ISOLATION AND IDENTIFICATION OF THE TOXIC PRINCIPLE OF GASTROLOBIUM GRANDIFLORUM

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SUMMARY

Gastrolobium grandiflorum **is one of the most toxic of Queensland plants and has caused serious stock losses in Central Queensland.**

The toxic principle was isolated from the leaf and identified as monofluoroacetic acid. Identity was established by gas chromatography, infra-red absorption spectroscopy, and micro-fluorine analysis.

An extract of *G. grandiflorum* **administered orally to both sheep and rats resulted in clinical symptoms, biochemical changes, and post-mortem findings similar to those obtained following the administration of an equivalent amount of authentic monofluoroacetic acid.**

I. INTRODUCTION

Gastrolobium grandiflorum F. Muell. is a shrub extending up to 6 ft in height, with several slender stems from a woody tuber just below ground level. It is known locally as heart-leaf poison bush but is not the true "heart-leaf", which grows only in Western Australia (Everist 1947). It is known also as desert poison bush and wallflower poison bush. The species grows on yellow sandy soil in poor forest country in northern and central Queensland. It occurs also in the Northern Territory and the north of Western Australia (Everist 1947). It is the only toxic species of *Gastrolobium* which extends beyond the boundaries of Western Australia and is the only toxic species found in the tropics (Gardner and Bennetts 1956, p. 70).

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The plant was first collected in the Whithrington Range in northern Australia by J. McD. Stuart on an exploration expedition and was characterized by F. von Mueller (1862). Its toxic nature was discovered early in its history, and Bailey (1900, p. 353) referred to it as "The most toxic of any in the Australian flora". Many instances of the toxicity have been recorded (Unpublished Reports, Official Records of the Queensland Department of Primary Industries). In these records many instances of sheep being killed with as little as 200 g or less are listed, while estimates of annual losses have been placed at 150–300 bullocks on one property and at 500 bullocks on another; the loss of 2,000 sheep, at one time, on another property in the affected area is also recorded.

In both sheep and cattle, poisoning is characterized by suddenness of death. Although several hours may elapse between exposure and the first apparent symptoms in the animal, it is frequently only minutes between onset of symptoms and death. Excitement, exercise or fright may precipitate symptoms.

The isolation and identification of the toxic principle in the leaf of *G. grandiflorum* is now reported.

II. MATERIALS AND METHODS

(i) *Source of Material.*—The leaf of *G. grandiflorum* was collected from widely separated areas in central Queensland. The samples were identified the Queensland Herbarium and the specimens were filed there under the numbers BRI 023743, BRI 025176, BRI 027202 and BRI 036495. Some specimens were air-freighted from the point of collection and were deep-frozen pending chemical examination. The bulk of the material was shade-dried, rail-freighted, hammer-milled and stored in sealed plastic or aluminium containers.

(ii) *Experimental Animals.*—Female albino rats of weight range 120–160 g and female white mice of weight range 25–30 g were used in experimental toxicity testing. Merino wethers of weight range 25–35 kg were used in the large-animal testing. Both rats and sheep were dosed by stomach tube with aqueous solutions which had been adjusted to pH 7. Mice were dosed by intraperitoneal injection. Sheep received volumes of up to 1 l, rats up to 10 ml, and mice up to 1·0 ml. All dosings were related to known quantities of air-dried leaf. Experimental toxicity testing with mice and/or rats was carried out after each chemical treatment of the extract.

Jugular blood samples from sheep were taken for biochemical testing directly into tared bottles containing 8 per cent. trichloroacetic acid solution. Samples of tissue were collected by routine necropsy methods immediately after death, weighed and macerated with 8 per cent. trichloroacetic acid solution.

(iii) *Citric acid Estimations.*—The estimation of citric acid was by a method developed in this laboratory and incorporating the better features of techniques reported by Weil-Malherbe and Bone (1949), Buffa and Peters (1949), Ettinger,

Goldbaum, and Smith (1952) and Taylor (1953). Samples of blood and tissues of sheep and organs of rats were examined. Delay in processing was avoided, as this caused a considerable reduction in the level of citric acid.

(iv) *Silicic Acid Chromatography.*—Two procedures were used. Method 1 was essentially that defined by the Association of Official Agricultural Chemists (1960, p. 359). Method 2, a modification of this method (Oelrichs and McEwan 1962) using the two mobile solvents fractionated chloroform and fractionated chloroform (950 ml) containing fractionated n-butanol (50 ml), was used to obtain a more complete separation of monofluoroacetic acid.

(v) *Esterification.*—Butyl esters were formed using butanol, free acid and concentrated sulphuric acid at 100°C for 1 hr in a sealed ampoule. After neutralization with aqueous sodium bicarbonate solution, the esters were extracted with a small volume of sulphuric ether, washed with water and dried.

2-chloroethanol esters were formed by the method of Oette and Ahrens (1961) by treating the acid with 2-chloroethanol containing 5–7 per cent. hydrogen chloride in a sealed tube for 1–2 hr. The mixture was then diluted with 5 volumes of water, extracted with hexane, the hexane washed with water and dried.

(vi) *Quantitative Estimation of Monofluoroacetic Acid.*—The method used was that of Oelrichs and McEwan (1962).

(vii) *Specialized Equipment.*—Micro-fluorine analyses were done by the method described by Harvey (1952). A Perkin-Elmer Model 154 D Vapor Fractometer with Flame Ionisation Detector and a 1 mV Honeywell Brown Strip Chart Recorder were used for vapour fractionation. In chromatography of free acids a metre stainless steel column was packed with acid-washed "Embacel" kieselguhr (7·5 g) coated with Tween 80 (2·5 g), a slight variation of the method of Emery and Koerner (1961). Esters were separated on a 2-m column packed with finely ground diatomaceous earth coated with polypropylene glycol—Perkin-Elmer Column R. For infra-red spectometry a Perkin-Elmer Model 21 instrument was used.

III. EXPERIMENTAL AND RESULTS

(a) Toxicity Testing

During the extraction and purification of the toxin from *G. grandiflorum,* solutions were toxicity tested with mice and rats. Mice were injected intraperitoneally with neutral aqueous solutions in a range of dosages, three mice being used at each dose rate. Initially, correlation of these results with toxicity to rats by oral dosage was good. However, as separation proceeded, particularly in relation to partition into butanol from aqueous solutions of varying pH, poor correlation indicated that the mouse test was not satisfactory.

In toxicity testing with rats using purified extracts, the symptoms of muscle tremors and convulsive spasms were very similar to those produced by monofluoroacetic acid. This directed attention towards the measurement of biochemical changes in blood and tissue.

Sheep were used in the terminal stages of the investigations to confirm that the extracted toxin produced clinical symptoms, biochemical changes and post-mortem findings similar to those obtained both with whole plant and with authentic monofluoroacetic acid. The residual plant material after extraction produced no clinical symptoms and no significant biochemical changes in sheep.

(b) Chemical Extraction

Preliminary work showed that the toxin was water-soluble. Investigation to determine a better extracting solvent, particularly from the point of view of obtaining complete extraction with minimum amounts of contamination, showed that methanol extracted the toxin readily, and that continuous extraction with ethanol in an all-glass Soxhlet apparatus yielded complete extraction. Ethanol extraction followed by concentration, addition of water, filtration, and further concentration of the neutralized solution provided a solid residue of maximum toxicity.

Acidification to approximately 1 per cent. with sulphuric acid, filtration, continuous extraction of the filtrate with alcohol-free sulphuric ether, and partition into N sodium bicarbonate solution yielded an extract which was extremely toxic to rats. Citric acid levels obtained from one rat dosed with this extract are recorded in Table 1 (Rat No. 2). A sheep dosed with a similarly prepared extract from 600 g of air-dried leaf died showing typical convulsions in 3½ hr; levels of citric acid in blood and tissue are included in Tables 2 and 3.

TABLE 1

CITRIC ACID LEVELS IN ORGANS OF RATS

Rat No.	Treatment	Organ	Citric Acid (p.p.m.)
1	Aqueous extract equivalent to 25 g leaf/kg	Kidney	1040
		Liver	102
2	Ether extract of aqueous solution equivalent to 28 g leaf/kg	Kidney	628
		Liver	254
3	Ether solution from silicic acid chromatography equivalent to 25 g leaf/kg	Kidney	1190
		Liver	141
4	Monofluoroacetic acid 3·5 mg/kg	Kidney	959
		Liver	112
5	Monofluoroacetic acid 3·0 mg/kg	Kidney	1096
		Liver	123
6	Undosed control—slaughtered	Kidney	37
		Liver	17
7	Undosed control—slaughtered	Kidney	42
		Liver	19

TABLE 2

CITRIC ACID LEVELS (P.P.M.) IN BLOOD OF SHEEP

Time of Sampling	Sheep No. 1. Dosed with Extract of 600 g of *G. grandiflorum* Leaf	Sheep No. 2. Dosed with 50 mg of Monofluoro-acetic Acid	Sheep No. 3. Dosed with 1,200 g of Extracted Leaf	Sheep No. 4. Undosed Control
Predosing ..	14·3	24·6	13·4	17·8
After 30 min ..	8·1	24·9	10·4	19·7
After 90 min ..	26·0	28·8	18·4	18·6
After 120 min ..	44·0	29·7	20·0	19·2
After 150 min ..	59·6	34·5	—	—
After 180 min ..	76·9	54·3	25·6	20·5
After 220 min ..	—	40·9	20·6	—
After 300 min ..	—	46·2	18·5	18·2
After 390 min ..	—	45·3	18·3	19·8

TABLE 3

CITRIC ACID LEVELS (P.P.M.) IN TISSUES OF SHEEP

Tissue	Sheep No. 1. Dosed with Extract of 600 g *G. grandiflorum* Leaf	Sheep No. 2. Dosed with 50 mg Monofluoroacetic Acid	Sheep No. 4. Undosed Control
Heart	304	400	39
Kidney	191	265	20
Diaphragm	76	100	39
Spleen	57	80	22
Liver	17	35	9
Cerebellum	15	58	25
Medulla	15	68	16
Cerebrum	23	95	12

Repetitive partition of the toxin obtained from 4 kg of leaf between ether and aqueous media by changing pH resulted in an ether solution containing 480 mg monofluoroacetic acid, estimated by gas chromatography. This solution (100 ml) was dried over anhydrous sodium sulphate (10 g) and activated carbon (5 g), filtered, and distilled through a semi-micro fractionating column, using an oil-bath temperature up to 180°C. Maximum distillation temperature obtained in this procedure was 130°C. Removal of volatile acids in this way reduced the total acid concentration by approximately 90 per cent. as estimated by titration with N sodium hydroxide. No appreciable toxicity was detected in the distillate.

The neutral solution obtained by titration of the residue was filtered acidified to 1 per cent. with sulphuric acid, extracted with ethanol-free sulphuric ether, extracted with sodium bicarbonate solution and carefully exaporated to dryness.

(c) Silicic Acid Chromatography

Half of the solid obtained by chemical extraction was acidified with 50 per cent. sulphuric acid, dried with a minimum quantity of anhydrous sodium sulphate and transferred to a silicic acid column (Method 1). The column was prepared by grinding 10 g silicic acid with 5·0 ml of 0·5N sulphuric acid and forming a slurry with the mobile solvent consisting of fractionated chloroform containing 10 per cent. by volume of fractionated n-butanol. Air bubbles were removed and the column packed under pressure. The eluate from the column was collected in 3-ml fractions. Examination of the fractions by gas chromatography revealed the presence of peaks corresponding to monofluoroacetic acid in fractions 10-12. These fractions were combined and the silicic acid chromatographic Method 2 used to obtain a more complete separation. In this procedure 15 g silicic acid was ground with 7·5 ml 0·5N sulphuric acid, mixed with fractionated chloroform and poured into a column. After preparation of the column and addition of the sample the acids were developed using fractionated chloroform (100 ml) and then eluted, using fractionated chloroform (95 ml) containing fractionated n-butanol (5 ml). Fractions (3 ml) were collected from the eluate and peaks corresponding to monofluoroacetic acid were found by gas chromatographic examination of fractions 28–30. These were combined, and quantitative gas chromatographic estimation indicated a recovery of 190 mg monofluoroacetic acid.

After evaporation to dryness from a sodium bicarbonate solution, the solid was dissolved in 1 per cent. sulphuric acid and extracted with a minimum quantity of distilled ethanol-free sulphuric ether. The ether solution was dried over anhydrous sodium sulphate and evaporated at −50°C, with precautions to prevent introduction of moisture, to a final volume of 0·5 ml.

(d) Gas-liquid Chromatography

In the preliminary studies gas chromatography was used to detect the presence of an acid in plant extracts and in fractions purified by silicic acid chromatography having the same retention time as monofluoracetic acid. Subsequently the gas chromatograph was used to prepare a pure, moisture-free sample of the toxin for infra-red absorption spectroscopy.

In this step the nitrogen carrier gas was thoroughly dried by passage through a mixture of phosphorus pentoxide and powdered asbestos. Comparison of the ether solution (0·5 ml) obtained from the silicic acid chromatography with monofluoroactic acid (Figure 1) was carried out to determine accurately the times of collection. To collect the pure sample a U-tube of pyrex glass of 7-mm internal diameter, 11 cm in height, packed with a dry purified sand and immersed in a freezing bath (−80°C) was connected to the column outlet at the predetermined times following three injections (each of 50 μl) into the gas flow. The sample was dissolved by heating with carbon tetrachloride (1ml) and was removed by means of a syringe. This solution was examined by infra-red spectroscopy.

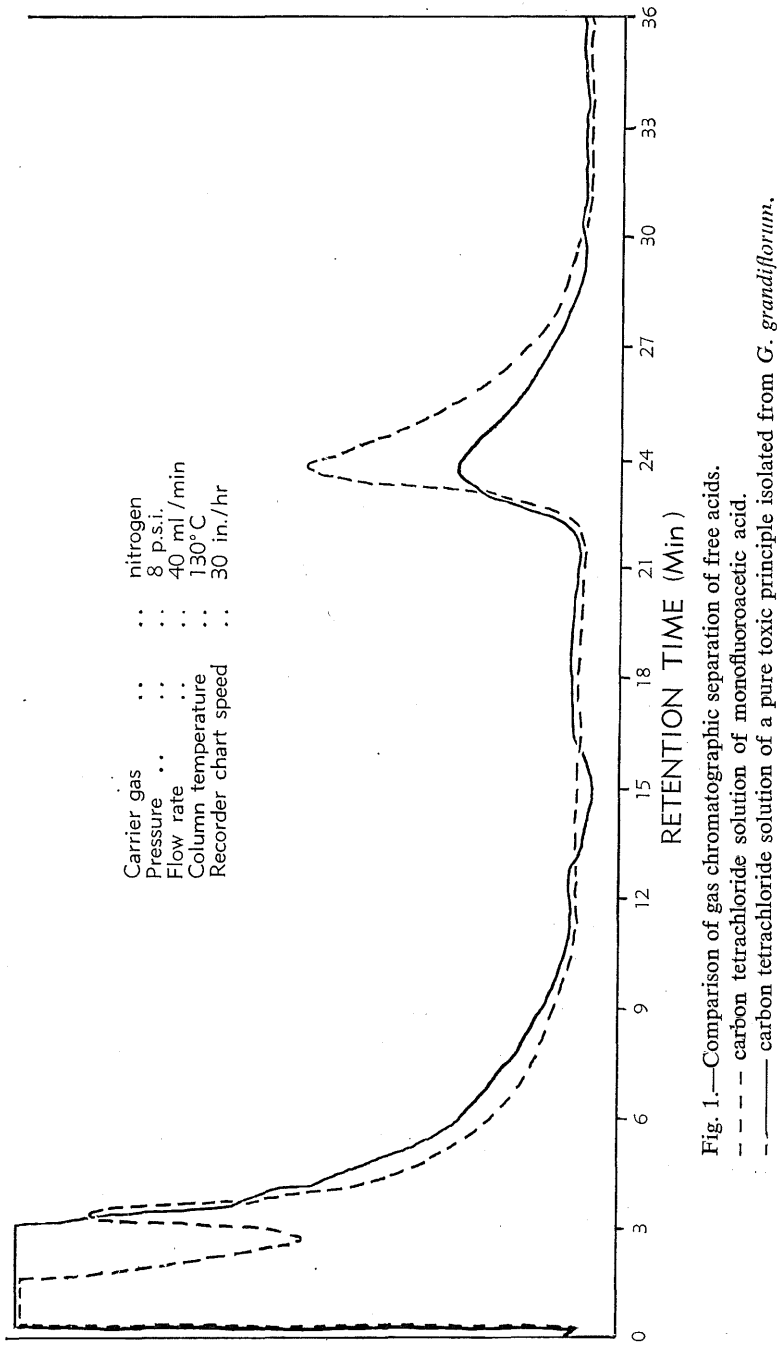

Fig. 1.—Comparison of gas chromatographic separation of free acids.
– – – – carbon tetrachloride solution of monofluoroacetic acid.
———— carbon tetrachloride solution of a pure toxic principle isolated from *G. grandiflorum*.

The remainder of the material adhering to the sand was removed with dry sulphuric ether and evaporated to dryness at —50°C. This material, together with the remainder of the ether solution obtained from silicic acid chromatography,

was used to form butyl and 2-chloroethanol esters for comparison with these esters of monofluoroacetic acid. Comparison by gas chromatography of the butyl esters is shown in Figure 2, and comparison of the 2-chloroethanol esters in Figure 3.

