whole seedlings between plants grown at 30 and 25°C than between those held at 25 and 20°C. For all three cultivars continuous temperatures greater than 25°C appear to have reduced root development.

3.3.2.2. The effect of cultivar

The growth behaviour of a cultivar is linked to its inherent photosynthetic characteristics which are related to its leaf area, leaf

So what?

anatomy, rates of dark and photorespiration, the photosynthetic ability of the fruit and other factors (Huffacker & Wallace, 1959; Bean & Todd, 1960; Todd, Bean & Propst, 1961; Clarke & Wallace, 1963; Thomson, Stolzy & Taylor, 1965; Kriedemann, 1968; Schneider, 1968).

Not in Ref. List

Singh & Ghose (1965) found that on the basis of vigour nursery seedlings could be grouped as follows:

- (i) Vigorous - lemons and limes
- (ii) Semi-vigorous - grapefruit and sweet orange
- (iii) Semi-dwarf - mandarins and citranges
- (iv) Dwarf - trifoliates

Australian?

From Table 3.4 it is evident that the mean root percentage of vigorous rough lemon seedlings (44,3) is less than that of the less vigorous Empress mandarin (49,0) or slow growing trifoliate (48,0). Greater seedling vigour is apparently accompanied by a smaller proportion of roots to shoots.

3.3.3. Derived data: relative growth and net assimilation rates

3.3.3.1. Relative growth rates

Substitution of dry mass data into formula (1) gave values for RGR presented in Table 3.5. Note that $t_2 - t_1$ values were not the same for all cultivar/temperature combinations. This arose through differences in transplant dates (see Table 3.1).

Bad mistake of exp procedure

TABLE 3.5 Calculated $\overline{\text{RGR}}$ for three citrus seedling cultivars grown at constant temperatures of 20, 25 and 30°C over time intervals $t_2 - t_1$ (g 100g⁻¹)

Tempe- rature	(g 100g ⁻¹)						Means
	Cultivars						
	Rough lemon	t ₂ -t ₁	E. mandarin	t ₂ -t ₁	Trifoliolate	t ₂ -t ₁	
		(days)		(days)		(days)	
20 ⁰ C	1,73	63	1,94	99	1,57	99	1,74
25 ⁰ C	1,31	125	2,00	64	1,58	121	1,63
30 ⁰ C	2,57	139	2,02	63	1,65	63	2,08
Means	1,87	109	1,99	75	1,60	94	1,82

$\overline{\text{RGR}}$ does not reflect differences in growth rate *per se* since here increases in dry mass are based on the dry mass of the plant at t_1 . As seedlings grow larger there is a tendency for the increase in mass per unit mass to slow down and therefore $\overline{\text{RGR}}$ calculated over long periods ($t_2 - t_1$) e.g. several years, would tend to have lower values than the $\overline{\text{RGR}}$ calculated over a shorter period during active seedling growth. The differences in $t_2 - t_1$ in Table 3.5 were found to have a negligible effect on $\overline{\text{RGR}}$.

At 30°C $\overline{\text{RGR}}$ was larger than at 25° or 20°C for all cultivars. This may be accounted for by the rapid growth of seedlings at that temperature (see Fig. 3.2), though the value would be expected to drop as seedling size increases. Taken over all three temperatures the $\overline{\text{RGR}}$ for Empress mandarin was higher than that of vigorous rough lemon or trifoliolate. This compares interestingly with the findings of Maggs & Alexander (1969) who measured a greater $\overline{\text{RGR}}$ in trifoliolate than in faster growing young Troyer citrange (*P. trifoliata* X *C. sinensis*) seedlings. Values obtained for sweet orange seedlings grown in summer

(2,85g 100g⁻¹ day) compared closely to that of rough lemon grown at 30°C (2,57g 100g⁻¹ day). $\overline{\text{RGR}}$ values obtained in the different temperature/cultivar combinations varied from 1,57 to 2,57g 100g⁻¹ day. Monselise (1951) recorded a range of 0,75 to 2,9g 100g⁻¹ day for sweet lime seedlings grown in shaded and unshaded plots during summer in Israel.