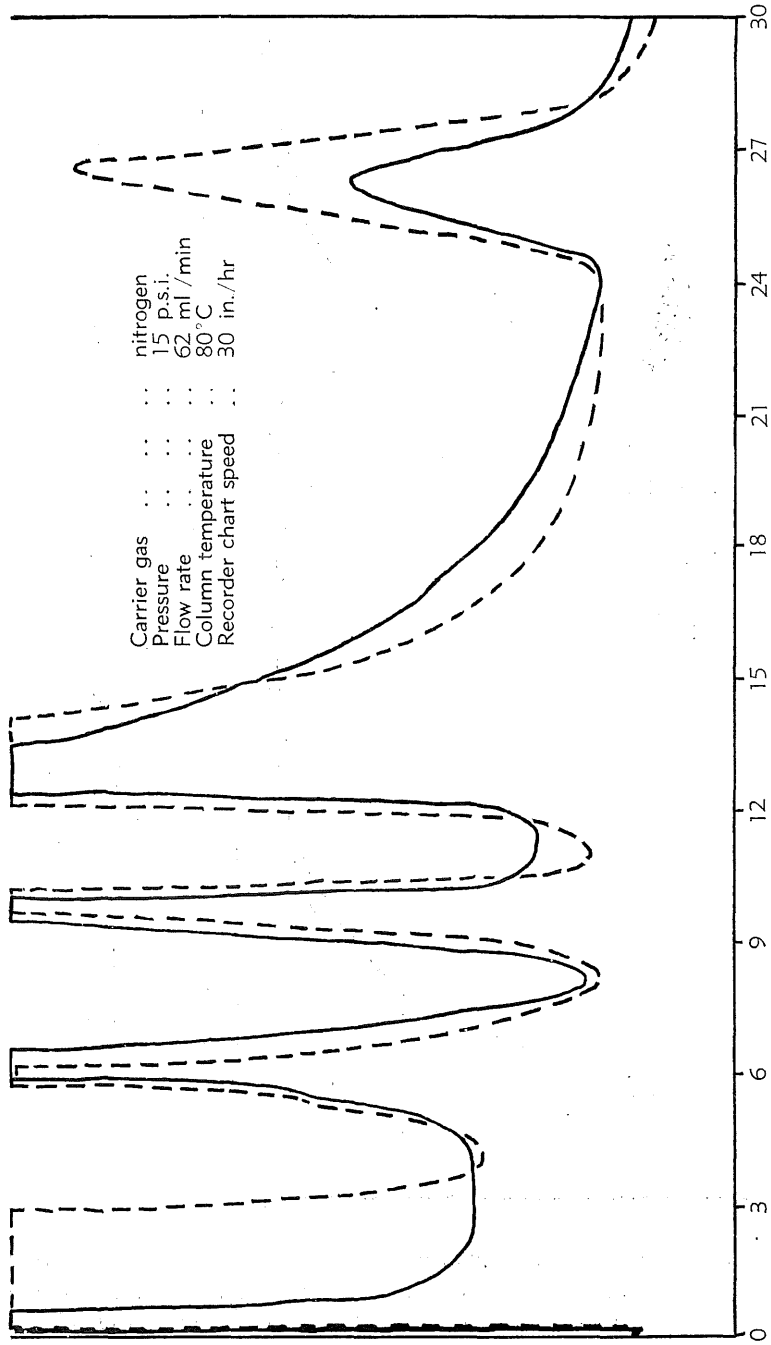

Fig. 2.—Comparison of gas chromatographic separation of butyl esters.
- - - - ether solution of ester prepared from monofluoroacetic acid.
——— ether solution of ester prepared from pure toxic principle isolated from *G. grandiflorum*.

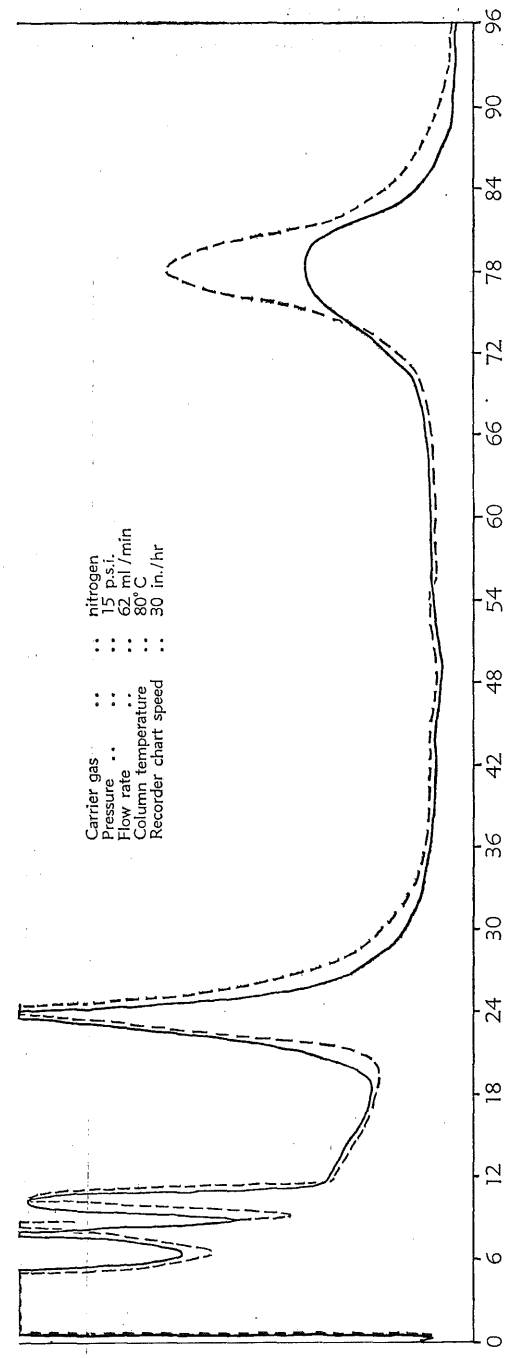

Fig. 3.—Comparison of gas chromatographic separation of 2-chloroethanol esters.
– – – – ether solution of ester prepared from monofluoroacetic acid.
——— ether solution of ester prepared from pure toxic principle isolated from *G. grandiflorum*.

(e) Infra-red Absorption Spectroscopy

The infra-red spectra of carbon tetrachloride solutions of the sample collected after gas chromatography and of monofluoroacetic acid are shown in Figure 4.

Both spectra show the absorption bands characteristic of a short-chain aliphatic carboxylic acid together with a band at 1095 cm^{-1} characteristic of a carbon-fluorine stretching mode.

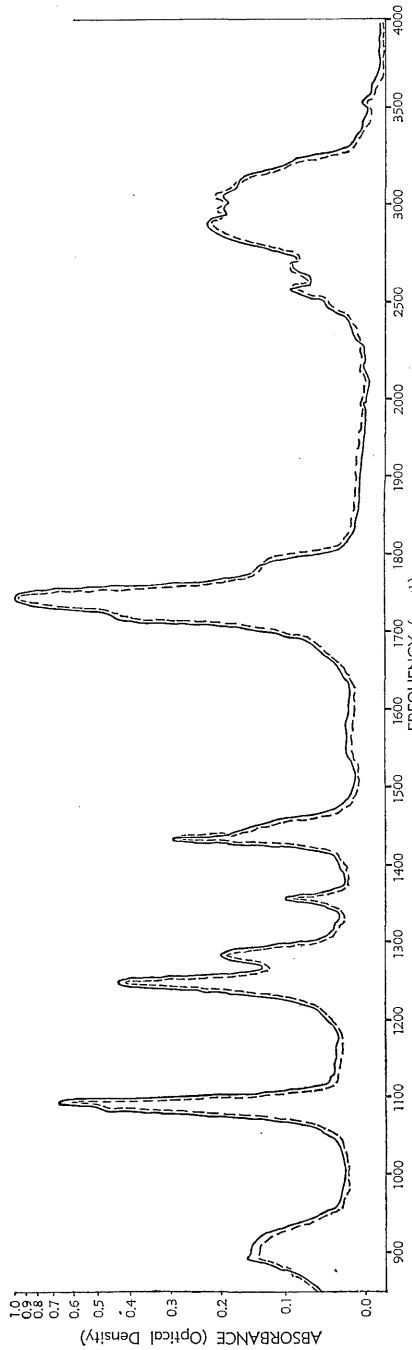

Fig. 4.—Infra-red absorption spectra of carbon tetrachloride solutions of monofluoroacetic acid – – – – and the acid isolated from *G. grandiflorum* ————

This evidence confirms the identification of monofluoroacetic acid in *G. grandiflorum*.

(f) Citric Acid Estimation

Estimations of citric acid concentration were made on the livers and kidneys of rats and on the blood and tissues of sheep. Samples were removed into 8 per cent. trichloroacetic acid solution immediately after death, weighed, macerated and filtered. Citric acid estimations were carried out on the filtrate; results are given in Tables 1–3.

(g) Fluorine Estimations

Quantitative fluorine estimations on monofluoroacetic acid and on the pure acid isolated from *G. grandiflorum* gave similar recoveries of fluorine.

(h) Quantitative Estimation of Monofluoroacetic Acid

Samples of *G. grandiflorum* from three different areas in Queensland were examined quantitatively. Results are given in Table 4.

TABLE 4

CONCENTRATION OF MONOFLUOROACETIC ACID IN *G. grandiflorum*

(mg/kg on air-dried basis)

Material	Area 1. Charters Towers District	Area 2. Clermont District	Area 3. Aramac District
Leaf air-freighted immediately after collection	150	185	—
Leaf air-dried in the field	120	160	105

IV. DISCUSSION

The large-animal toxicity testing of *G. grandiflorum* (Official Records of the Queensland Department of Primary Industries) showed that the mature plant was toxic whether fed fresh or air-dried. To facilitate the identification of the toxic principle, it was necessary to find a suitable laboratory test animal which could be used at least in the initial isolation procedures.

Considerable variation was found in the reaction of mice to extracts of *G. grandiflorum* and oral administration to rats proved much more satisfactory. Toxicity testing with rats was also a guide to the nature of the toxic principle. Symptoms of muscle tremors and tetanic spasms, following the administration of partially purified extracts, closely resembled those found when rats were dosed with purified extracts of *Acacia georginae* (Oelrichs and McEwan 1962). Biochemical analysis or organs showed the presence of high levels of citric acid and suggested that the toxin of *G. grandiflorum* might also be the monofluoracetic acid radical.

Using the procedure already developed for *A. georginae,* an extract was obtained from 600 g of leaf which produced characteristic monofluoroacetic acid poisoning and elevated levels of citric acid in the blood and organs of a sheep. The lower levels of citric acid in the brain of the sheep dosed with plant extract could be due to the rapidity of death ($3\frac{1}{2}$ hr as compared with 6 hr in the sheep which received monofluoroacetic acid), or to the slightly greater time lapse between death and the sampling of brain tissue in the sheep poisoned with *G. grandiflorum* extract. Dosing of a sheep with massive doses of residual plant material showed that extraction had removed virtually all the toxic principle.

Examination of samples of the toxic extract by gas chromatography revealed the presence of an acid identical in retention time with monofluoroacetic acid. Further extraction, silicic acid partition chromatography and passage through a gas chromatographic column provided a solution in carbon tetrachloride which gave an infra-red absorption spectrum identical with that of pure monofluoroacetic acid in saturated solution in carbon tetrachloride. Micro fluorine analysis confirmed the presence of fluorine in concentration equivalent to monofluoroacetic acid.

All evidence is in agreement with the conclusion that monofluoroacetic acid is the toxic principle of *G. grandiflorum.*

The first report of monofluoroacetic acid as the active principle of a toxic plant was that of Marais (1944) in the leaf of *Dichapetalum cymosum* in South Africa. Serious stock losses from this plant had been reported by Rimington (1935), and Marais (1943) had succeeded in isolating the toxin. A level of 15 mg monofluoroacetic acid per gram of the dry plant has been quoted by Watt and Breyer-Brandwijk (1962, p. 380).

Naturally occurring monofluoroacetic acid toxicity was next reported by Oelrichs and McEwan (1961), who identified the fluoroacetate ion as the toxic principle in the leaf and pod of *Acacia georginae* in Queensland. Subsequently, Murray, McConnell, and Whittem (1961) recorded the suspected presence of the acid in *A. georginae* in the Northern Territory. Oelrichs and McEwan (1962) reported levels usually ranging from 10 to 40 mg/kg in leaf, pod and seed, with one sample of young, immature seed showing 390 mg/kg on an air-dried basis.

The levels found in *G. grandiflorum* ranged from 105 to 185 mg/kg in the air-dried leaf, but some field reports suggest that under some circumstances these levels must be greatly exceeded.

The symptoms and post-mortem changes reported by Bennetts (1935) for sheep poisoned with four species of *Oxylobium* and 16 species of *Gastrolobium* in Western Australia are strikingly similar to those associated with *G. grandiflorum* toxicity and with varying doses of monofluoroacetic acid. It seems most probable that the same toxin—monofluoroacetic acid—or a toxin containing this radical is responsible for the toxicity of these species.

V. ACKNOWLEDGEMENTS

The author wishes to acknowledge with thanks the assistance of officers of the Veterinary Services Branch and Mr. M. Neill (Shire Clerk, Aramac) for collection of plant samples; Mr. S. L. Everist (Government Botanist) and his staff for identification of plant specimens; officers of the Pathology Branch, in particular Mr. L. Laws, for dosing and pathological examination of sheep and for valuable advice in other aspects of this work; Mr. H. J. Schnitzerling (C.S.I.R.O. Veterinary Parasitology Laboratory) for infra-red spectra; and Dr. J. M. Harvey for fluorine estimations and for his help and advice throughout this work and in the preparation of this paper.

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ADDENDUM

Since completion of this manuscript information has been received from Dr. J. R. Cannon, Chemistry Department, University of Western Australia, that he and his co-workers have recently obtained evidence independently that monofluoroacetic acid is present in the toxic Western Australian plants *Gastrolobium callistachys* and *Oxylobium parviflorum.*

(Received for publication October 11, 1963)

MELIOIDOSIS IN ANIMALS IN NORTH QUEENSLAND. 3. BACTERIOLOGY

By L. LAWS, B.V.Sc.*

SUMMARY

The bacteriology of 74 strains of *Pseudomonas pseudomallei* was studied. All strains had a characteristic earthy odour, showed marked bipolar staining and were motile with a single polar flagellum. When incubated at 37°C all strains grew well on MacConkey agar, nutrient agar, and 10 per cent. sheep blood agar. On incubation at 21°C on nutrient agar and 10 per cent. sheep blood agar and at 42°C on nutrient agar growth occurred but was slower.

Colonial type was variable. Rough and smooth forms and forms intermediate between these two were present.

All strains produced acid in litmus milk with slow digestion of the clot, liquified gelatin, reduced methylene blue, produced ammonia, formed catalase and fermented glucose with the production of acid, but without gas. Indole was not formed by any strains nor was hydrogen sulphide produced. In addition, the methyl red and Voges-Proskauer tests were negative. Growth occurred in the presence of potassium cyanide, in Koser's citrate and Levine's medium. Agglutination did not occur in 1/1000 trypaflavine or 0·4 per cent. or 0·85 per cent. saline. No pigmented colonies were produced.

The strains varied in their ability to ferment lactose, sucrose, maltose and mannite, to reduce nitrate, to liquify Leoffler's medium, to form urease and to grow on SS agar.

There was no demonstrable toxin in 24-hr broth cultures. The growth of *Ps. pseudomallei* was not inhibited by increased concentrations of neutral red, crystal violet or bile salts in MacConkey agar.

***In vitro, Ps. pseudomallei* was most sensitive to chloramphenicol, Aureomycin and tetracycline and partially sensitive to Terramycin and sulphafurazole.**

Forty-three guinea-pigs died from melioidosis following intraperitoneal inoculation with emulsions of pus or tissue, two after inoculation with cultures from muddy water and six after inoculation with broth cultures of *Ps. pseudomallei*. Invariably these showed abscesses of the liver, spleen and omentum and a peritonitis. Sometimes, also, there were subperitoneal abscesses and abscesses of the lungs and kidneys.

I. INTRODUCTION

The causative organism of melioidosis, originally described as *Bacillus pseudomallei* by Whitmore (1913) and since named *Bacillus whitmori* by Stanton and Fletcher (1921), *Pfeifferella whitmori* (Wilson and Miles 1955),

Animal Research Institute, Yeerongpilly (Queensland Department of Primary Industries).

Malleomyces pseudomallei (Anon. 1948), *Leofflerella pseudomallei* (Brindle and Cowan 1951), has been classified by Bergey (Anon. 1957) as *Pseudomonas pseudomallei.* Wetmore and Gochenour (1956) comment on the *in vitro* similarity between *Ps. pseudomallei* and the achromogenic forms of *Ps. aeruginosa.*

During investigation at Oonoonba Animal Health Station of the incidence and epidemiology of melioidosis in domestic animals in North Queensland, 194 strains of *Ps. pseudomallei* were recovered from 116 naturally infected animals and two strains were recovered from muddy water. This paper reports the examination of 67 of these, including two from water, two National Collection Type Culture strains (N.C.T.C.), three strains isolated by Lewis and Olds at the Animal Health Station (Oonoonba) and two strains from humans in Townsville.

II. MATERIALS AND METHODS

The method of examination of abscesses and central nervous system (C.N.S.) tissue by culture onto 10 per cent. sheep blood agar and MacConkey agar and by intraperitoneal inoculation of guinea pigs was described by Laws and Hall (1963).

Of the 65 strains recently isolated from animals, 40 were from pigs, 16 from sheep, 5 from goats, 3 from cattle and 1 from a horse. Both strains from muddy water were recovered from a swamp near Townsville. The two N.C.T.C. strains were No. 1688 from a rat and No. 8018 from a sheep. The origin of two of the strains of Lewis and Olds is not known. The third was Oonoonba ("O") strain recovered from a lamb (Case 1, Lewis and Olds 1952). It was used for the preparation of antiserum in rabbits and the preparation of antigens for serological tests. The two human cases were Rimington's Cases V and VI (1962).