3.3.3.2. Net assimilation rates

As stated in 3.2.2. $\overline{\text{NAR}}$ can only be determined from substitution into equation (2) provided a linear or near linear relationship exists between A & M. Where A and M are not linearly related other formulae can be used such as those of Evans & Hughes (1962) and Whitehead & Myer^scough (1962). *Cited by Radford*

Fig. 3.4 shows the relationship and correlation coefficient (r) for each temperature/cultivar combination. Six of the combinations had r values greater than 0,9. Those with lower values were trifoliate at 20 and 25°C and Empress mandarin at 30°C. Values of r for the latter however, all exceeded 0,8 and the use of equation (2) was therefore considered suitable.

The $\overline{\text{NAR}}$ values obtained from substituting the dry mass and leaf area data (Table 3.2) into equation (2) are given in Table 3.6.

Most of the $\overline{\text{NAR}}$ values presented fall within the range of 1,5 to 6,7 g m⁻² day calculated for sweet lime seedlings over a five month period by Monselise (1951).

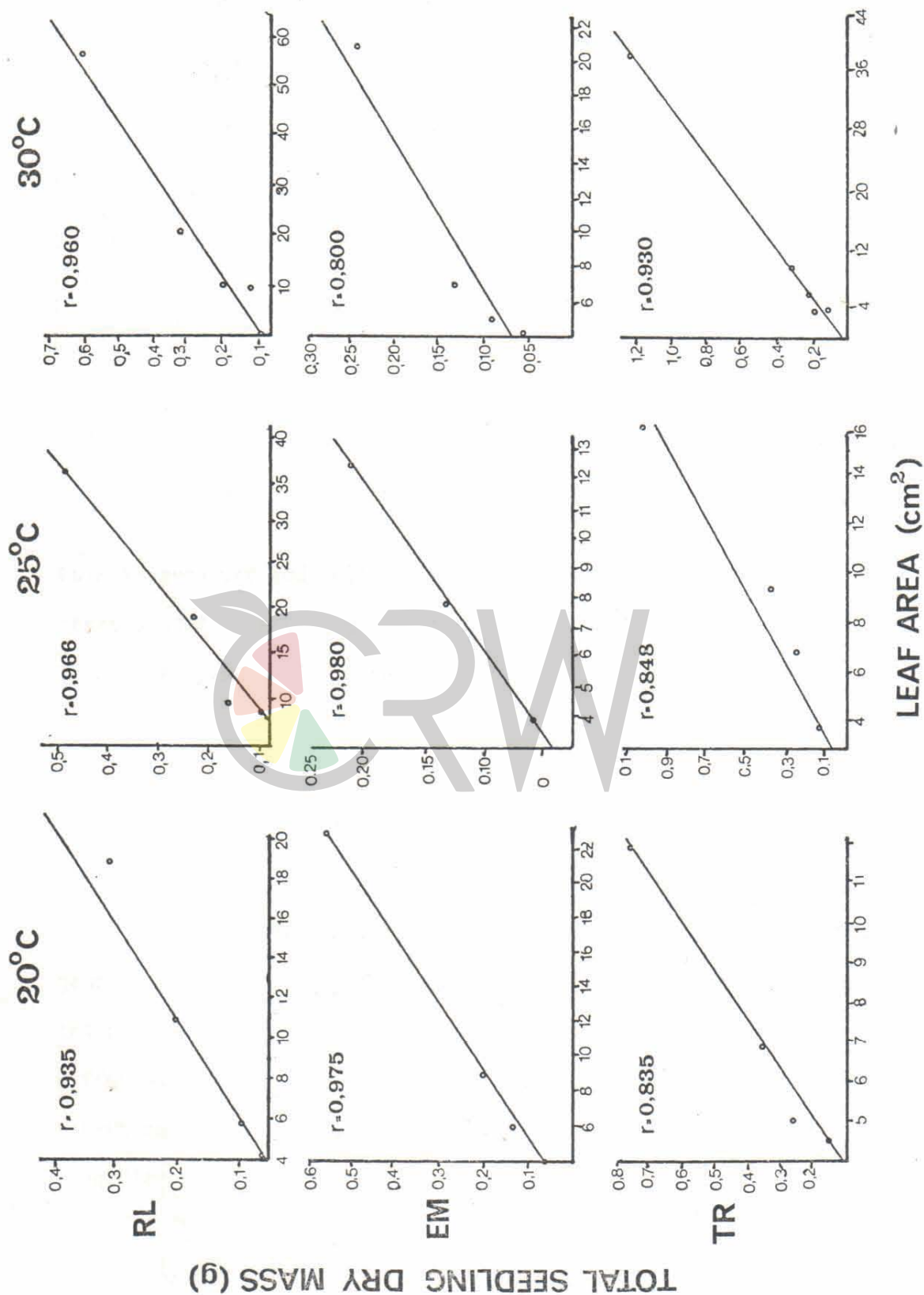


FIG. 3.4 Relationship between total leaf area (A) and total dry mass (M) for seedlings of 3 rootstock cultivars grown at 3 constant temperatures

TABLE 3.6 Calculated $\overline{\text{NAR}}$ of values over time intervals (t_2-t_1) of seedlings of 3 citrus rootstock cultivars grown at constant temperatures of 20, 25 and 30°C. Values of (t_2-t_1) are the same as those used in the determination of r for A X M

Temperature	(g m ⁻² day)						
	Cultivar						Mean
	Rough lemon	t_2-t_1	E. mandarin	t_2-t_1	Trifoliolate	t_2-t_1	
20°C	4,55	63	4,16	99	8,01	99	5,57
25°C	1,72	125	3,28	64	7,56	121	4,18
30°C	1,65	139	2,66	63	4,96	63	3,09
Mean	2,64	109	3,37	75	6,85	94	4,29

Both temperature and cultivar clearly influenced $\overline{\text{NAR}}$. Lowest values corresponded to seedlings giving the fastest growth while the highest $\overline{\text{NAR}}$ s were given by the slow growing trifoliolate seedlings at the lowest temperature.

3.4. GENERAL DISCUSSION

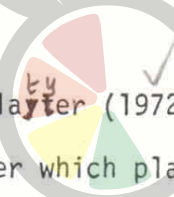
Determination of absolute growth rates confirmed the wide differences in growth characteristics between rough lemon, Empress mandarin and trifoliolate seedlings. The growth response of all three cultivars to different temperatures follows the same pattern observed in commercial citrus nurseries, where Empress mandarin and trifoliolate lag far behind rough lemon in reaching required transplant and budding size (Esselen, 1976). These differences were greater at 30°C than at 25°C or 20°C, so that under the mild growth conditions which prevail in South Africa's cooler production areas such as the Eastern Cape, nurserymen could expect to be less troubled by the differential growth rate of nursery

seedlings. However, since the growth rate of the seedlings tested was so much more vigorous at the higher temperature (30°C) there seems to be no case for recommending cool areas above warmer areas for the production of nursery trees.

The maintenance of constant day and night temperatures during the experiment produced artificial conditions. According to the observations made and the findings of other workers (Crider, 1927; Reed & MacDougal, 1937; Webber, 1948; Krishnamurthi & Kulasekharan, 1957; Randhawa & Khanna, 1963; Wutcher, 1973 and Reuther, 1973) greater root growth than at 30°C could be expected to result from day and night temperatures in the range $20-25^{\circ}\text{C}$ or temperature fluctuations to levels of 20°C and below at night. Overall seedling growth may also be enhanced by diurnal temperature fluctuations because high night temperatures assist high rates of dark and photorespiration (Reuther, 1973). Since the development of a strong fibrous root system is basic to the successful transplanting of nursery stock, the need for fluctuating temperatures in commercial operations is probably essential. This presents an argument against the concept of producing citrus seedlings in artificial environments in which high night temperatures are maintained. Nevertheless plastic tunnels may prove useful in this regard since the high daytime temperatures which develop inside the tunnel are usually followed by night time temperatures which drop to within a few degrees of the ambient temperature.

However, a possible disadvantage of the use of plastic tunnels during warm weather was shown by the NAR values obtained for all cultivars at $20, 25$ & 30°C . Contrary to expectations these values showed that the temperatures of greatest photosynthetic efficiency are not the same as those for optimum growth rate. These findings are backed by the work

✓ of Thomson *et al.* (1965), Kriedemann (1968) and Mikaberidze (1975) who found that the rate of orange leaf photosynthesis decreases as temperatures increase above 21°C. This implies that at 30°C where growth rate is near optimum for most citrus cultivars (Reuther, 1973), the utilization of photosynthetic products is rapid while the rate of synthesis of these products is not optimum. Under these conditions only a small portion of the photosynthate is available for storage so that a plant grown at 30°C has a low level of carbohydrate reserves. This could account for the poor transplant ability at certain times of the year of nursery trees grown in hot areas (Esselen, 1976). Nursery trees grown in cooler areas where a greater portion of photosynthetic products are stored in the leaves, stems and roots (Lewis, Coggins & Hield, 1963, and Kriedemann, 1968), may transplant more successfully as a direct result of higher carbohydrate reserves being present.