Of the 67 strains isolated by the author, 65 were the original culture or the first, second or third subculture, while the other two, A234 and A709, were freeze-dried cultures. The N.C.T.C. strains were freeze-dried cultures also. The number of times the last four strains were subcultured is not known. Although "O" strain has been subcultured several times, the exact number is not known. The history of the other two strains of Lewis and Olds is not known. One had been stored for at least seven years at room temperature on a glycerol agar slope. The two human strains examined were the first and second subcultures respectively. Table 1 gives the subculture tested and the length of storage of the 74 strains.

All strains were inoculated into Difco nutrient broth, incubated for 24 hr and the resultant growth used to inoculate all test media. Nutrient agar, MacConkey agar and SS agar were prepared according to the Difco Manual (Anon. 1953). Blood agar plates were 10 per cent. sheep blood in Difco nutrient agar base. Glycerol agar plates were 5 per cent. glycerol in Difco nutrient agar base.

For growth of *Ps. pseudomallei* at different temperatures of incubation and on various media the following were tested:—

At 21°C—all cultures on nutrient agar plates and blood agar plates and 15 cultures on glycerol agar plates.

At 37°C—all cultures on nutrient agar plates, blood agar plates, MacConkey agar plates and SS agar plates.

At 42°C—all cultures on nutrient agar plates only.

For incubation at 21°C a thermostatically controlled refrigerated incubator was used. All plates were labelled and stored in the incubator for several hours before use. They were removed two at a time, sown and immediately returned to the incubator. Ten to 15 cultures were examined on any one day. The inoculum was at room temperature. Electric bacteriological incubators were used for incubation at 37°C and 42°C. A similar technique to the above was adopted.

To ensure that nutrient to support growth on SS agar was not being carried over with the inoculum of nutrient broth, the inoculation of many of the strains was done from either saline-washed organisms or the top of single colonies on nutrient agar.

Motility was determined by dark-ground microscopy on a drop of 24-hr broth culture incubated at 37°C. Flagella were stained by Kirkpatrick's method (Mackie and MacCartney 1938). If the examination was unsatisfactory on 24-hr cultures the organism was subcultured several times at 24-hr intervals and examined again. The fresh suspension of organisms in distilled water was examined. This was then incubated at 37°C overnight and the supernatant smeared again.

The tests for indole, hydrogen sulphide on triple sugar iron agar, methyl red, Voges-Proskauer and potassium cyanide were done by the methods given in the International Bulletin of Bacteriological Nomenclature and Taxonomy (1958). Where two methods are given the one used is indicated.

Methods given by Wilson and Miles (1955), using media prepared from Difco products, were used for methylene blue reduction, catalase production, the detection of hydrogen sulphide by the use of lead acetate paper and reduction of nitrate.

The methods given in the Difco Manual (Anon. 1953) were used for detecting urease, the liquefaction of gelatin and preparation of Koser's citrate medium. The test for liquefaction of gelatin was also done by Smith's Method (Smith 1946). Levine's medium was described by Levine *et al.* (1954). Litmus milk and Loeffler's medium were prepared according to Mackie and MacCartney (1938). Horse serum was used for the Loeffler's medium. Ability to ferment glucose, sucrose, maltose, mannite, and lactose in peptone water (Wilson and Miles 1955) was observed during 14 days' incubation at 37°C.

To test for agglutination in trypaflavine or 0·4 per cent. or 0·85 per cent. saline, the organisms were grown for 72 hr on nutrient agar at 37°C and suspended in each of the three solutions. These were incubated overnight at 37°C.

For observations on pigment production all strains were sown onto nutrient agar incubated at 37°C for 4 days then left at room temperature in the light for 7 days.

Growth of the organism on MacConkey agar containing either added crystal violet, neutral red or bile salts was tested. Growth was also tested in MacConkey broth containing added crystal violet and/or penicillin and streptomycin. The MacConkey agar was prepared according to Difco Manual (Anon. 1953) but with the following concentrations of the inhibiting substances: crystal violet 0·0002 per cent., 0·0003 per cent., 0·001 per cent., 0·01 per cent., 0·02 per cent., 0·1 per cent.; neutral red 0·006 per cent., 0·009 per cent., 0·03 per cent., 0·06 per cent.; bile salts 0·075 per cent., 0·15 per cent., 0·3 per cent. The MacConkey broth contained the constituents of MacConkey agar (Anon. 1953) without the agar, adjusted to pH 7·1 and with the following concentrations of the inhibiting substances: crystal violet 0·001 per cent., 0·02 per cent.; crystalline sodium penicillin 25 Units per ml and streptomycin sulphate 50 Units per ml; crystal violet 0·02 per cent. and penicillin and streptomycin as above.

Both the solid and the liquid media were inoculated with a drop of a 48-hr nutrient broth culture of *Ps. pseudomallei,* then incubated at 37°C. After 48 hours' incubation, a drop of the liquid medium was plated on MacConkey agar.

In vitro sensitivity to chemotherapeutic agents was done with tablets and discs on 10 per cent. sheep blood agar. Strain A234 (bovine) was tested with Evans Sentest Tablets and "O" strain with Evans Sentest Tablets, Multo Discs (Oxoid) and tablets of Vancomycin and Ilosone (Eli Lilly).

To test the pathogenicity of four strains which were recovered by culture, and not by guinea-pig inoculations, and of two strains of Lewis and Olds, a 24-hr broth culture of each strain was inoculated intraperitoneally into a guinea pig.

To test for toxin production, bacteria-free filtrates of 24-hr broth cultures were prepared by filtration through Hormann-Ekwip D9 filters. These were inoculated subcutaneously into two sheep and intraperitoneally into two guinea pigs. Each sheep was given 1 ml and the guinea pigs 0·1 and 0·2 ml.

III. RESULTS

The results are summarized in Table 1.

All cultures of *Ps. pseudomallei,* whether primary isolations or subsequent subcultures, had the characteristic earthy aromatic odour, which was well developed after 24 hours' incubation.

TABLE 1

SOURCE, LENGTH OF STORAGE, AND SOME OF THE VARIABLE CHARACTERISTICS OF 74 STRAINS OF *Ps. pseudomallei*

Pigs

Strain No.	56/125	56/130	56/290	A 272	A 233	A 252	A 393	A 314	A 331	A 424	A 447	A 450	A 697	A 713	B 303	B 586	C 107	D 286	D 412	D 411
Subculture tested	1st	1st	3rd	1st	2nd	1st	3rd	2nd	1st	3rd	3rd	2nd	3rd	1st	1st	1st	1st	1st	1st	1st
Length of storage (months)	51	51	49	40	40	40	38	39	39	37	37	37	34	33	27	21	18	4	2	2
Lactose fermentation†	–	A5	–	A5	A3	A6	–	A5	A7	–	A7	A5	A13	A3	A3	A6	A7	A7	–	A5
Nitrate reduction	+	–	+	–	+	+	+	–	–	+	+	–	+	–	–	–	+	–	+	–
Liquefaction of Loefflers	+	+	+	–	+	+	+	+	+	–	+	+	+	–	+	+	–	+	+	+
Growth on SS agar	–	+	+	+	+	–	+	+	+	+	–	+	–	+	–	–	+	+	+	+

Strain No.	D 282	D 425	D 348	D 208	D 470	D 460	D 413	D 299	A 709*	A 714	E 218 (2)	E 218 (3)	E 218 (4)	E 222	E 230	E 273	E 286	E 288	E 298	E 300
Subculture tested	1st	1st	1st	1st	1st	1st	1st	1st	2nd	1st	orig.	1st	1st	orig.	orig.	orig.	orig.	orig.	orig.	orig.
Length of storage (months)	4	2	3	6	1	1	2	4	33	40	–	1	1	–	–	–	–	–	–	–
Lactose fermentation†	A3	A5	A2	–	A3	A3	A3	A1	A5	A7	A4	A4	A4	A14	A5	A5	A4	A4	A9	A5
Nitrate reduction	–	–	+	+	+	+	+	–	+	+	+	+	+	–	+	+	+	+	–	+
Liquefaction of Loefflers	+	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Growth on SS agar	–	+	+	+	+	+	+	+	+	+	+	–	+	+	+	–	–	–	–	–

Sheep

Strain No.	56/440	A 120	B 469	C 113	116	G 91	D 361	D 50	F 68	D 46	D 207	J 53	D 88	F 67	"O"	E 156 (1)	N.C.T.C. 8018*	E 156 (3)
Subculture tested	2nd	2nd	3rd	1st	3rd	1st	1st	1st	1st	1st	1st	1st	1st	1st	?	orig.	?	orig.
Length of storage (months)	46	42	24	18	18	6	3	8	8	8	6	5	7	8	104	–	?	–
Lactose fermentation†	A3	A5	A5	A3	A7	A7	–	A5	A3	A3	A3	A7	A5	–	A11	A4	A5	A4
Nitrate reduction	–	+	+	–	+	+	–	–	–	+	–	+	+	–	+	–	+	+
Liquefaction of Loefflers	+	+	+	–	+	+	+	+	+	–	+	–	+	+	+	+	+	+
Growth on SS agar	+	+	+	+	–	–	+	+	+	+	+	+	+	+	–	+	+	+

	Goats					Cattle			Horse	Human		Unknown (Lewis & Olds)		Rat	Soil	
Strain No.	D 136	D 196	T 878	D 131	D 212	A 234	A 234*	B 297	C 119	D 238	S	No. 6	No. 121	N.C.T.C. 1688*	5	10
Subculture tested	1st	1st	1st	1st	1st	1st	2nd	1st	1st	1st	1st	?	?	?	1st	1st
Length of storage (months)	6	6	3	6	6	40	40	27	18	5	4	>84	>84	?	1½	1
Lactose fermentation†	A3	A2	A5	A3	A4	–	A5	A4	A3	A3	A4	–	A5	A9	A14	A4
Nitrate reduction	–	+	–	+	+	–	+	+	–	–	+	+	+	+	–	+
Liquefaction of Loefflers	–	+	+	–	+	+	+	+	–	+	–	–	+	+	+	+
Growth on SS agar	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+

? Not known.
* Freeze dried.

† A followed by a numeral indicates acid production after this number of days' incubation.
orig. Original culture.

In all freshly isolated strains, the organisms were short Gram-negative rods with marked bipolarity. On subculture they became pleomorphic and bipolarity decreased. The large elements 15-20 μ in length mentioned by Stanton and Fletcher (1932) and not observed by Cottew (1950) were seen occasionally in subcultures on solid media. They were not observed in primary isolations.

After 24 hours' incubation in nutrient broth all strains produced turbidity with a thin surface pellicle. On further incubation the pellicle thickened and a ropy deposit developed at the bottom of the tube.

On primary isolation, most of the strains had the colonial form of Cottew's (1950) "type A." A high percentage of these colonies showed concentric rings of growth. After prolonged growth on both blood and MacConkey agar, radiate wrinkling sometimes appeared. This is described by Cottew as occurring on glycerol agar. All of these "type A" colonies were of glutinous consistency. They were opaque, frosty white on blood agar and pink on MacConkey agar. Several strains produced round, smooth, moist, mucoid colonies with an entire edge. They were translucent on blood agar and red on MacConkey agar. By successive subculturing all could be changed to "type A" colonial form. This change occurred after a variable number of subcultures. At times both "type A" and smooth forms or forms intermediate between these two appeared on the same plate. Regardless of colonial type, the ability to ferment lactose on MacConkey agar was sometimes lost. On three occasions, these lactose nonfermenting organisms were inoculated into guinea pigs. They fermented lactose normally on MacConkey agar on recovery from the guinea-pig tissues.

Broth subcultures inoculated from either "type A" or "smooth" colonies and incubated for 24 hr were pathogenic on intraperitoneal inoculation of guinea pigs.

Fifteen strains were tested for growth on glycerol agar at 21°C. These included the N.C.T.C. strains and all grew under these conditions.

The following characteristics were possessed by all 74 strains examined: Growth on nutrient agar occurred at 21°C, 37°C and 42°C. Growth occurred on 10 per cent. sheep blood agar at 21°C and 37°C. (No tests were done on blood agar at 42°C.) Growth occurred on MacConkey agar at 37°C. The bacteria were motile with a single flagellum at one pole. Acid was produced in litmus milk with clot formation and then slow digestion of the clot. Gelatin was liquefied when tested after seven days (Difco and Smith's gelatin). Methylene blue was reduced. The catalase test was positive. Ammonia was produced. Indole was not formed (Erlich-Boehme). Hydrogen sulphide was not produced in triple sugar iron agar or by the lead acetate paper method. Methyl red and Voges-Proskauer tests (Barritt) were negative. Growth occurred in the presence of potassium cyanide, in Koser's citrate (Difco) and Levine's medium. Glucose was fermented without gas formation. Agglutination did not occur in 1/1000 trypaflavine or 0·4 per cent. or 0·85 per cent. saline. No yellow colonies were seen.

The strains varied in their ability to ferment lactose, reduce nitrate, liquefy Loeffler's medium, grow on SS agar. These results are given in Table 1.

The variable cultural characteristics were: Acid was produced from lactose by 64 strains, from sucrose by 44 strains, from maltose by 61 strains, from mannite by 66 strains. No gas was produced. Nitrate was reduced to nitrite by 45 of the strains. Loeffler's medium was liquefied by 60 strains. An indefinite, faint pink, urease reaction was seen with 10 strains (Kauffmann). Growth on Difco SS agar was demonstrated in 57 of the strains but it was variable, with only a few colonies developing from a loop of inoculum on some of the plates. Both lactose and non-lactose fermenting colonies were seen.

Ps. pseudomallei grew on MacConkey agar containing crystal violet in concentrations from 0·0002 per cent. to 0·02 per cent. inclusive. It did not grow on MacConkey agar containing 0·1 per cent. crystal violet. The colonies on agar with 0·02 per cent. crystal violet were umbonate and even after prolonged incubation did not develop greater than 1-mm dia. The colonies on the remaining plates had the same colonial form as those on MacConkey agar. They were purple instead of pink. At all concentrations of neutral red and bile salts, growth of the organism was comparable to that on MacConkey agar. The colonies on plates containing added neutral red were red. Growth occurred in the MacConkey broths containing added crystal violet, penicillin and streptomycin and crystal violet plus penicillin and streptomycin.

Strain A234 was sensitive to chloramphenicol, Aureomycin and tetracycline, partially sensitive to Terramycin and resistant to penicillin, streptomycin and erythromycin.

Strain Oonoonba was sensitive to chloramphenicol, tetracycline and Aureomycin, partially sensitive to sulphafurazole and Terramycin, and resistant to Vancomycin, Ilosone, bacitracin, neomycin, nitro-furantoin, penicillin, polymyxin B, streptomycin, erythromycin, novobiocin and oleandomycin.

One hundred-and-sixty-five samples of pus or tissue from animals were inoculated into guinea pigs to check for melioidosis. Of these, 43 died with lesions indicative of melioidosis and from which *Ps. pseudomallei* was recovered. The two strains recovered from muddy water were isolated from fatally infected guinea pigs. A further 6 guinea pigs inoculated with cultures of strains "O", G91, A234, D207, J53, and Lewis and Olds No. 6 died.

All of the infected guinea pigs had multiple abscesses of the liver and spleen, peritonitis and multiple abscess formation and folding of the omentum into a firm mass along the greater curvature of the stomach. The Strauss reaction was seen in male guinea pigs. On a few occasions abscesses were also detected in the lungs, kidneys and subperitoneally on the ventral abdominal wall.

Inoculation of the bacteria-free filtrate produced no ill-effects in the sheep or guinea pigs.

IV. DISCUSSION

The bacteriological findings agreed with those described by Bergey (Anon. 1957) and Cottew (1950) with the exception that not all of the strains reduced nitrates or liquefied Loeffler's medium. Some strains grew on SS agar. Glucose was regularly fermented with the production of acid but without the production of gas. The variation in fermentation of the other carbohydrate media tested, limits the use of these tests for identification. Fermentation of lactose when it occurs helps to distinguish *Ps. pseudomallei* from *Ps. aeruginosa.*

Kirkpatrick's method for staining flagella was most satisfactory on organisms which had been subcultured on nutrient agar several times at 24-hr intervals. The best results were obtained if the bacterial suspension in distilled water was left at 37°C overnight and the supernatant smeared.

The marked variation of colonial type reported by Nigg, Ruch, Scott, and Noble (1956) was seen. Some colonies resembled those of the coliform bacillus. Broth cultures prepared from either "type A" or smooth forms were pathogenic for guinea pigs on intraperitoneal inoculation.

Wetmore and Gochenour (1956) examined 15 strains of *Ps. pseudomallei* and 20 strains of *Pseudomonas aeruginosa.* They found that *Ps. aeruginosa* grew on glycerol agar at 21°C and on Difco SS agar at 37°C, while *Ps. pseudomallei* did not. Fifty-two of my strains and the N.C.T.C. strain 8018 grew on Difco SS agar at 37°C. All strains grew on blood agar at 21°C and all of the 15 strains tested, including the two N.C.T.C. strains, grew on 5 per cent. glycerol agar at 21°C.

Cottew (1950) and Brygoo and Richard (1952) described yellow pigmented colonies. No yellow colonies were seen in either primary isolations or subsequent subcultures on any of the media used. For storage, cultures were incubated for 48 hr at 37°C on nutrient agar slopes, in air-tight bottles, then placed in a dark cupboard at room temperature. One of these stored cultures developed a salmon pink colour.