✓  Downton & Slatyer (1972), however, have shown that the climatic conditions under which plants are grown can affect their temperature-photosynthesis response. Hence caution should be exercised in interpreting the temperature response curves presented by Kriedemann (1969) and Possingham & Kriedemann (1969).

The decreasing $\overline{\text{NAR}}$ of citrus leaves as temperatures rise above 21°C is being used as the basis of research work aimed at developing citrus cultivars with high rates of photosynthesis under hot dry conditions (Khairi & Hall, 1976). Results presented in the text are in accordance with those of Monselise (1951) who found that deciduous trifoliate seedlings have a higher $\overline{\text{NAR}}$ than more vigorous ever-green cultivars. This agrees with the listing of deciduous trees as having a greater photosynthetic efficiency than evergreen plants (Kriedemann, 1971). A general problem with breeding higher photosynthetic capacities into

Abst.
Not in Ref list.

✓ commercial cultivars is that the assimilation rates of closely related species are usually very similar. In citrus however, it is possible to cross trifoliolate types with sweet oranges, lemons and so on giving rise to hybrids which may combine both vigour and higher photosynthetic capacities. This may partially account for the success and wide acceptance of citrange (*C. sinensis* X *P. trifoliata*) rootstocks.

All values of $\overline{NAR}$ and $\overline{RGR}$ given in the text represent the mean values over a time period $t_2 - t_1$. Within that time interval these values may vary widely depending upon the cyclic nature of the plant's growth pattern and other factors. In cases where more information concerning changes in $\overline{NAR}$ and $\overline{RGR}$ over small time intervals is required a different approach can be used for presenting results. Called the 'new approach' to growth analysis by Radford (1967), this involves mapping out the relationships between A & t and then deriving functions which fit these relationships best. The traditional methods of analysing growth were considered adequate in this study since the experiment was not designed to trace the variation of RGR and NAR with the physiological development of the plant.

So what?

What about Root mean

OVERALL DISCUSSION AND CONCLUSIONS

The germination of Empress mandarin seed, both in respect of rate and final percentage, was found to be determined more by the basic seed storage and pre-germination treatments to which they were subjected than by any hereditary peculiarities on the part of the seed. Experimental results suggest that Empress mandarin seeds may be more sensitive to certain adverse pre-germination conditions than other more commonly used rootstock cultivars such as rough lemon. This would explain the prejudice many nurserymen have built up against the use of Empress mandarin rootstock, often at the expense of providing growers with rootstock of a cultivar better suited to their specific conditions.

Extracted Empress mandarin seed rapidly loses its viability if allowed to dry out at ambient temperatures (20-25°C) for as little as 72 hours. Accordingly, provision must be made to either plant the seed out soon after extraction or to store the seed in a high humidity environment.

A third possibility, that of permitting the seed to dry out slowly at the normal seed storage temperature of 5°C prior to storing it in a sealed container at that temperature was not investigated. This may prove useful in prolonging seed life but may not be practical for all nurserymen since cold room facilities are required.

The planting of freshly extracted seed also presents practical problems especially where this follows storage of the seed in the fruit. It was found that freshly extracted Empress mandarin seed is surrounded by a tightly adhering gelatinous mucilage to which a range of fungi and bacteria are attracted. These micro-organisms eventually invade the seed causing loss of viability.

So why
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it

Should have been included.

Benomyl?

In order to remove the mucilage, seeds need to be rinsed in a sieve under a tap for 4 minutes to achieve satisfactory results. It is a practical method capable of application on a large scale by nurserymen. Rinsed seed should be soaked for 8 minutes in a 10 per cent. solution of 8-hydroxyquinoline sulphate (Mergal), be allowed to surface dry and thereafter can either be planted directly or be stored in a sealed container at 5°C. Of all storage treatments, low temperature (5°C) proved best for the maintenance of seed viability.

why not?

Although extracted and rinsed seed was preserved by 8-hydroxyquinoline sulphate it is likely that other chemicals could be used with similar effect. For example, tetramethylthiuram disulphide ('Arasan') is recommended in the U.S.A. although it was not available for experiments reported on in the text. Also, benomyl ('Benlate') gave good results. However, the seed fungicide commonly used in South Africa, phenyl mercuric acetate ('Trigosan'), was found to actually depress Empress mandarin seed viability during storage. Its effect on the viability of other rootstock cultivars was not tested but the possibility exists that Empress mandarin seed is more sensitive to the harmful side-effects of mercuric fungicides than the seed of other cultivars.

Agar

Difficulties in quantitatively determining the content of GA-like substances in developing Empress mandarin seeds were encountered. However, it was possible to show that changes in the amount of GA-like substances do occur during Empress mandarin seed ontogeny. Nevertheless, attempts to isolate and quantify the GA present in developing citrus seeds were not entirely successful. In future attempts, use should be made of the alkaline hydrolysis of seed extract prior to purification. Alternately, or in addition to this seed homogenates should be treated with proteolytic enzymes to convert bound forms of GA into measurable acidic

ethyl acetate-soluble GA-like substances. These bound forms of GA may account for a large proportion of the GA present in mature seeds.

X
Photofluorometric methods were not successful for GA detection in seed extracts owing at least in part to difficulties in purifying GA from all the interfering compounds present. Bioassays should preferably be used for such analyses though gas-liquid chromatography may prove even more reliable. It is suggested that future studies follow changes in the neutral, acidic ethyl acetate and acid-butanol fractions of maturing seeds. The nature of bound gibberellins and their interconversions to free forms during seed maturation will also constitute an important future study.

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The necessity for finding pre-germination treatments aimed at improving Empress mandarin seed germination became less urgent once the importance of conditions during seed storage were demonstrated. Nevertheless, experiments showed that germination rates can be accelerated by treating seeds with GA at various concentrations and by germinating excised embryos. In both cases, however, final germination percentages were not affected and in practice neither of these techniques appear to have much future. It is apparent that the faster germination rate of excised embryos may result from the unimpeded entry of oxygen and water to the embryo.

Not tested.

In determining the optimum germination temperature for Empress mandarin seed, care must be taken not to be misled by the high initial germination rate of seeds incubated at 36°C. At this high temperature a lower final germination percentage can be expected owing to increases in seed waste and other factors including the risk of desiccation. Furthermore, under most commercial seed-bed situations the

Not tested!

maintenance of regular watering to prevent seeds incubated at temperatures of 36°C from drying out could be expected to create difficulties. A sufficiently rapid germination rate was achieved at 30°C without this being accompanied by a high incidence of seed mortality. A suitable means of artificially creating seed-bed soil temperatures in this range without the risk of rapid soil desiccation occurring would be by the use of plastic tunnels. These would be particularly useful in the cooler production areas such as Rustenburg and the Eastern Cape where temperatures during the commercially ideal seed germination period (winter) are frequently below the germination minimum ($\pm 13^{\circ}\text{C}$).

! The nature of early seedling growth is also very dependent on temperature. Fluctuating temperatures probably favour healthy seedling growth since there is evidence that root development is stimulated by temperatures lower than the optimum for the aerial plant parts.

The dramatic effect of temperature upon the rate of seedling growth as shown in the growth analysis study places further emphasis on the need for temperature modification to be introduced into nursery seedbeds.

Growth checks? The constant temperature conditions under which the experimental seedlings were grown may have exaggerated growth rate differences of seedlings produced at 20, 25 and 30°C. Nevertheless, it is likely that nurserymen in the cool production areas would benefit greatly by being able to raise daytime temperatures in their seedbeds during late winter and spring to between 25 and 30°C.

However, where seedbed temperatures are allowed to remain constantly in the 25-30°C range under conditions of low humidity and high light intensity, weak though rapid seedling growth may occur because under such

✓ conditions increases in the rates of dark and photorespiration cause rapid utilization of synthesized carbohydrates leading to diminished available reserves. This could partly account for the poor transplant ability at certain times of the year of nursery trees grown in hot areas such as Messina and Malelane. Temperatures in unsealed plastic canopies are known to drop steadily with ambient temperatures at night. Thus the depletion of carbohydrates would probably be a less serious problem in citrus seedlings grown in cool areas under plastic canopy than where seedlings are grown in hot production areas in the open. Further growth analysis studies on a wide variety of citrus rootstock and scion cultivars would be valuable in classifying their relative photosynthetic characteristics. This information could be used in designing breeding experiments aimed at developing cultivars with a good bearing potential for arid tropical areas to which the common commercial citrus cultivars are not well suited.