Gallie (1942) obtained good inhibition of most lactose-fermenting coliform bacilli by modifying the constituents of MacConkey agar. Tests were done to determine the inhibition of *Ps. pseudomallei* by increase in concentration of the inhibitive substances in MacConkey agar. No tests were done on the inhibition of other Gram-negative bacilli or Gram-positive organisms. As an increase in concentration of bile salts or neutral red did not inhibit the growth of *Ps. pseudomallei,* and as it grew on MacConkey agar containing up to 0·02 per cent. crystal violet, media with increased concentration of these dyes were prepared for examination of soil and water samples for *Ps. pseudomallei.*

Chambon, De Lajudie, and Fournier (1954) stated that chloramphenicol was the only antibiotic effective against melioidosis and Aureomycin and Terramycin were useless. Moustardier, Dulong de Rosnay, and Salvat (1959) reported that a strain of *Ps. pseudomallei* from a man was sensitive *in vitro* to chloramphenicol, novobiocin and the tetracyclines. Hezebicks and Nigg (1958)

demonstrated the efficacy of chloramphenicol, sulphonamides and chlortetracycline in prolonged doses on melioidosis in mice. Novobiocin was effective in a low percentage of cases and the other tetracyclines had a suppressive action on this infection. Laws and Hall (1963) showed temporary clinical improvement in sheep experimentally infected with melioidosis following treatment with chloramphenicol, tetracycline or sulphadiazine, but their tests were not critical. *In vitro,* both strains A234 and Oonoonba were sensitive to chloramphenicol, tetracycline and Aureomycin and partially sensitive to Terramycin. Oonoonba strain (the only strain tested) was also partially sensitive to sulphafurazole but resistant to novobiocin.

The lesions in guinea pigs were similar to those described by Whitmore (1913) and Cottew (1950). Abscess formation, with folding of the omentum, was a constant feature of melioidosis produced by intraperitoneal inoculation. Cultures or tissue suspensions containing *Brucella suis, Corynebacterium ovis* or *Chromobacterium violaceum* produced visceral abscesses in guinea pigs on intraperitoneal inoculations. In these latter infections there were no abscesses in the omentum. Francis (1958), quoting Griffith (1911), stated that guinea pigs dying following intraperitoneal inoculations of 0·1 mg of avian *M. tuberculosis* showed discrete nodules filled with yellow pus in the omentum.

Colling, Nigg, and Heckly (1958) stated that their early work in which mice and hamsters died in 1 or 2 days with no gross lesions after inoculation of viable organisms suggested that death was due to a lethal toxin and subsequently they proved that *Ps. pseudomallei* produced a toxin. Heckly and Nigg (1958) demonstrated two thermolabile exotoxins and a thermostable endotoxin in bacteria-free filtrates of cultures of *Ps. pseudomallei* incubated for 7 days. The rapid rise in rectal temperature of experimentally infected sheep (Laws and Hall 1963) suggested that perhaps a toxin was produced even though cultures for inoculation had been incubated for 24 hr only. Bacteria-free filtrates of broth cultures similar to those used to infect the experimental sheep produced no ill-effect when inoculated into sheep or guinea pigs. These sheep did not produce detectable C.F. antibodies.

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(Received for publication August 27, 1963)

STUDIES ON FACTORS IN BEEF CATTLE PRODUCTION IN A SUBTROPICAL ENVIRONMENT. 2. GROWTH TO WEANING

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SUMMARY

Data from 973 calves from 259 cows during the years 1955-1960 at "Brian Pastures" Pasture Research Station in south-eastern Queensland were analysed. The inter-relationships between birth weight, weaning age, daily gain to weaning, weaning weight and weaning score were studied and the effects of sex, year of birth, age of dam, weight of dam and time of birth on them were evaluated.

The mean birth weight of all calves was 72·3 lb, with very significant sex and year differences. The mean suckling gain was 1·52 lb per day but was the most variable character studied, year differences, age and precalving history of the cow having significant effects. The repeatability of suckling gain was high. Weaning weight averaged 339 lb at 172 days, with significant year, sex and dam influences. Weaning score was not nearly so variable as the other characters measured and was only influenced by whether a cow had had a calf the previous year or not.

Birth weight exerted its major effect on weaning weight, which in turn was closely associated with suckling gain and weaning score.

I. INTRODUCTION

The seasonal nature of the growth curve of beef cattle under Queensland conditions has been studied quite extensively (Chester 1952; Alexander and Chester 1956; Shelton 1956; Sutherland 1959). However, these investigations have been devoted almost exclusively to the description of growth after weaning Very little information is available on the performance of beef animals prior to weaning. The first paper in this series (Alexander *et al.* 1960) dealt with birth weight of beef calves. In this paper, it is proposed to examine the period from birth to weaning.

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II. EXPERIMENTAL PROCEDURE

The investigation recorded in this paper was based on the growth rates of 793 calves from 259 cows during the years 1955 to 1960, inclusive, at "Brian Pastures" Pasture Research Station in south-eastern Queensland. Owned by the Australian Meat Board, the station is operated by the Queensland Department of Primary Industries as a pasture research station with emphasis on beef cattle production. The property is situated about 10 miles from Gayndah in latitude 25° 40′S. and has an average rainfall of about 29 in., mainly of summer incidence. The property consists of ridges of varying slopes and broken areas of river bank and flood plain regions along the small creeks flowing into Barambah Creek, which forms the eastern boundary of the property. Originally the vegetation was open eucalypt forest and the pasture grasses now are *Heteropogan contortus* and species of *Dichanthium* and *Bothriochloa.*

Seasonal mating is practised on the property, so the calving usually extended over approximately 10 weeks from late October to early January. The cows were either Hereford or Poll Hereford cows of known age mated to Poll Hereford bulls.

The inter-relationships between birth weight of calf, weight of dam, weaning age of calf, daily gain in weight of calf from birth to weaning, weaning weight, and weaning score were studied. Weaning was considered to be the weighing date nearest to the time when the average age of the calves was six months. The actual weaning dates were somewhat variable; they were close to eight months during the early years but since 1957 have been standardized at six months of age.

Preliminary analyses indicated that interactions were unimportant and regressions could be regarded as homogeneous from year to year. An additive linear model was therefore fitted by least squares, constants being years, sex, previous history of cow (i.e. calved or did not calve in previous year) and cows. The computational techniques of Rao (1955) were used.

The weight of the dam was taken to be that at the time of weaning of the calf, while the weaning score was the average of scores placed on each calf at weaning by four independent scorers. Two scorers were beef cattle producers and two were Departmental officers; the scoring method used was that described by Wagnon, Albaugh, and Hart (1960).

III. RESULTS

Birth Weight.—The mean birth weight based on the dam as an adult having a calf in the previous year and averaged over years, sexes and cows was 72·3 lb. There were very significant year and sex differences in birth weight (Tables 1 and 2). Differences in the birth weights of calves from heifers, cows not having calves in the previous year and cows which did, were not significant (Table 2). The repeatability of birth weight was significant but of relatively small magnitude (Table 3).

TABLE 1

MEAN VALUES FOR BIRTH WEIGHT, WEIGHT OF DAM, WEANING AGE, GAIN, WEANING WEIGHT AND WEANING SCORE

—	Overall Mean and Standard Deviation	Coeff. of Variation (%)	Year					
			1955	1956	1957	1958	1959	1960
Birth weight (lb) ..	72 3 ± 9·4	13·0	71·7	72·3	75·8	68·4	73·9	71·7
Weight of dam (lb) ..	883 ± 105	11·9	879	895	824	912	933	855
Weaning age (days) ..	172 ± 17·7	10·3	172	169	168	174	171	178
Daily gain to weaning (lb)	1·54 ± 0·22	14·6	1·50	1·60	1·38	1·66	1·55	1·43
Weaning weight (lb) ..	339 ± 48	14·2	331	345	311	386	338	323
Weaning score ..	72·6 ± 3·4	4·6	69·4	72·4	72·6	73·9	74·0	73·3

TABLE 2

EFFECT OF SEX AND HISTORY OF DAM ON THE PREWEANING AND WEANING PERFORMANCE OF THE CALF

—	Male–Female (Mean difference and Standard Error)	Difference Adult without Calf Previous Year—Adult	Adult–Heifer
Birth weight (lb)	4·94 ± 0·70***	+ 1·08 ± 0·99	2·58 ± 1·35
Weight of dam (lb) ..	2·70 ± 3·69	52·3 ± 5 3***	57·3 ± 7·19***
Weaning age (days) ..	−0·11 ± 1·47	8·15 ± 2·10***	−10·5 ± 2·86***
Daily gain to weaning (lb)	0·025 ± 0·013***	0·095 ± 0·019***	0·149 ± 0·026***
Weaning weight (lb) ..	10·52 ± 2·96	32·0 ± 4·2***	+20·4 ± 5·8***
Weaning score	0·173 ± 0·227	1·40 ± 0·32***	+0·629 ± 0·442

*** Significant at the 0·1 per cent. level of probability.

TABLE 3

REPEATABILITY OF VARIOUS FACTORS

—	Repeatability	95% Fiducial Intervals
Birth weight	0·23	+0·15 to +0·31
Weight of dam	0·83	+0·79 to +0·85
Weaning age	0·02	−0·05 to +0·09
Daily gain to weaning	0·51	+0·43 to +0·57
Weaning weight	0·47	+0·39 to +0·54
Weaning score	0·35	+0·27 to +0·43

Weight of Dam.—Very significant year differences around the mean of 883 lb were recorded in the weights of the cows, due particularly to the lower weights in the drought years of 1957 and 1960 (Table 1). No effect of sex of calf on weight of dam at weaning was recorded but significant differences were obtained in the different classes of cow. Heifers were significantly lighter than cows which had a calf the previous year ($P < 0·001$) and the difference in body-weight between cows calving the previous year and those which did not was also significant at the 0·1 per cent. level (Table 2). On eliminating weaning age, these differences were still significant at the 0·1 per cent. level. The repeatability of body-weight of cows was high (0·83).

Daily Gain to Weaning or Suckling Gain.—The suckling gain of the calves was among the most variable of the characteristics studied, having a coefficient of variation of 14·6 per cent. Year differences were very significant ($P < 0{\cdot}001$) but no significant effect of sex on preweaning gain was observed (Tables 1 and 2). Differences significant at the 0·1 per cent. level were recorded between heifers and adult cows and between adult cows and those which did not have a calf in the previous year. The repeatability of suckling gain was 0·51, which was significant and of a reasonably high order (Table 3).

Weaning Weight.—Weaning weight varied similarly with suckling gain and had a similar order of coefficient of variation (Table 1). Year differences were also very significant ($P < 0{\cdot}001$). There was a very significant sex difference in weaning weight although the sex difference in gain did not quite attain significance (Table 2). The three classes of dam had calves with significantly different weaning weights (Table 2) and the significance increased with the elimination of weaning age. Weaning weight had very nearly as high a repeatability as suckling gain (Table 3).

Weaning Score.—Weaning score was not nearly so variable as the other characteristics measured, the coefficient of variation being only 4·6 per cent. (Table 1). Significant year differences in weaning score appeared to show a time trend, with an increase in value with time. While no significance was observed in the score of heifer calves and cows which had a calf in the previous year, there was a significant difference in the score of calves from cows which did and did not have a calf the previous year.

Phenotypic Correlations and Regressions.—The phenotypic correlations between the five characters were not markedly affected by weaning age, so there was little change in the order of the correlations and regressions when weaning age was eliminated (Tables 4 and 5). Birth weight exerted its major effect on weaning weight, while weight of dam was most closely associated with the birth weight of the calf. Suckling gain was markedly associated with weaning weight and score, which were inter-related.

TABLE 4

PHENOTYPIC CORRELATION COEFFICIENTS BETWEEN BIRTH WEIGHT, WEIGHT OF DAM, WEANING AGE, GAIN, WEANING WEIGHT AND WEANING SCORE OF THE CALVES

—	Birth Weight	Weight of Dam	Weaning Age	Gain	Weaning Weight	Weaning Score
Birth weight ..		+0·26***	−0·14*	+0·27***	+0·35***	+0·19**
Weight of dam ..	(+0·26)***		−0·003	+0·09	+0·13*	−0·01
Weaning age ..				−0·18**	+0·29***	+0·13*
Daily gain to weaning	(+0·20)***	(+0·10)			+0·78***	+0·56***
Weaning weight ..	(+0·42)***	(+0·14)*		(+0·89)***		+0·61***
Weaning score ..	(+0·21)***	(−0·01)		(+0·72)***	(+0·60)***	

Values in parentheses are partial correlation coefficients with weaning age eliminated.
* Significant at 5 per cent. level.
** Significant at 1 per cent. level.
*** Significant at 0·1 per cent. level.

TABLE 5

REGRESSION COEFFICIENTS BETWEEN BIRTH WEIGHT, SUCKLING GAIN AND OTHER CHARACTERS

—	Standard Error of Estimate	Regression Coefficient and Standard Error	95% Fiducial Interval for Regression Coefficient
Birth weight on—			
Weight of dam	±8·3	+0·0059 ± 0·008	−0·010 to +0·022
(Weight of dam)	(± 8·2)	(+0·0034 ± 0·0083)	(−0·013 to +0·020)
Weaning age	± 8·2	−0·049 ± 0·021	−0·089 to −0·008
Suckling gain on—			
Birth weight	± 0·156	+0·0017 ± 0·0082	+0·0001 to +0·0033
(Birth weight)	(± 0·150)	(+0·0013 ± 0·00079)	(−0·0003 to +0·0029)
Weight of dam	± 0·145	+0·0013 ± 0·00014	+0·0010 to 0·0016
(Weight of dam)	(± 0·143)	(+0·0013 ± 0·00014)	(+0·0010 to 0·0016)
Weaning age	−0·153	−0·0017 ± 0·00038	−0·0024 to −0·0010

Figures in parentheses are those after weaning age is eliminated.

The regression of weaning age on each characteristic is taken to indicate the effect of time of birth on performance of the calf. The regression of birth weight on weaning age was −0·05 ± 0·021 lb per day. Those calves born later in the calving season were slightly heavier at birth. They also appeared to gain faster prior to weaning, but had lower weaning weights and scores (Tables 4-6).

TABLE 6

REGRESSION COEFFICIENTS BETWEEN WEANING WEIGHT AND WEANING SCORE AND THE OTHER CHARACTERS

—	Standard Error of Estimate	Regression Coefficient and Standard Error	95% Fiducial Interval for Regression Coefficient
Weaning weight on—			
Birth weight	± 34·5	+0·85 ± 0·18	+0·50 to 1·21
(Birth weight)	(± 30·0)	(+1·07 ± 0·16)	(+0·75 to 1·38)
Weight of dam	± 34·2	+0·19 ± 0·033	+0·13 to +0·26
(Weight of dam)	(± 29·4)	(+0·25 ± 0·030)	(+0·19 to +0·30)
Weaning age	± 31·3	+0·93 ± 0·077	+0·78 to +1·08
Daily gain to weaning ..	± 28·2	+136 ± 7·9	+120 to +151
(Daily gain to weaning) ..	(± 19·1)	(+162 ± 5·4)	(+151 to +172)
Weaning score on—			
Birth weight	± 2·70	+0·018 ± 0·014	−0·010 to +0·046
(Birth weight)	(± 2·65)	(+0·024 ± 0·014)	(−1·003 to +0·052)
Weight of dam	± 2·69	+0·0054 ± 0·0027	+0·0001 to +0·0107
(Weight of dam)	(± 2·64)	(+0·0070 ± 0·0026)	(+0·0019 to +0·0121)
Weaning age	± 2·66	+0·0279 ± 0·0066	+0·0149 to +0·0409
Daily gain to weaning ..	± 2·52	6·21 ± 0·71	+4·83 to +2·06
(Daily gain to weaning) ..	(+ 2·43)	(7·07 ± 0·69)	(5·71 to 8·43)

Figures in parentheses are those after weaning age is eliminated.

IV. DISCUSSION

In a previous study of this herd, Alexander *et al.* (1960) reported a mean birth weight of 70·4 lb. The figure of 72·3 lb in this study is based on a much larger number of calves and over a greater number of years. Both these figures are generally in accord with those reported by Dawson, Phillips, and Black (1947), Nelms and Bogart (1956), Koch and Clark (1955) and Clark *et al.* (1958). A significant sex effect was observed in the birth weight of the calf and is of similar order to those reported in the literature. Knapp, Lambert, and Black (1940) considered that 25-35 per cent. of the variation in birth weight between the sexes was accounted for by differences in the length of the gestation period. The gestation length of male calves is generally slightly longer than that of female calves (Dawson, Phillips, and Black 1947; Joubert and Bonsma 1959).

While the birth weights of calves from heifers and adults in this study were not significantly different, significant differences have usually been reported in the literature. These have been associated with shorter pregnancies in heifers. Indirect evidence of the shorter pregnancy in heifers is obtained in this study by the older weaning age of heifers' calves, although it may be merely an indication that heifers did not come into oestrus and conceive as quickly as cows in the mating season. Another factor influencing the birth weight of the calf is the time of birth. Calves born late in the season are generally heavier than calves born early (Koch and Clark 1955; Davenport and Neil 1958; Alexander *et al.* 1960). This is due to the combined influences of better conditions later in the calving season and of longer gestations. Condition of the dam has also been shown to influence the birth weight of the calf (Blaxter 1957; Ryley 1961; Ryley and Gartner 1962; Neville 1962; Wiltbank *et al.* 1962).

Gain during the suckling period is a reflection of the milk production of the cow and the ability of the calf to utilize the available nutrients. This gain has a greater influence on weaning weight than does birth weight and is more variable than birth weight (Clark *et al.* 1958; Knapp and Black 1941). The repeatability of suckling gain of 0·51 in this study is supported by the observation of Botkin and Whatley (1953) and Knapp and Black (1941) that the milk production of the cow exerts a major influence on suckling gain. The gains reported in this study were of similar order to those reported by Clark *et al.* (1958) and Heyns (1960).

Weaning weight in turn is strongly influenced by suckling gain and birth weight. The latter influence would appear to be responsible for the sex difference in weaning weight, since these are the only two performance criteria showing sex differences. Sex and year-to-year differences in weaning weight have also been reported by other workers (Clark *et al.* 1958; Knapp *et al.* 1942).

The influence of time of calving on suckling gain and weaning weight presents some interesting possibilities in interpretation. The regression of suckling gain on weaning age is $-0{\cdot}0017 \pm 0{\cdot}00038$, while that of weaning weight on weaning age is $+0{\cdot}93 \pm 0{\cdot}077$. This indicates that the younger calves at weaning were growing faster but the older calves at weaning were heavier. An examination

of the preweaning growth curve for the calves on this property as reported by Burns and Alexander (1956) shows that the growth rate tends to slow down about weaning. Thus, if the older calves at weaning were gaining more slowly at weaning than the younger calves, this would explain the relationships.

Weaning score has been used in this study as an attempt to reproduce a commercial evaluation of the calves. If it can be presumed that this was successful, then an assessment can be made of the relationship the more objective criteria bear to the weaning score. Calves with high suckling gains and weaning weights scored well (suckling gain b = 6·21 ± 0·71). When weaning age is eliminated, approximately 50 per cent. of the variation in weaning score is associated with suckling gain and 36 per cent. with weaning weight. Only about 4 per cent. of this variation was associated with birth weight. Since weaning weight and suckling gain are very closely associated (r = 0·89, weaning age eliminated), it may be inferred that much of the other half of the variation in weaning score is associated with conformation evaluation. These relationships are of similar magnitude to those reported by Koch and Clark (1955), who concluded from a genetic analysis of their data that selection of calves on the basis of their weaning score would lead to increased genic value affecting weaning score directly and to a lesser extent genic value for maternal environment affecting weaning score. This conclusion could also hold for the present study.

In the selection of beef cattle on performance to weaning it seems desirable that emphasis should be placed upon the milking ability of the cow, growth rate of the calf and conformation grade of the calf. The inter-relationships between the performance criteria describing these three features are important in deciding which should be used and the relative stress to be placed on each. The information presented in this study provides some basic information which can assist in this decision.

V. ACKNOWLEDGEMENTS

The authors wish to acknowledge indebtedness to the following officers of the Queensland Department of Primary Industries: Messrs. M. A. Burns, P. J. Round, J. J. Daly, P. F. Wall (for assistance in the collection of the data) and Miss G. P. Davey and Miss P. M. Pepper for assistance in the statistical analyses.

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(Received for publication August 29, 1963)

AN EXPERIMENTAL PINEAPPLE JUICE CONCENTRATE PLANT INCORPORATING FLAVOUR RECOVERY

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SUMMARY

In order to investigate the concentration of Queensland-produced pineapple juice an experimental plant incorporating an ester recovery section has been designed and constructed. Pulp in the fresh juice is reduced to less than 1 per cent. by screening and centrifuging and then the juice is fed into a turbulent thin film evaporator which strips off the esters during concentration under vacuum. Esters and water are condensed together in a refrigerated surface condenser. The distillate is partially vaporized and then fractionated at atmospheric pressure to produce ester concentrate of 250-fold which is reincorporated with the concentrated juice (50–60° Brix). A detailed description of construction and operation of the plant is given.

I. INTRODUCTION

The production of single-strength canned and bottled fruit juices in Australia amounts to approximately 3½ million gallons annually, of which about 3 million gallons are pineapple juice. The freight and container costs involved in exporting pineapple juice from Queensland to other Australian States and overseas is relatively high when it is considered that the single-strength juice contains over 80 per cent. of water. In 1958 a project was initiated to investigate concentration as a means of reducing these costs in order to assist Queensland's major horticultural industry.

In Queensland, pineapple juice is expressed from the shell (comprising the skin, the flesh up to 1-in. thick, which is removed during the peeling operation), the core and the trimmings from the cylinders of flesh. Since this method differed from that used in Hawaii, where skins are not pressed, and this juice may have contained undesirable constituents extracted from the skin (Seale 1953), it was considered essential to install a small experimental juice concentration plant to undertake this work.

Although the production of frozen concentrated juices, particularly orange juice, has become a well-established industry in many countries, frozen concentrated pineapple juice is manufactured only in Hawaii.

Anon (1952), Smyser (1952), Jefferson and Lloyd (1952), Seale (1953), Tressler and Joslyn (1954) and Kefford (1954) reported that it was necessary to recover and reincorporate the esters† volatilized during the concentration of pineapple juice. The Hawaiian plant was described as a triple-effect evaporator through which the juice was passed twice. During the first pass 25 per cent. of the juice was evaporated, while in the second stage it was concentrated to about 66° Brix. Practically all esters were recovered from the calandrias of the second and third effect in the first pass.

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† "Ester" in this paper refers to volatile flavouring constituents, including esters, ketones, aldehydes, alcohols, etc.

In order to simplify production of juice concentrates on a laboratory scale, it was decided to use a flash evaporator similar to that described by Dimick and Makower (1951) as the first effect to strip off the esters with 20–25 per cent. of the water, and a single-pass turbulent thin-film exaporator for the second effect. This equipment, which was described briefly by Leverington (1962), has been exhaustively tested and modifications made until satisfactory operation has been attained.

II. INVESTIGATIONS LEADING TO MODIFICATIONS

Since Haagen-Smit *et al.* (1954) and Connell (1964) had reported that ethyl acetate was generally present in much higher concentrations than all other esters in pineapple, preliminary experiments with the pilot plant were carried out with an aqueous solution of ethyl acetate before proceeding with freshly expressed pineapple juice. The effectiveness of stripping methods was judged by the amount of esters recovered when determined as ethyl acetate by a modification of the method of Thompson (1950). Variables investigated with regard to ester stripping included feed rate, steam pressure, degree of vaporization, vacuum, cooling brine temperature, heating surface and diameter of vaporizing tubes. Ester recoveries of 60–70 per cent. could be obtained on runs of up to 2 hr. However, the fine stainless-steel preheating tubes (0·078 in. I.D.) readily fouled up, which is in contrast to the work reported by Walker and Patterson (1955), who used similar sized tubes. It appeared therefore that the recommendation made by Claffey *et al.* (1958), that a velocity of 20 ft/sec for pectinaceous juices through the heat exchanger tubes was necessary, may also apply to pineapple juice, which is very low in pectin. As the plant was designed to process 4-5 Imp. gal of juice per hour, it was obvious that to obtain this high velocity the diameter of the preheating tubes would have been smaller than practical limits. It was therefore decided that the juice would be evaporated to the required concentration in one pass of the turbulent thin-film evaporator and all the distillate fractionated. This modified technique necessitated the installation of a pasteurizer operating at 165°F. This reduction in temperature of pasteurization reduced the likelihood of adverse heating effects on the organoleptic qualities of the juice.

Since experiments had shown that there was a loss of aroma-bearing constituents in the column bottoms and that the maximum obtainable fold of the essence was rather limited, the height of the fractionating column was extended so that the number of theoretical plates would approach the 22 plates used commercially (Tressler and Joslyn 1954). Due to the difficulties experienced with reflux heating by either electrical means or heat exchangers and the small advantages gained in using this principle in a plant of this size, its use was discontinued.

The operation of the fractionating column under vacuum and the maintenance of the system at equilibrium proved very difficult. This was due to considerable pressure drop through the column as well as intermittent flooding of the scrubber and column caused by small fluctuations in vacuum. Although Smyser (1952), Seale (1953), Tressler and Joslyn (1954) and Wood (1961)

described vacuum fractionation plant for pineapple esters, the necessity for low-temperature fractionation was not indicated. Hugo (1959) described an experimental plant operated entirely under vacuum which he used for ester recovery and the concentration of pineapple juice, but in subsequent private communications he has described the difficulties experienced in recovering these esters fractionated under vacuum.

Since Milleville and Eskew (1944), Philips *et al.* (1951), Eskew *et al.* (1951*a*, 1951*b*, 1951*c*), Dimick and Simone (1952), Eskew *et al.* (1952), Walker *et al.* (1954), Walker and Patterson (1955), Eisenhardt *et al.* (1958), Claffey *et al.* (1958) and Eskew *et al.* (1959) have reported the successful fractionation at atmospheric pressure of a number of fruit juices, including apple, peach, pear, berry and grape, it was decided to investigate the feasibility of fractionating pineapple esters under the same conditions. The fractionating system was therefore converted to atmospheric pressure. Results to date have been quite satisfactory.

III. OUTLINE OF PROCESS

Photographs of the plant are shown in Figures 1 and 2 and a flow diagram of the process is illustrated in Figure 3. The commercially expressed juice is pasteurized at 165°F, cooled, screened, chilled to 35°F, centrifuged to less than 1 per cent. insoluble solids, homogenized at 2,500 lb/in.^{2} and then evaporated under vacuum in a single pass to approximately 60° Brix. During evaporation, esters are distilled off and condensed with the water. This distillate is then fractionated at atmospheric pressure and the esters removed from the system at a concentration of approximately 250-fold. These are blended with the concentrated juice, which is then canned and quick-frozen.

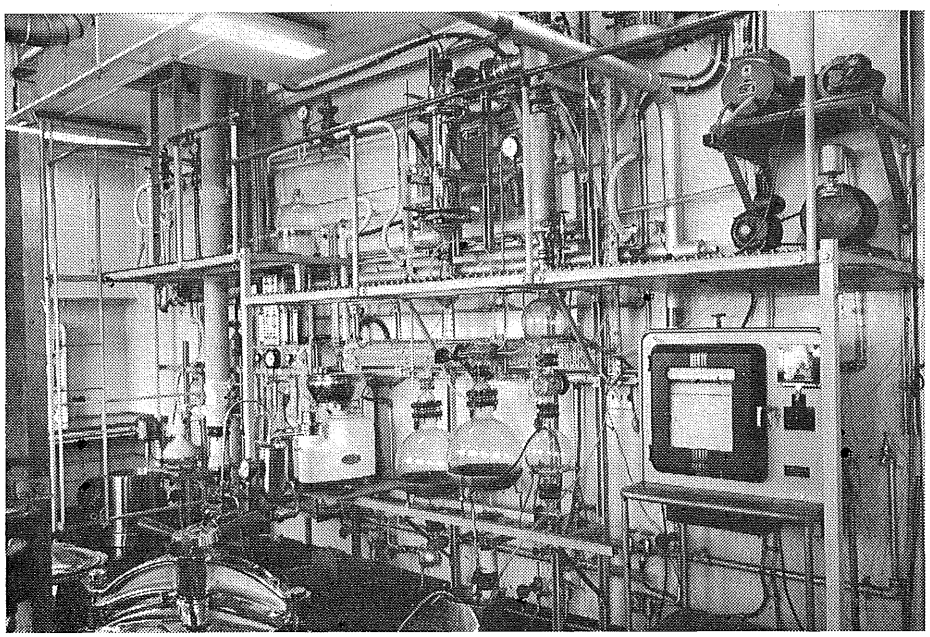

Fig. 1.—General view of pineapple juice concentration plant.

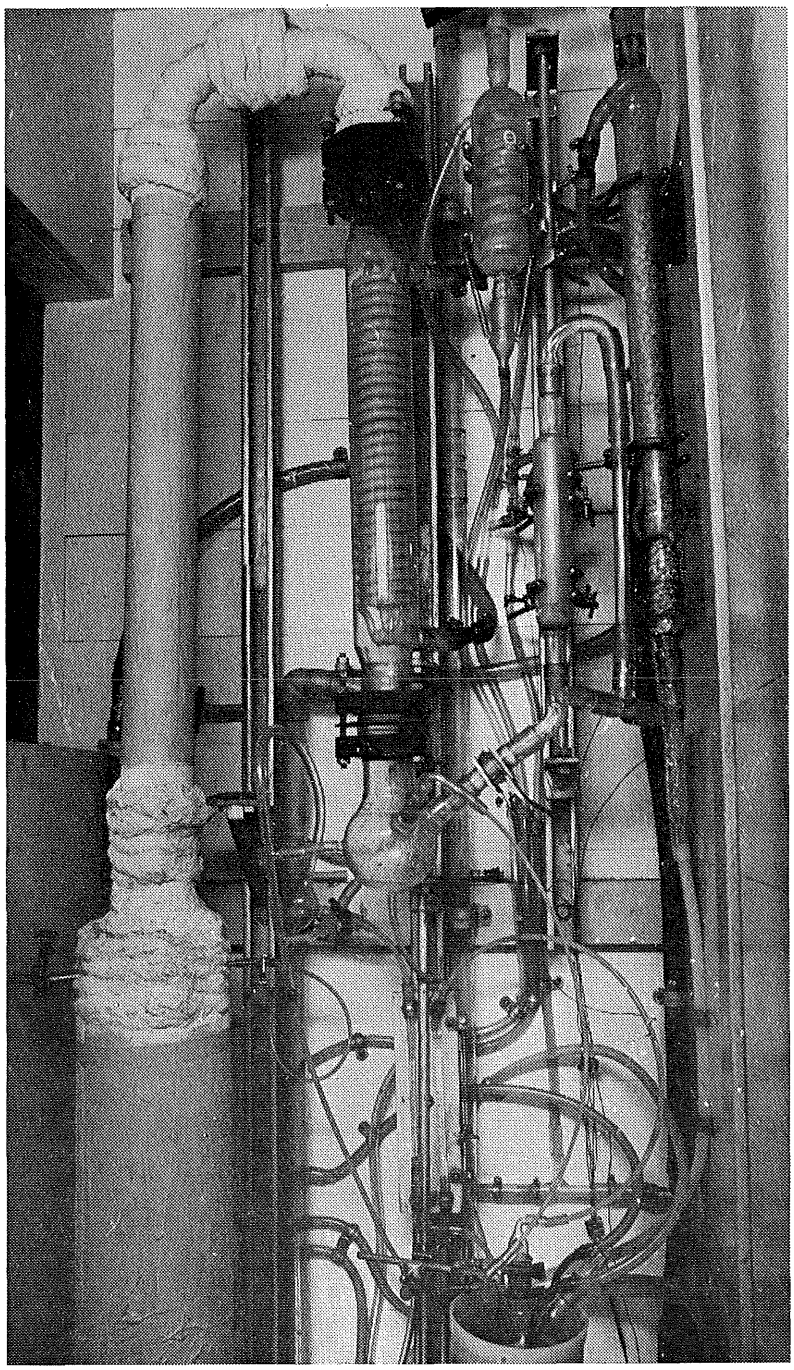

Fig. 2.—Upper section fractionating system showing fractionating column, essence condenser, reflux splitter, vent cooler, scrubber, scrubber liquid cooler and essence receiver.

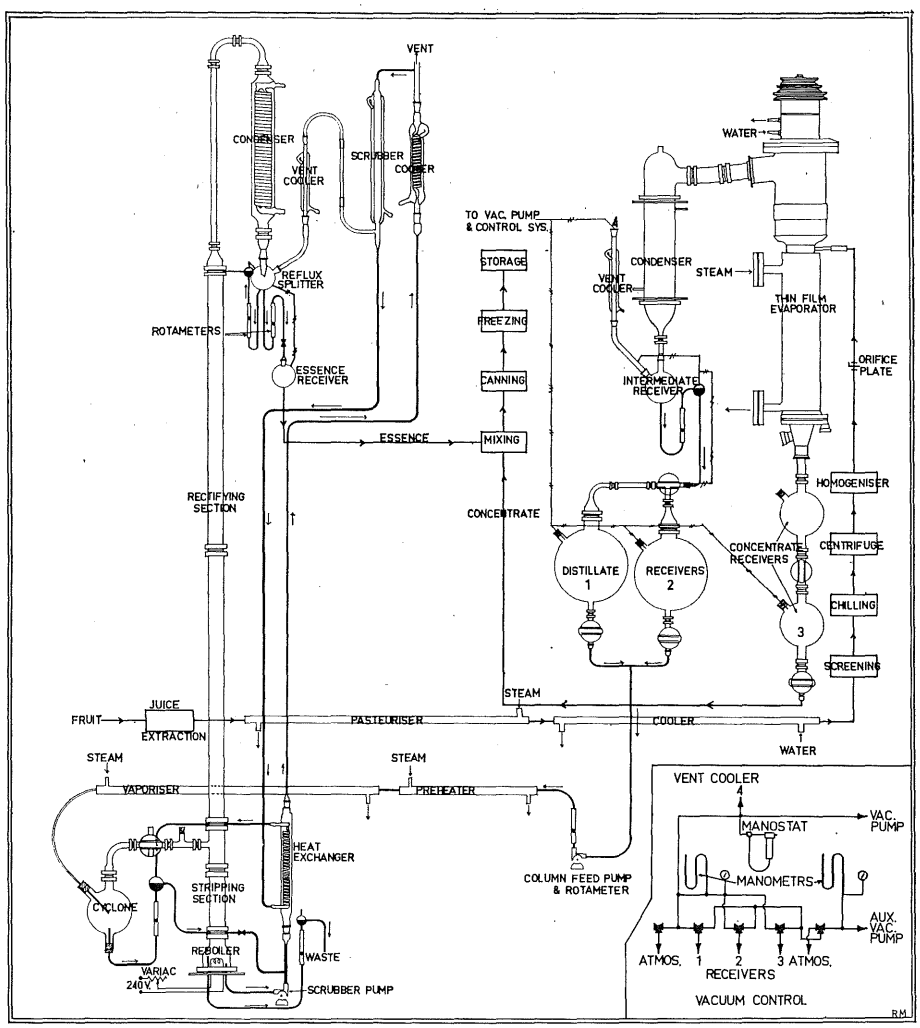

Fig. 3.—Flow diagram of pineapple juice concentration plant.

IV. CONSTRUCTION AND METHOD OF OPERATION

Juice Preparation.—Freshly expressed pineapple juice containing 20 per cent. pulp is drawn from a nearby cannery and pasteurized promptly at 165°F to prevent fermentation during processing. Continuously agitated juice is fed by a Mono* pump at 20 Imp. gal/hr through an 8-ft shell and tube heat exchanger consisting of a ½-in. O.D. 20-gauge stainless-steel† tube in which is centrally

* Mention of trade name or company in this paper does not imply recommendation or endorsement by the Queensland Department of Primary Industries over those not mentioned.