As a result of the studies reported on in the preceding chapters it should now be possible for nurserymen to achieve both rapid and even Empress mandarin seed germination. This will have a direct bearing on the availability to growers of a rootstock which in terms of its vigour and tolerance to gummosis is ideally suited to certain conditions.

Attempts to extract and quantify the levels of GA in Empress mandarin seeds were not entirely successful. Nevertheless, the study illustrated many of the difficulties which can be encountered in an investigation of this kind. By so doing, it has provided future workers in this specific field with a list of hints which, if followed, should prove of some value in improving the accuracy of their results.

? The seedling growth analysis work has provided a foundation upon which to base sound methods for future investigation of this kind. Important work still to be conducted includes the determination of the effect of exogenously applied GA upon shortening the time required for seedlings of slow growing cultivars, such as Empress mandarin, to reach budding height. It has also set the stage for detailed studies to be made on the effect of temperature fluctuations upon the rates of root and shoot growth, the frequency and duration of growth flushes and the levels of carbohydrate reserves present at different stages of seedling growth. Work of this kind should lead to explanations for the differences in the transplant success achieved at different times of the year and enable nurserymen to plan their operations with this in mind.

Work of the kind conducted in this study should be continued in a further effort to make a meaningful contribution towards fulfilling the primary objective of South Africa's Citrus Improvement Programme, viz., to achieve the highest standards in citrus nursery tree production.

SUMMARY

Seed of the cultivar Empress mandarin, *Citrus reticulata* (Blanco) was germinated in soil and on an artificial medium after receiving various storage and pre-germination treatments designed to uncover the major factors responsible for its characteristically poor germination. Concurrently, attempts were made to estimate the significance of changes in GA levels in the developing seed and their possible link with seed germinability. Finally a comparison between the seedling growth characteristics of Empress mandarin and two other common citrus rootstock cultivars was made.

Seed storage conditions and pre-germination treatments were found to dominate the response of Empress mandarin seeds to conditions favorable for germination. Seed stored in the fruit germinated better than seed stored after being extracted. Chemically untreated seed or seed stored in fruit which had not received any post-harvest waste control treatment gave a poor mean germination percentage (50,5 per cent.) but not as low as that of either treated or untreated seed stored at ambient temperature (45,1 per cent.). Conversely highest mean germination percentages were given by seeds stored at 5°C (84,6 per cent.).

Unlike the seeds of many other plant species, the viability of Empress mandarin seed was found to be best retained by the prevention of desiccation. This was achieved by the use of freshly extracted seeds from fresh fruits or from fruits stored under suitable conditions. Good results were also obtained from extracted, moist, seed which had been stored in sealed containers. The latter method, however, usually led to the build-up of and invasion by seed-destroying fungi. Fungal

development was reduced and seed viability over extended periods increased by first rinsing from the seed surface the sticky fruit pulp mucilage which characterizes freshly extracted Empress mandarin seed. Fungal development was further restricted by the chemical treatment of seed prior to storage with 10 per cent. 8-hydroxyquinoline sulphate (Mergal) followed by storage at a low temperature (5°C). The storage of seed in its own juice or after treatment with phenyl mercuric acetate (Trigosan) depressed its germinability.

Benomyl?

Also don't full

Pre-germination GA seed soak treatments at 50, 250, 1 000 and 5 000 ppm increased the rate of seed germination but did not improve the final germination percentage. Similar effects were produced by 24 hour leaching of seed in running tap water and by removal of both seed coats. None of these treatments, however, were considered of future practical value.

Temperature was found to have a marked effect on the rate of Empress mandarin seed germination. Fastest initial germination was given at a constant temperature of 36°C but the final germination percentage of seeds germinated at 30°C was higher. Only 20 per cent. of seeds incubated at 15°C germinated over a 38 day period. In cooler citrus production areas, plastic tunnels may offer a means of attaining satisfactorily high germination temperatures during those times of the year that temperatures normally drop below the growth minimum.

The spectrophotofluorometric measurement of GA extracted from Empress mandarin seeds was unsuccessful but better results were obtained by bioassay methods. Of these, the barley endosperm bioassay proved most useful. Changes in the GA content of developing seeds from 1.8×10^{-4} to 8.0×10^{-6} $\mu\text{g GA g}^{-1}$ mass fresh seed were measured but

extraction methods did not account for bound gibberellins. Measurements of changes in the latter together with changes in the neutral acid ethyl acetate and acid butanol fractions would have given more realistic results.