† Stainless-steel in this paper refers to 18/8 Mo grade.

placed a core of $\frac{5}{16}$-in. stainless-steel rod to ensure rapid flow over the hot surface. Steam at 25 lb/in.^{2} raises the temperature of the juice from 70°F to 165°F and then it is cooled to about 100°F in a similar 11-ft shell and tube heat exchanger. All connections are made with nylon pipe and compression fittings.

The cooled juice is then passed through a vibrating screen (shown in Figure 4) to remove about 80 per cent. of the pulp and some of the precipitated protein. Vibration of the screen is obtained by a variable speed cam (750–1450 r.p.m.), the stroke of which can be adjusted between $\frac{1}{32}$ and $\frac{6}{32}$ in. The upper screen is 20 mesh and the lower 42. Careful adjustment of the slope of the screen is essential to ensure efficient separation and to prevent excessive loss of juice with the discharged pulp. The maximum capacity of the unit is about 40 gal per hr when fed with juice containing 20 per cent. of pulp.

The screened juice runs into a refrigerated tank, where it is cooled rapidly to approximately 35°F to minimize microbial activity and ester losses. Due to the variation in total soluble solids of pineapples as pointed out by Leverington (1962), it is necessary to adjust the sugar concentration to 13° Brix by the addition of cane sugar to produce a standard product. Agitation of the chilled juice and suspended pulp is essential to ensure that the centrifuge is fed with homogeneous product.

The juice is pumped to a De Laval laboratory centrifuge, which not only removes fine pulp and particles of foreign matter but also most of the precipitated protein. Unfortunately, during this stage a considerable amount of froth is produced which is difficult to eliminate even with the addition of various antifoam agents. Provided foam does not accumulate in the juice feed no difficulties are experienced.

Approximately 1 per cent. protein is precipitated after centrifuging. To prevent this settling in the concentrate, thereby detracting from the appearance, the juice is homogenized at 2500 lb/in.^{2} in a Manton Gaulin 2-stage homogenizer.

Juice Concentration.—Although the juice was originally pumped into the evaporator using a positive delivery pump with by-pass for flow control, intermittent fluctuations in flow caused evaporation difficulties. A steadier flow has been obtained by drawing the juice via a rotameter into the evaporator by vacuum through a stainless-steel 0·04-in. capillary or alternatively through a $\frac{3}{32}$-in. needle valve.

The original evaporator described briefly by Leverington (1962) did not meet specification requirements and was replaced by a Luwa Model 020 turbulent thin-film evaporator. This unit, which has a 1·4-sq. ft. heating surface and a 2000 r.p.m. 4-bladed rotor with $\frac{1}{32}$-in. clearance, is shown in Figure 1. A modified $\frac{1}{2}$-in. pilot-operated reduction valve has been used to provide steam at $\frac{1}{2}$–15 lb/in.^{2} in conjunction with a $\frac{1}{2}$-in. ball-float steam trap.

The separation head of the evaporator has proved effective in preventing entrainment. To prevent any possible distortion of the shell during operation, which would in turn cause the rotor blades to scrape the wall, flexible metal bellows-type hoses are used for steam connections. Due to vibration of the evaporator, flexible connections in the form of Tygon tubing between the glass condenser and concentrate receivers have been essential. The lower carbon

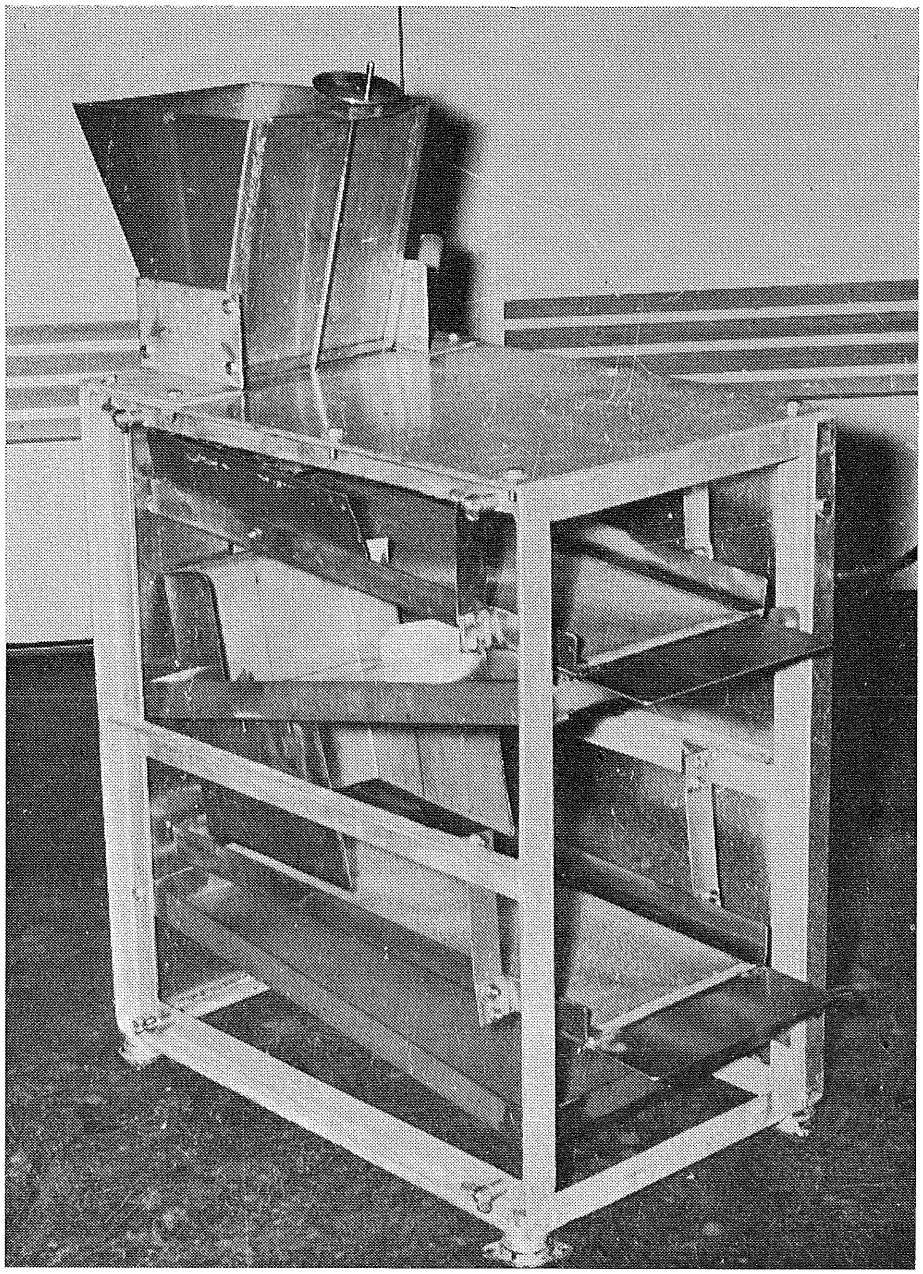

Fig. 4.—Vibrating screen.

bearing, which is lubricated by water metered in at 2 ml/min, has had to be frequently replaced, as small chips of carbon tend to break off and contaminate the products. An experimental Teflon bearing is now under trial. The concentrate receivers consist of two 5-l Q.V.F. flasks connected in series by means of a 1½-in. stopcock. This permits the lower flask to be discharged while the plant

is in operation. Since refrigeration is not provided for these flasks, there is a tendency for the concentrate to boil in these receivers on a hot day if the plant is operated at pressures below 25 mm Hg.

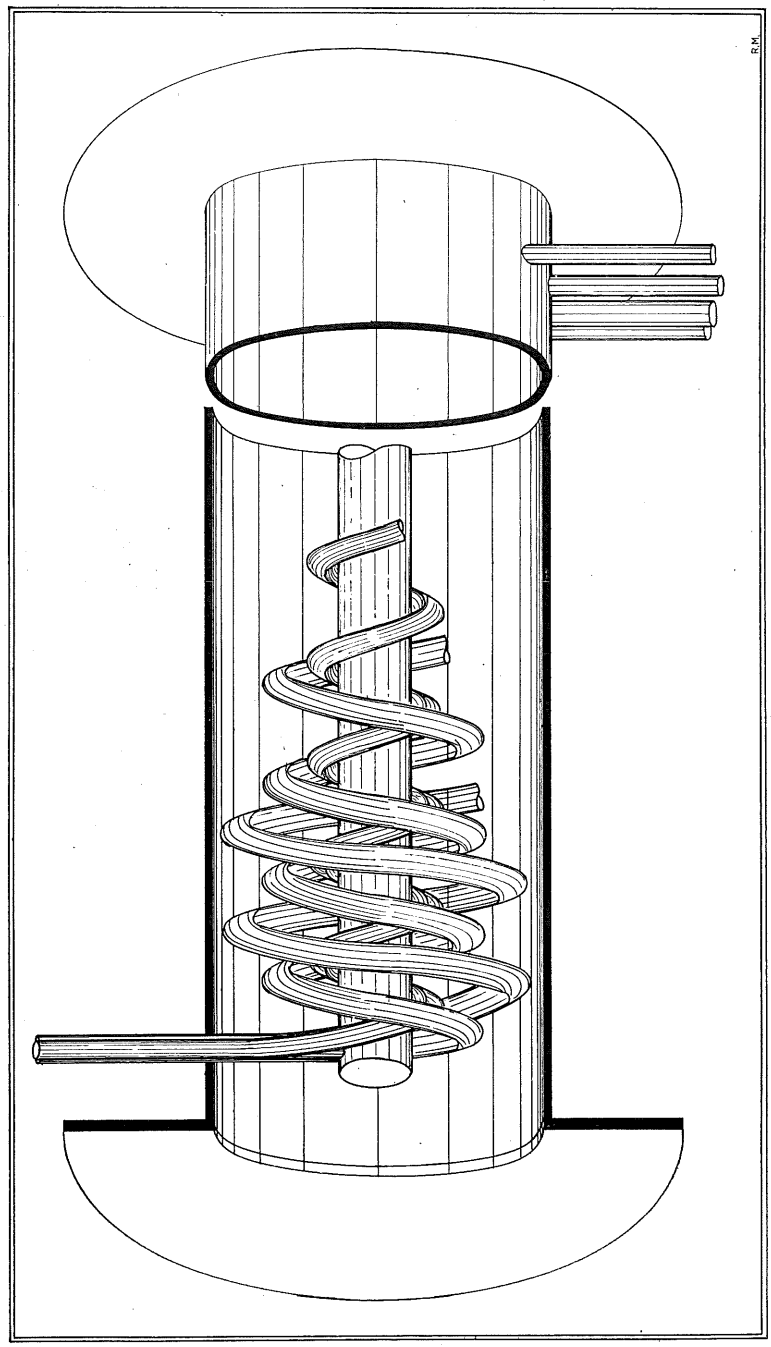

Fig. 5.—Main condenser.

The vapour outlet of the evaporator is connected to the stainless-steel condenser by means of a 3-in. line in which is fitted the bulb of a distant-reading thermometer. In order to reduce pressure drop in the condenser to a minimum, it was designed so that it had a free cross-sectional area equivalent to a 3-in. pipe. The cooling surface, comprising two ½-in. coils, one ⅜-in. coil and a 1¼-in. tube, has a total surface area of 9 sq. ft., which has proved adequate when using 20 per cent. glycol brine at 32°F. The brine is distributed in parallel to each cooling tube by means of a manifold, which is provided with screw clip valves to ensure that the temperature rise in each coil is constant. The design is shown in Figure 5. The intermediate condensate receiver consists of a modified 1-l flask to which is attached a 1¼-in. side arm and a 2 sq. ft. glass surface condenser which acts as a vent cooler. Every precaution is taken to ensure that water vapour is not visibly being condensed on this vent cooler and therefore that loss of esters to the vacuum is at an extreme minimum. The condensate receiver is normally half full, so that any slight variation in condensate rate due to hold up of condensed water on the coils does not cause fluctuations in the rotameter readings. A tube from the bottom of the intermediate condensate receiver is connected via a rotameter to a 1-in. 2-way T stopcock which permits distillate at 50°F to be directed to either of the two 20-l distillate receivers, which have ⅝-in. draincocks. To avoid loss of esters, vacuum is not applied directly on these flasks during filling, but a constant pressure is maintained by means of an 8-mm balancing tube which is looped from just ahead of the 2-way stopcock to the head of siphon breaker and in turn to the vent cooler line. This siphon breaker is fitted above the rotameter to ensure a steady flow and therefore accurate readings.

Ester Fractionation.—The ester-containing distillates, which are stored overnight in stainless-steel drums at 35°F, are pumped via a rotameter into the preheater, which consists of a 5-ft length of 0·078-in. I.D. stainless steel tubing encased in a steam jacket. The heated fluid then discharges into the vaporizer, which is a steam-jacketed 5-ft length of ½-in. O.D. stainless steel tubing. Each heat exchanger has its own pilot-operated reducing valve which can be regulated between ½ lb/in.^{2} and 15 lb/in.^{2} depending upon the degree of vaporization required. Normal feed rate is 240 c.c. per min, of which approximately 30 per cent. is vaporized. Liquid/vapour separation occurs as the mixture enters the 5-l cyclone separator, the vapour passing over into the vapour feed plate of the fractionating column. A 2-way T stopcock has been fitted between the column and the cyclone to enable samples of vapour to be withdrawn if required. The stripped water discharging from the cyclone passes through a rotameter and is fed into the bottom feed plate of the fractionating column in order to distill off any esters which may have been retained. To balance pressures in this part of the system, the head of the siphon breaker on top of the rotameter is connected to a feed plate on top of the stripping section of the column. The cyclone (which is heavily insulated above the liquid level) normally contains about 1½ l of water.

The fractionating column has been constructed of 3-in. Q.V.F. glass pipe sections and packed with ¼-in. glass raschig rings, the free volume being 74 per cent. The stripping and rectifying sections contain 18 in. and 100 in. of packing

respectively. The reboiler consists of a 6 in. x 3 in. pipe reducer with a ¼-in. stainless-steel base plate in which there is fitted two ¼-in. liquid discharge tubes and a 1800W 240V nichrome element wound on a ceramic bobbin. This element is mounted on ⅛-in. stainless-steel conductors which are insulated with nylon bushes as they pass to the junction box on the lower side of the reboiler plate. The heat output of this element is controlled by a 10A Variac, which is in the active line of the power supply to prevent any heating effects due to conductivity of the water. Column bottoms are discharged by an overflow system and flow rates are indicated in a rotameter. A series of experiments have been conducted at various reboiler settings and 110V has been found to be the maximum to ensure that the column does not flood when the system is fed with water at the rate of 240 ml/min, of which 30 per cent. is vaporized. The vapour feed plate consists of a 1-in. pipe discharging into the column. All liquid feed plates are constructed of ½-in. stainless-steel rings through which pass ¼-in. lines, the discharge point of which is exactly in the centre of the column and facing downwards. Three such plates are fitted, viz. one at the bottom of the stripping section to receive the cyclone liquid discharge, one at the vapour feed level where scrubber liquid is discharged back into the column, and the third at the top above the rectifying section to feed reflux liquid back into the system. The whole of the fractionating column and the 1-in. vapour pipe leading to the condenser is covered with 1½-in. magnesia insulation or tightly bound 1-in. asbestos rope. A 1-in. dia. inspection hole is cut in the insulation at the bottom of the rectifying section to observe any column flooding.

The glass coil condenser has a surface area of 3½ sq. ft. and a free cross-sectional area of 0·8 sq. in. The condensed liquid containing the esters is received in a reflux splitter, which is a modified 500-ml flask with take-off tubes for reflux and ester. To equalize pressures in this section of the plant, one side arm is connected to a siphon breaker on the reflux line and also to the essence receiver. Another arm is connected to a 650-sq. cm. glass condenser which acts as a vent cooler. The essence (ester concentrate) take-off is controlled by throttling the line, the balance of the distillate passing back as reflux. Both reflux and essence flow rates are indicated by rotameters. The scrubbing tower consists of a 350-sq. cm. Liebig condenser packed with ¼-in. ceramic berl saddles up through which vent gases pass counter-current to the scrubbing liquid. For scrubbing purposes, column bottoms are drawn from the reboiler and pumped through one side of a heat exchanger and then through a refrigerated cooler before being fed to the top of the refrigerated vent gas scrubber at 40 c.c./min. The liquid then passes through the other side of the heat exchanger and is discharged into the second column feed plate mentioned above. This arrangement results in water being fed into the scrubber at 35°F and it is discharged back into the column at about 120°F. To ensure that cavitation does not occur in the centrifugal scrubber pump, most of the boiling liquid it handles is bypassed back into the lower feed plate. The fractionating system is virtually closed, the only openings to atmosphere being through the liquid lock on the column discharge and the top of the scrubber.

In addition to dial-type and mercury thermometers placed at strategic points throughout the plant, 25 copper constantan thermocouples are fixed in thermometer pockets and on liquid lines. The thermocouples are connected to an electronic recorder which can be seen in Figure 1. All condenser refrigerant inlet and outlet lines have short sections of metal tube to which the thermocouple is bound with tape and asbestos rope.

Ancillary Equipment.—The evaporator, along with all the other associated equipment, is mounted on galvanized Unistrut scaffolding which is bolted to the brick wall as well as being supported by the catwalk which forms an integral part of the framework. This structural material was selected because of the infinite adjustment available in a channel of this type. The decking of the catwalk is galvanized heavy-gauge expanded metal welded to the framing. Small pieces of equipment (such as condensers) are fixed with laboratory retort clamps which are attached to vertical ½-in. aluminium alloy rods bolted to the main framework. Pipework, which is polyvinyl chloride, polythene, nylon, glass and butyrate, is generally mounted on galvanized cable tray and given distinctive coding for identification.

As shown in Figure 3, there are two independent vacuum systems in use. The main system, which maintains the reduced pressure in the evaporator and receivers, utilizes a 5 c.f.m. water-cooled oil-type gas ballast pump. A close check on air leaks is imperative to eliminate the risk of esters being carried over in the non-condensables (Tressler and Joslyn 1954). To control vacuum at the desired level, a Cartesian manostat was installed in series in the vacuum line as recommended by Walker and Patterson (1951), but it was soon found that a laboratory manostat was entirely inadequate due to the limited orifice size. The exhaust orifice of the manostat has therefore been modified in a manner recommended by Spadaro, Vix, and Gastrock (1946). It is connected in such a way that control is by means of a bleed into the vacuum line close to but just after the vent cooler of the evaporator. A mercury manometer with a scale of ± 90 mm is connected into the vacuum line.

The auxiliary vacuum system operated by a 1 c.f.m. oil-type vacuum pump with its associated manometer is installed to evacuate receivers after their contents have been discharged. These receivers are connected over to the main system when the auxiliary manometer reading corresponds with the main manometer reading. The sizing of vacuum lines is of extreme importance and to minimize pressure drops clear rigid 1¼-in. O.D. butyrate pipe is used for all the main lines.

The primary refrigeration system consists of a 5-ton sealed unit compressor, a 7½-ton evaporative cooler and two shell and tube heat exchangers through which passes the secondary refrigerant, which is an aqueous 20 per cent. ethylene glycol solution. This brine is circulated through condensers, vent coolers and the scrubbing system by a centrifugal pump at approximately 250 Imp. gal/hr. Adequate safeguards, including a control thermostat, safety thermostat as well as a differential pressure switch to prevent freezing of the glycol in the heat exchanger, have been installed. To avoid fluctuations in brine temperature as the compressor cuts in and cuts out, a 6 Imp. gal. header tank has been installed

and fitted with a 2kW element and thermostat so that the refrigeration capacity can be balanced against the heat load. In this way temperature of the brine can be controlled to ± 1°F.

V. NATURE OF DATA OBTAINED

It has not been practicable in a paper of this type to present all the data obtained during the development of this plant, but typical operating conditions now in use are as follows:—

Evaporation

Juice feed; 4–5 Imp. gal/hr; Brix 13°; temperature 50°F; pulp >1 per cent.
Evaporator jacket temperature: 212–240°F.
Product vapour—temperature: 70–140°F; pressure 20–150 mm Hg.
Distillate rate: 240–400 c.c./min.
Concentrate Brix: 50–70°.

Fractionation

Column feed: 250 c.c./min; 27 p.p.m. ester.
Preheater steam pressure: 5½ lb/in.^{2}; vaporizer steam pressure: 6 lb/in.^{2}.
Degree of vaporization: 37 per cent.; Reboiler voltage: 110.
Reflux ratio: 36:1; Essence fold: 250.
Recovery of essence based on column feed: 82 per cent.
Reflux rate: 51 c.c./min; Ester rate: 1·3 c.c./min.
Cyclone discharge rate 158 c.c./min; Reboiler discharge rate: 249 c.c./min.
Scrubber flow rate: 16 c.c./min; Ester in scrubber liquid: 9 p.p.m.

A suitable pilot plant having been developed, experimental work has been commenced to determine the suitability of Queensland-produced pineapple juice for concentration. Factors to be investigated include pasteurization temperature, enzyme destruction, protein precipitation, evaporation temperature, ester variation between and within season, importance of ester losses, desirability of cut-back juice, optimum ester level for summer and winter juices, feasibility of blending summer and winter esters, storage properties of concentrates and pasteurized concentrates.

VI. ACKNOWLEDGEMENTS

The authors wish to acknowledge the assistance of Messrs. N. Herriman and G. Harrison of the Public Works Department and Mr. P. C. Brookes of the University of Queensland for assistance in design, the Pineapple Sectional Group Committee of the Committee of Direction of Fruit Marketing for making the initial supplies of juice available, and the contributions of various members of the staff of this Laboratory, particularly Mr. R. G. Prince for assisting in construction and operation and Mr. C. J. Bagley for construction and modification.

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(Received for publication September 30, 1963)

CHANGES IN THE PECTIC SUBSTANCES OF STORED ELBERTA PEACHES

By J. B. WATKINS, B.Sc.*

SUMMARY

Total and soluble pectic substances were determined in Elberta peaches ripened for 7 days at 70°F following storage at 30°F and 34°F for 7, 14, 19, 24 and 29 days. Some samples were gassed with ethylene (1000 p.p.m.) prior to storage. All fruit was mature-green when harvested.

A considerable loss of total pectic substances occurred in fruit which ripened normally following storage. Abnormal ripening of overstored fruit was associated with little or no loss in total pectic substances during ripening. A possible inactivation of the enzyme pectin methylesterhase is discussed in relation to this abnormal ripening.

A detailed account is given of an accurate and rapid method for the determination of total pectic substances in small quantities of marc prepared from fruit tissue. In this method, an infra-red gas analyser and a potentiometric recorder were used to measure the carbon dioxide evolved from fruit marc decarboxylated with hydrochloric acid.

I. INTRODUCTION

Fruit texture and juiciness appear to be related primarily to the composition and structure of the cell wall, of which the pectic substances are an important constituent. A characteristic change associated with normal ripening is a decrease of insoluble and a corresponding increase of soluble pectin. In overstored peaches, abnormal ripening occurs on removal from storage, resulting in fruit of a mealy texture, a condition known as woolliness. Practically no free juice can be expressed from such fruit. On sections of woolly peaches stained with ruthenium red, it can be observed that the intercellular spaces are filled with red masses of jelly-like pectins (de Haan 1957). It should be noted, however, that ruthenium red is not specific for pectic substances (Kertesz 1951). Working with several varieties of peaches, including Elberta, de Haan (1957) found that woolliness could be avoided if the fruit was ripened before storage to a stage where the ratio of soluble to insoluble pectin was approximately 2 : 1. Storage at as low a temperature as possible for the shortest period was recommended. In some samples of Boland and Beregrine peaches stored at 31° and 37°F, he found an increase in both insoluble and soluble pectin. Date and Hansen (1953) found an increase in total pectin in three varieties of pears stored at 30°–31°F.

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Elberta peaches grown in the Queensland Granite Belt are frequently cool-stored at temperatures approximating 34°F. A marked loss in quality due to woolliness often occurs in fruit stored longer than two weeks. In view of the evidence suggesting that the metabolism of the pectic substances is an important factor in determining the quality of fruit in relation to juiciness and texture, trials have been carried out over two seasons to determine the changes in the pectic substances of stored Elberta peaches.

II. EXPERIMENTAL DETAILS

Source and Treatment of Fruit.—Mature-green Elberta peaches were obtained from the one grower for each of the two seasons involved (1961 and 1962). Half-bushel case samples were subjected to the following prestorage and storage treatments:

(i) immediate storage at 30°F
(ii) immediate storage at 34°F
(iii) gassed with ethylene (1000 p.p.m.) for one day prior to storage at 30°F (designated as E1)
(iv) gassed with ethylene (1000 p.p.m.) for two days prior to storage at 30°F (designated as E2).

Removals from storage were carried out after 14, 19, 24 and 29 days' storage during the 1961 season and after 7, 14, 19 and 29 days' storage during the 1962 season. Each removal consisted of samples of 10 fruit per treatment and this fruit was held at 70°F for 7 days prior to analysis. Samples of gassed and non-gassed fruit were analysed for total pectic substances prior to storage during the 1962 season only. Both the total and the soluble contents of the fresh fruit were determined during the 1961 season. Total pectic substances only were determined during the 1962 season, using marc prepared by the method of Gee, McComb, and McCready (1958).

Analytical Methods, 1961 *Season.*—The 10 fruit comprising each sample were halved and peeled and macerated in a Waring blendor for 3 min. Duplicate samples (approximately 5 g) of macerated pulp were weighed for each of the soluble and total pectin determinations. To each sample was added 30–50 ml distilled water. The samples for soluble pectin determination were allowed to stand for 2 hr with occasional shaking and then filtered through a fast filter paper (Whatman No. 4). To the samples for total pectin determination were added four drops of concentrated hydrochloric acid (to bring the pH to approximately 1·5) and the samples boiled for 1½ hr; hot water was added periodically to maintain the initial volume. The samples were filtered through a fast filter paper.

To each of the filtrates from both the soluble and total pectin determinations were added two volumes of 95 per cent. alcohol; the alcohol was added slowly with constant stirring and the mixture allowed to stand overnight. The mixture was then filtered through a fast filter paper having appreciable wet strength

(Whatman No. 541). The pectin precipitate was washed several times with 70 per cent. alcohol and finally once with 95 per cent. alcohol. The washed precipitate was dissolved off the paper with hot water and the pectin solution de-esterified by adding 10 ml N sodium hydroxide. This solution was made up with distilled water to a final volume of 200 ml in a volumetric flask. The solution was allowed to stand at least 30 min at room temperature before being analysed by the colorimetric method of McCready and McComb, (1952).

Analytical Methods, 1962 *Season.*—According to McComb and McCready (1954), most of the conclusions based upon the role of total pectic substances in fruit texture have been drawn from the characterization of less than 50 per cent. to about 70 per cent. of the total pectic substances present. This is because it has not been possible to extract the pectic substances from the three-dimensional lattice of the cellular framework without changing their molecular and chemical composition. Gee, McComb, and McCready (1958) have outlined a procedure for the quantitative determination and partial chemical characterization of the total amount of the pectic substances without extraction from the plant tissue. This procedure was used for the analysis of the 1962 samples. The method is a titrimetric one and is valid only if the titratable acidity of the fruit marc is due to pectin only. This can be ascertained by analysing a sample of marc by a method specific for total pectic substances.

The determination of galacturonic acid by measuring the carbon dioxide evolved by decarboxylation with hydrochloric acid is one of the most dependable procedures used in analysing pectic substances (Kertesz 1951). This is the basis of a method that was used to check the titrimetric method. The evolved carbon dioxide was determined with an infra-red gas analyser and potentiometric recorder by the following procedure.

Procedure.—Nitrogen was passed at a known flow rate, approximately 300 ml per min, through a round-bottomed long-necked boiling flask A (Figure 1), above which was a refluxing condenser B. The effluent gas stream from the condenser was passed through a zinc trap C and thence through a U-tube D containing silica gel and connected to the inlet side of a flow-meter E; the outlet was connected to an infra-red gas analyser (1·17 per cent. CO_2 full-scale deflection). The analyser output was fed to a potentiometric recorder (O–4mV) with a chart speed of 24 in. per hr.

The boiling flask containing some boiling chips and 100 ml of 19 per cent. (w/w) hydrochloric acid was heated by an electrothermal heating mantle adjusted to keep the acid boiling moderately. The whole apparatus and infra-red analyser were purged with nitrogen until the system was free of carbon dioxide (indicated by a constant baseline on the recorder chart). A known amount (about 20–30 mg) of air-dried marc was weighed into a small receptacle and dropped into the boiling acid in the flask either through a side arm or down the neck of the flask (the refluxing condenser was momentarily raised). The receptacle used was a short section, approximately $1\frac{1}{2}$ in. of a 10-mm test-tube flared at the open end.

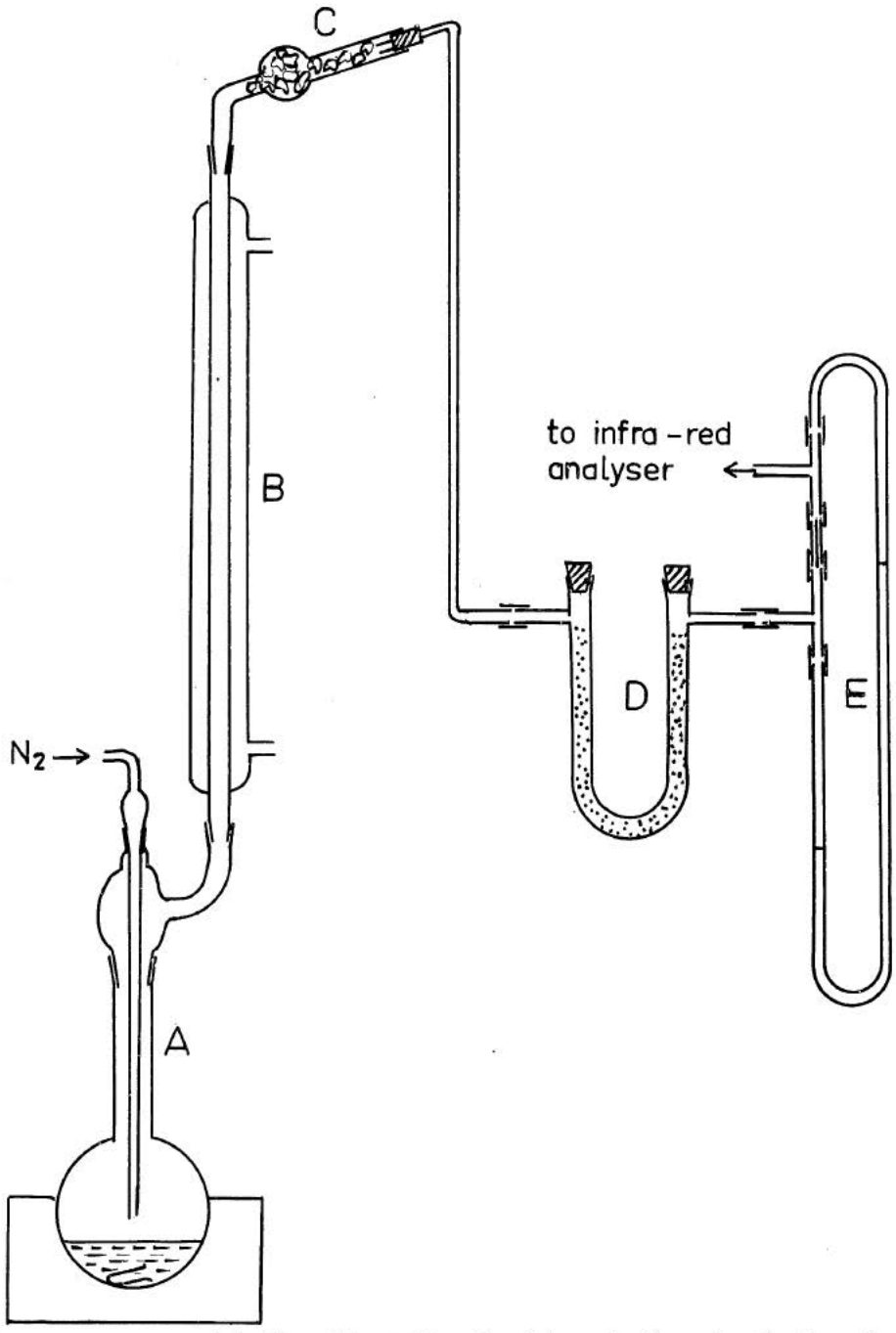

Fig. 1.—Apparatus used for determining total pectic substances by the carbon dioxide method.

The reaction took from 45–60 min. to reach completion as indicated by the recorder trace returning to the baseline. The area under the curve traced during the reaction represented the total amount of carbon dioxide evolved. This

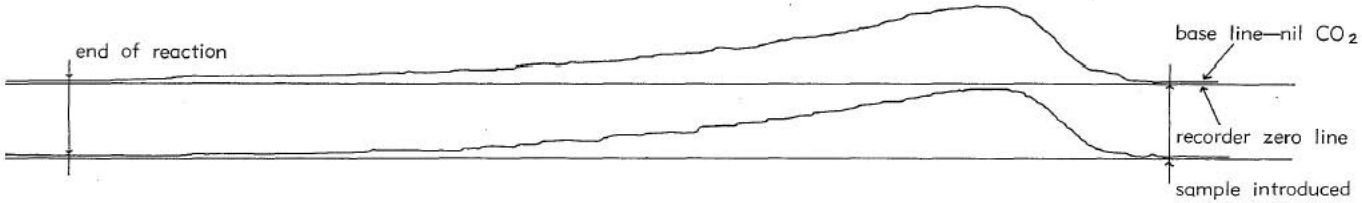

Fig. 2.—Recorder tracings of duplicate 20-mg samples.

was calculated by carefully cutting out the curve and weighing the recorder paper. A unit area of recorder paper was also cut out and weighed. The height of the unit area represents a known percentage of carbon dioxide and the length represents the time of evolution. The weight of carbon dioxide thus represented was calculated from the known flow rate. The amount evolved from the pectic substances was calculated from the weight of paper under the curve and the weight of the unit area. The weight of carbon dioxide multiplied by 4·0 gives the weight of anhydrouronic acid in the sample.

The method was checked with pure galacturonic acid and found to give theoretical yields of carbon dioxide within 1 per cent. This method gave consistently lower results than the titrimetric method. Esau, Joslyn, and Claypool (1962) found that the titrimetric method used for determining the pectic content of pears gave considerably higher results than those determined by the colorimetric versene pectinase extraction procedure.

A reproduction of the curves obtained from duplicate 20-mg samples of marc is shown in Figure 2. The percentage total pectic substances determined from these curves were 16·8 and 16·9 respectively.

The output signal from the infra-red analyser (Grubb-Parsons, Model SB2) was 1 mA at 0·5 V with an output resistance of 500 ohms, provided by a 500-ohm potentiometer from which a voltage can be fed to a potentiometric recorder. The potentiometer adjustment was too coarse to provide the 4 mV full-scale deflection required for the recorder. Consequently a 10 ohm wire-wound potentiometer was wired in series with the 500-ohm potentiometer, which was bridged with a 27,000-ohm resistor to reduce the resistance of the potentiometer and parallel resistor to 490 ohms. The recorder was fed from the 10-ohm potentiometer.

As the method was found to be reliable and suited to the serial determination of pectic substances, the 1962 samples were determined by this method as well as by the titrimetric method.