Using modified growth analysis techniques the absolute growth rates, the mean relative growth rates ($\overline{RGR}$) and mean net assimilation rates ($\overline{NAR}$) were calculated for Empress mandarin, rough lemon and trifoliate orange seedlings raised over a 4 month period at constant growth temperatures of 20, 25 and 30°C. Over all temperatures the absolute growth rate of rough lemon was higher than that of the other two cultivars. However, taken over all three temperatures the $\overline{RGR}$ values for Empress mandarin were higher than those of rough lemon or trifoliate orange. All $\overline{RGR}$ values obtained fell within the range of 0,75 to 2,90g 100g⁻¹ day obtained by Monselise (1951).

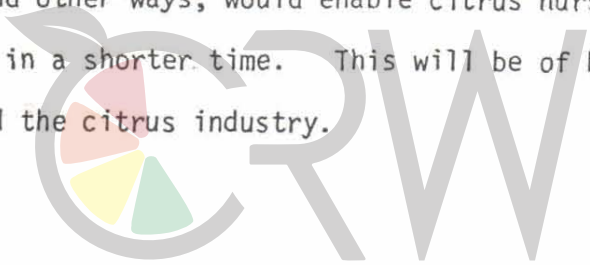
$\overline{NAR}$ s were lowest for the rough lemon seedlings which grew fastest (1,65g m⁻² day) and highest for slow growing trifoliate orange seedlings grown at the lowest temperature (8,01g m⁻² day). The latter value indicates that the photosynthetic efficiency of trifoliate orange is significantly greater than that of rough lemon although the utilization of assimilates as measured in terms of absolute growth rate was greater for rough lemon. A combination of greater photosynthetic efficiency and rapid utilization of assimilates within a single cultivar could produce a rootstock cultivar of exceptional quality.

Arising from the germination studies, it should now be possible for all nurserymen to achieve both rapid and even Empress mandarin seed germination. This will serve to make more readily available to

growers a rootstock which is ideally suited to certain environmental and soil conditions.

Attempts to extract and quantify GA in Empress mandarin seeds were not altogether successful. However, as a result of the study any future work conducted on this specific subject should benefit from the suggested means of overcoming some of the difficulties which were encountered.

The growth analysis study has cleared the way for future investigations into the many factors influencing the patterns of seedling growth. Such studies would be useful in determining optimum transplant times and in this and other ways, would enable citrus nurserymen to produce healthy trees in a shorter time. This will be of benefit to both the nurserymen and the citrus industry.



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APPENDIX TABLE 1 Comparison between the seed viability obtained by the 2, 3, 5-Triphenyl Tetrazolium chloride (T T C) method and a standard germination method on two rootstock cultivars (angular transformed data) *after 3 days.*

(Percentage Germination)			
<i>Poncirus trifoliata</i>		Empress mandarin	
T T C	Standard	T T C	Standard
100	100	100	85
95	90	100	100
95	75	95	90
100	70	100	70
75	95	90	75
85	75	95	95
95	90	100	90
90	80	100	100
95	70	100	85
90	75	100	80
Mean 92,0	82	98	87
$t (18 \text{ D.F.}) = \frac{9,2}{4,48} \text{ NS}$		$t (18 \text{ D.F.}) = \frac{14,1}{4,27} **p = 0,01$	

APPENDIX TABLE 2 Mean germination percentages of Empress mandarin seeds stored in their own juice at ambient temperature or 5°C for 0, 2, 4, 6 and 8 days

S T O R A G E		(Percentage Germination)																	% wasty
		D A Y S A F T E R P L A N T I N G																	
Temperature	Time (days)	3	5	7	9	11	13	15	17	19	21	22	23	24	25	26	27	28	
Ambient 5°C	0	7,5	12,5	12,5	32,5	42,5	65,0	77,5	92,5	97,5	97,5	97,5	100						0,0
	2	4,9	8,3	23,2	-	26,6	43,2	74,7	88,0	88,0	-	92,7	-	96,3	-	97,9	-	100	0,0
	2	3,3	6,6	11,6	-	28,2	44,8	73,0	84,7	91,3	-	94,6	-	96,3	-	97,9	-	100	0,0
Ambient 5°C	4	3,3	6,6	-	18,3	24,9	38,2	53,1	64,7	78,0	-	79,7	-	86,3	-	96,3	-		3,7
	4	3,3	8,3	-	24,9	31,5	53,1	66,4	78,0	91,3	-	96,3	-	97,9	-	97,9	-	100	0,0
Ambient 5°C	6	5,0	-	13,3	19,9	39,8	54,8	61,4	76,4	84,7	86,3	-	92,9	-	92,9	-	94,6	-	5,4
	6	2,0	-	6,6	9,7	24,9	38,2	53,1	69,7	84,7	91,3	-	96,3	-	97,9	-	97,9	-	2,1
Ambient 5°C	8	-	-	3,3	9,9	31,5	41,5	58,1	76,4	78,0	81,3	-	81,3	-	81,3	-	89,6	-	10,4
	8	-	4,9	9,9	21,6	34,7	44,8	59,8	66,1	73,0	88,0	-	88,0	-	88,0	-	89,6	-	10,4

APPENDIX TABLE 3 The effect of water leaching Empress mandarin seed for 24 hours upon germination rate

Treatment	(number of seeds germinated out of 150)										Means
	DAYS AFTER PLANTING										
	FROM START OF EXPT.										
	36	38	40	42	44	46	48	50	53	57	
2 min. rinse	17	26	49	57	81	92	102	113	125	136	79,8 a
24 hr. leach	26	38	59	68	90	99	108	114	130	136	86,8 b
Means	21,5a	32,0b	54,0c	62,5c	85,5d	95,5e	105,0f	113,5f	127,5g	136,0g	

Totals in any vertical or horizontal column not followed by the same letter are significantly different at $P = 0,05$.

S.E.'s and L.S.D.'s

	S.E.	L.S.D. (5%)	L.S.D. (1%)
Body of table	0,9	2,6	3,4
Leach treatments	1,4	4,1	5,4
Days after planting	3,2	9,1	12,0

CV = 12,24%

If this is taken into account to diff

APPENDIX TABLE 4 Effect of seed soaks in solutions containing various levels of GA₃ upon the germination rate of Empress mandarin seeds

Treatments	(No. of seeds germinated out of 150)							Means
	Days after planting							
	32	34	38	42	46	50	55	
Control	9	13	26	57	92	113	129	62,7a
50 ppm	22	29	51	75	111	130	140	79,7bc
250 ppm	14	24	49	86	119	133	140	80,7bc
1 000 ppm	8	17	48	76	119	136	145	78,4b
5 000 ppm	21	30	56	91	120	134	139	84,4c
Means	14,8a	22,6b	46,0c	77,0d	112,2e	129,2f	138,0g	

Means in any vertical or horizontal column not followed by the same letter are significantly different at $P = 0,05$.

S.E.'s and L.S.D.'s

	S.E.	L.S.D. (5%)	L.S.D. (1%)
Body of table	1,79	3,00	4,0
GA treatment	2,03	5,7	7,5
Days after planting	2,40	6,7	8,9

APPENDIX TABLE 5a Germination rates of Empress mandarin seed at three temperatures

TEMP.	REPL.	(No. of seeds germinated out of 150)								
		DAYS AFTER PLANTING								
		14	18	22	26	30	33	37	44	47
20°C	1	-	-	4	19	49	80	104	128	134
	2	-	-	2	15	42	84	106	136	143
	G%	-	-	1,9	11,2	30,0	54,1	69,3	87,1	91,4
25°C	1	-	1	11	22	51	68	92	118	118
	2	-	-	7	28	60	86	107	136	136
	G%	-	0,3	5,9	33,0	36,6	50,8	65,7	83,8	83,8
30°C	1	4	27	75	119	135	136	-	-	-
	2	3	17	59	108	126	133	-	-	-
	G%	4,6	14,5	44,2	74,9	86,1	88,7	-	-	-

APPENDIX TABLE 5b Percentage germination of Empress mandarin seed at 7 temperatures

TEMP.	DAYS AFTER PLANTING											S.E.'s
	5	7	9	12	14	16	18	20	22	24	28	
15°C	1	2	6	6	7	7	8	9	9	9	14	4,1
18°C	-	-	3	3	7	9	12	15	20	20	26	9,5
21°C	2	3	5	9	9	16	17	22	25	40	59	9,1
24°C	-	4	9	17	29	46	67	74	86	90	98	8,7
30°C	5	13	20	37	50	70	84	92	99	99	100	8,0
33°C	5	12	23	24	41	51	59	76	89	91	96	8,6
36°C	10	19	25	39	43	51	59	69	83	83	88	6,3

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