III. RESULTS

Results for both seasons appear in Tables 1 and 2 and are expressed graphically in Figures 3–6. The 1961 results, as determined by the colorimetric carbazole method, are expressed as optical density. As the results are for purely comparative purposes, it was not necessary to plot a standard curve for galacturonic acid to determine the results as percentage pectin. Results for both seasons can be compared from the slope, but not from the levels of the graphs in Figures 3–6. The 1962 results are expressed as percentage of total pectic substances in the fruit marc.

Although no account has been taken of any change in fresh fruit weight during storage and subsequent ripening, it is improbable that any such change could invalidate the results. Some loss in weight would undoubtedly occur during storage and the 1961 results probably show a higher level of total pectic substances than was actually present. The same trends, however, would still

exist and these same trends are evident in the 1962 season, in which the results would be independent of any change in fresh weight due to loss of moisture during storage and ripening.

All results in Tables 1 and 2, except those for the carbon dioxide method, are the means of duplicate samples. Both duplicates are shown for the carbon dioxide method to indicate the degree of accuracy obtained.

TABLE 1

TOTAL AND SOLUBLE PECTIN CONTENT OF FRUIT HELD FOR 7 DAYS AT 70°F FOLLOWING COOL STORAGE

Storage Temperature (°F)	Storage Time (days)	Total Pectic Substances			Soluble Pectin (1961 only) (Expressed as optical density)
		1961 Method	1962 Methods		
		Carbazole (optical density)	Titrimetric (%)	Carbon dioxide (%)	
30	7	—	22·8	17·9–18·2	—
	14	·315	19·7	12·0–12·1	·300
	19	·326	—	—	·249
	24	·362	27·6	19·4–19·7	·188
	29	—	29·1	19·7–20·2	—
34	7	—	—	20·1–20·4	—
	14	·354	23·4	18·8–18·9	·240
	19	·380	—	—	·178
	24	·494	30·5	22·9–23·3	·180
	29	—	32·8	25·0–25·4	—
E1 30	7	—	20·8	15·8–15·9	—
	14	·336	20·0	13·5–13·7	·274
	19	·253	—	—	·202
	24	·343	28·5	16·9–16·9	·221
	29	·298	—	16·6–16·7	·205
E2 30	7	—	20·8	15·1–15·1	—
	14	·381	23·1	14·1–14·4	·341
	19	·263	—	—	·222
	24	·235	21·0	12·7–12·9	·228
	29	·263	24·9	16·8–16·9	·232

TABLE 2

PERCENTAGE TOTAL PECTIC SUBSTANCES IN FRUIT PRIOR TO STORAGE (CARBON DIOXIDE METHOD)

Non-gassed	Gassed with Ethylene (1000 p.p.m.) for 1 Day	Gassed with Ethylene (1000 p.p.m.) for 2 Days
24·6	22·2	22·9

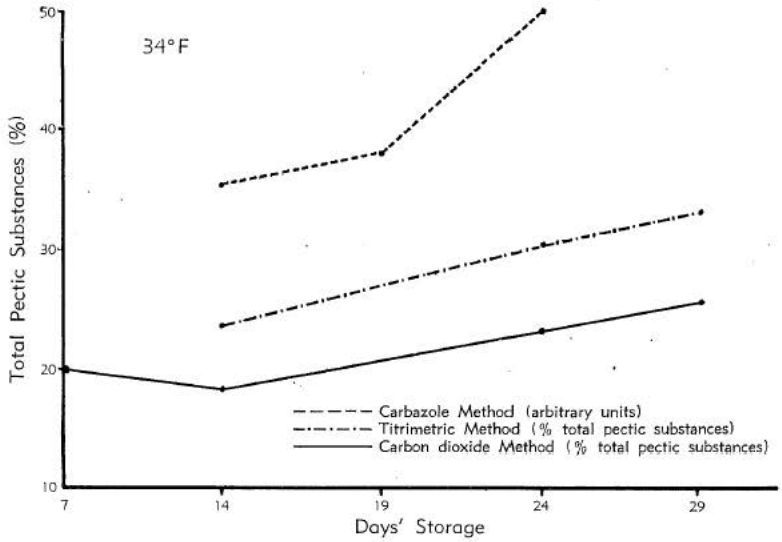

Fig. 3.—Total pectic substances in Elberta peaches ripened for 7 days at 70°F following storage at 34°F.

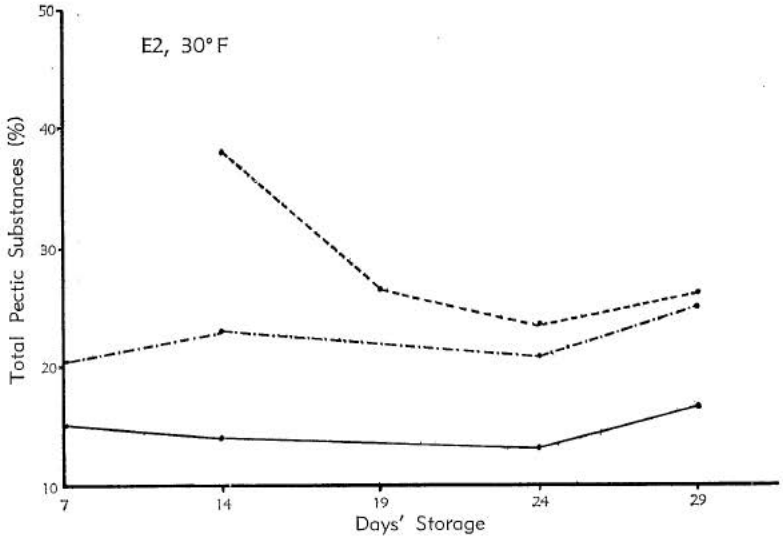

Fig. 4.—Total pectic substances in Elberta peaches ripened for 7 days at 70°F following storage at 30°F. Legend as for Fig. 3.

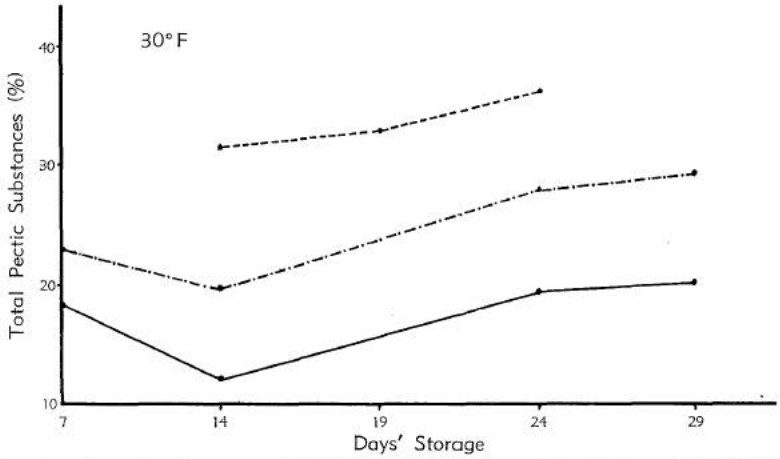

Fig. 5.—Total pectic substances in Elberta peaches ripened for 7 days at 70°F following storage at 30°F. Gassed with ethylene for 1 day prior to storage. Legend as for Fig. 3.

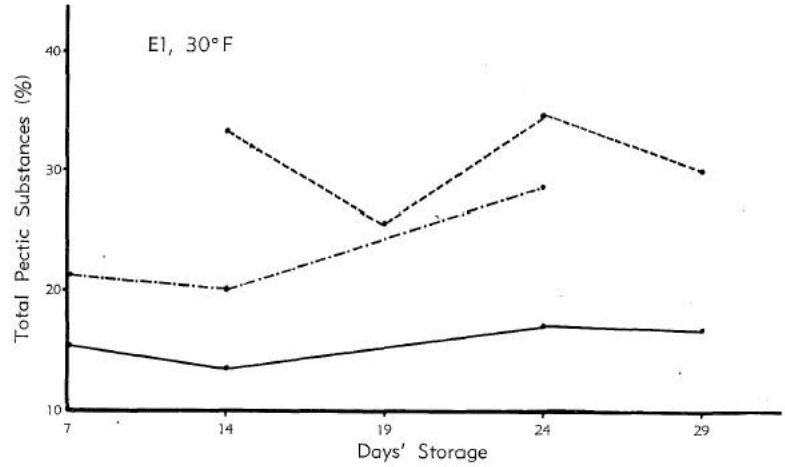

Fig. 6.—Total pectic substances in Elberta peaches ripened for 7 days at 70°F following storage at 30°F. Gassed with ethylene for 2 days prior to storage. Legend as for Fig. 3.

IV. DISCUSSION

Previous trials (unpublished data) carried out by the author have shown that the storage life of mature-green Elberta peaches grown in the Queensland Granite Belt is approximately 2 weeks at 32–34°F. Beyond 2 weeks' storage, the incidence of woolliness increased to 100 per cent. after 4 weeks' storage. Holding the harvested fruit at air temperatures for 2 days prior to storage decreased the incidence of woolliness to some extent and in fruit gassed with ethylene (1000 p.p.m.) for 2 days at 70°F prior to storage, the incidence of woolliness was negligible. Overstorage of this fruit resulted in a mushy breakdown of the flesh.

In the trials under discussion here, the condition of the fruit ripened after storage confirmed the observations made in previous trials. Unless otherwise stated, fruit referred to in the following discussion is fruit which has been ripened for 7 days at 70°F following storage. From the results, it is apparent that the storage life of mature-green Elberta peaches grown in the Queensland total pectic substances in the ripe fruit compared with the level in fruit which ripens normally following storage. In both seasons, fruit which was stored at 34°F contained the highest level of total pectic substances, irrespective of the storage time. Some degree of woolliness was evident in this fruit after 14 days' storage. With the exception of fruit gassed for 2 days prior to storage, the lowest level of total pectic substances for each treatment occurred after 14 days' storage, but rose as the storage time increased further. The highest level occurred in fruit stored for 29 days at 34°F; total pectic substances in this fruit was approximately the same level as in the green-mature fruit prior to storage and all fruits were in a woolly condition. The lowest level of total pectic substances after 29 days' storage occurred in both samples gassed with ethylene. The incidence of woolliness in these samples was negligible.

According to McCready and McComb (1954), the anhydrouronic acid contents of ripe peaches, pears and avocadoes were essentially the same as those of unripe fruits. Reference to Table 1, however, shows that the anhydrouronic acid (total pectic substances) content of peaches ripened at 70°F after storage for 2 weeks at 30°F, irrespective of the prestorage treatment, was approximately only half that of the green fruit. This finding is in agreement with the work of Ash and Reynolds (1954), who reported the presence of free galacturonic acid in fruit ripened at 20°C when removed from the tree but not in tree-ripened fruit. Two significant points emerged from their work. Firstly, a number of samples of pears and peaches ripened in a constant-temperature room at 20°C contained much higher concentrations of free galacturonic acid than any previously recorded. Secondly, where there was an appreciable variation in picking maturity, the amount of free galacturonic acid present after ripening was greater in fruit which was originally less mature and consequently required a longer time to ripen at 20°C. Ash and Reynolds postulated that the most probable mode of formation of free galacturonic acid in fruit would be by the enzymic degradation of pectin, which would require the presence of both pectin methylesterase (PME) and polygalacturonase (PG).

The increasing activity of PG as the methyl ester is progressively removed from pectins has been demonstrated by several authors (Colowick and Kaplan 1955). Glasziou, Sacher, and McCalla (1960), in studying the effect of auxins on membrane permeability, considered it possible that middle-lamella pectins are attacked by a combined PME-PG action and that PME activity may be a prerequisite for middle-lamella dissolution; one of the several types of PG which have been described acts very slowly or not at all on high-ester pectins. It appears then that the action of polygalacturonase must be preceded by the de-esterification of pectin by pectin methylesterase.

It is interesting to note that although the incidence of woolliness was negligible in fruit gassed for two days prior to storage, there was little or no loss of total pectic substances in this fruit prior to storage (see Table 2), although a considerable loss occurred during subsequent ripening after removal from storage, irrespective of the storage time. This suggests that during at least the first two days of the ethylene-induced ripening of the detached green-mature fruit, the pectic substances are de-esterified by pectin methylesterase allowing a subsequent breakdown to galacturonic acid by polygalacturonase during the ripening which occurs following storage.

From the foregoing discussion it appears that the normal ripening process in detached Elberta peaches involves the breakdown of part of the pectic substances to free galacturonic acid. The 1962 results show a lower level of total pectic substances in fruit ripened after 14 days' storage than after 7 days' storage. A possible explanation is that as the ripening period at 70°F was a constant 7 days in each case, fruit ripened after 14 days' storage may have been in a more advanced stage of ripeness than fruit ripened after 7 days' storage. (Unpublished data of the author have shown that the number of days taken to reach the climacteric in pears ripened at 70°F following storage decreases with increasing storage time). With the exception of fruit gassed for two days prior to storage, the rise in total pectic substances with further storage suggests that one aspect of overstorage in peaches is a partial to complete failure of the mechanism, probably enzymatic, whereby the pectic substances are catabolized to galacturonic acid.

In the 1961 season, soluble pectin in fruit stored immediately at 30°F and 34°F decreased, with increasing storage time, as the total pectin increased. The lowest level of soluble pectin occurred in fruit stored at 34°F for 19 days, the level remaining constant with further storage. Approximately the same level occurred in fruit stored at 30°F after 24 days' storage. The soluble pectin levels also fell in fruit gassed with ethylene prior to storage but this fall paralleled the fall in total pectin which occurred after 14 days' storage. As in the 1962 season, fruit stored for 24 days may have been in a more advanced stage of ripeness than fruit stored for 19 days when both samples were held for 7 days at 70°F. A fall in total pectin resulting from a more complete breakdown to alcohol-soluble constituents could also result in a lower level of soluble pectin in the riper fruit.

Glasziou (1957*a*), in studying the effect of indole acetic acid (IAA) on the binding of pectin methylesterase (PME) to the cell walls, considered it possible that the binding of PME to the cell wall would lower the activity of the enzyme in the cell, decreasing the rate of hydrolysis of pectins. Glasziou (1957*b*), when carrying out similar studies on the tubers of the Jerusalem artichoke, found a very low non-specific adsorption of PME to cell-wall preparations from freshly dug tubers but similar preparations from the same batch of artichokes showed high non-specific adsorption after storing in damp sand in a cold room at 5°C for 1 week.

If an increased degree of adsorption of PME to cell walls is general for any cool-stored tissue, then one aspect of the abnormal ripening of overstored Elberta peaches may be an irreversible binding, beyond a certain critical storage time, of PME to the cell walls, the degree of binding increasing with storage time. The increasing level of total pectic substances with increasing storage time beyond 14 days in fruit stored immediately at 30°F and 34°F supports this theory. The theory is tenable only if PME is inactive in the bound state. Jansen, Jang, and Bonner (1960), in studying orange pectinesterase (PE) binding and activity, considered that their results clearly indicated that cell walls firmly bind PE and that, in the bound state, the enzyme is inactive at the pH of orange juice on the *in situ* pectic material.

V. ACKNOWLEDGEMENT

The author wishes to acknowledge the assistance of Miss M. Nugent in carrying out the chemical analyses.

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(Received for publication August 13, 1963)

CORRECTIONS

Vol. 21, page 56: Line 5 should read "that the development of woolliness is associated with an increase in the level of"

S. G. REID, Government Printer, Brisbane

FLOW OF WATER IN A CHANNEL LINED WITH KIKUYU GRASS

By J. ROSSER, B.Sc.Agr.*

SUMMARY

The retardance effect of kikuyu grass was found to be similar to that of Bermuda grass. In terms of the Stillwater retardance curves, a thick stand of long green kikuyu grass fitted curve B and the same stand of kikuyu grass grazed to an average height of 3·5 in. fitted between curves C and D.

I. INTRODUCTION

Hydraulic problems relating to the capacities and velocities of grassed waterways lined with various types of vegetation have been the subject of experimentation in the United States, notably by the Soil Conservation Service at Spartanberg from 1937 to 1941 and at Stillwater since 1941. This work was reported by various workers (Cox 1942; Palmer 1945; Smith 1946; Cox and Palmer 1948; Ree and Palmer 1949) and culminated in the development of a handbook pertaining especially to the design of channels lined with vegetation (Anon. 1947).

These American workers demonstrated that a constant value of the retardance coefficient used in flow formulae (e.g. the Manning formula) is not applicable in grassed channels as it is in channels lined with artificial linings such as concrete. Under the influence of velocity and depth of flow, vegetation tends to bend and oscillate continuously. The retardance to flow varies as these factors change. They found that the Manning retardance coefficient n varies with VR, the product of velocity and hydraulic radius, and from experimental results with different vegetal linings they developed five n-VR curves. These curves were designated A,B,C,D and E for very high, high, medium, low and very low vegetal retardance respectively.

The objectives of the present study were to find out if kikuyu grass (*Pennisetum clandestinum*) would follow these retardance curves and to determine the applicable curve for various growth stages. Due to limitations of site and limited availability of water for test flows, these objectives were only met in part,

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but some evidence was produced to show that the Stillwater retardance curves do apply to kikuyu grass waterways and a guide to the selection of curve to meet various growth stages was produced.

While the study was concerned principally with the effect of the kikuyu grass lining on the capacity of the channel, observations were also made on the ability of the grass to protect the channel from erosion.

II. EXPERIMENTAL CONDITIONS AND PROCEDURES

The waterway selected for observation was located below the spillway of a spring-fed dam. It had carried a complete kikuyu grass cover for at least five years. Water was impounded in the dam above normal spillway level by means of a control gate (Figure 1) made of $\frac{1}{8}$-in. steel plate. The control gate consisted essentially of a rectangular weir with an opening 16 ft by 2 ft. The opening was closed by a $\frac{1}{8}$-in. steel plate which could be raised and lowered by levers. Industrial sponge rubber was used to obtain a seal between the plate and the weir.

It was thus possible to pond water in the dam above spillway level and let it out as desired and to exercise control over the rate of discharge by raising or lowering the levers.

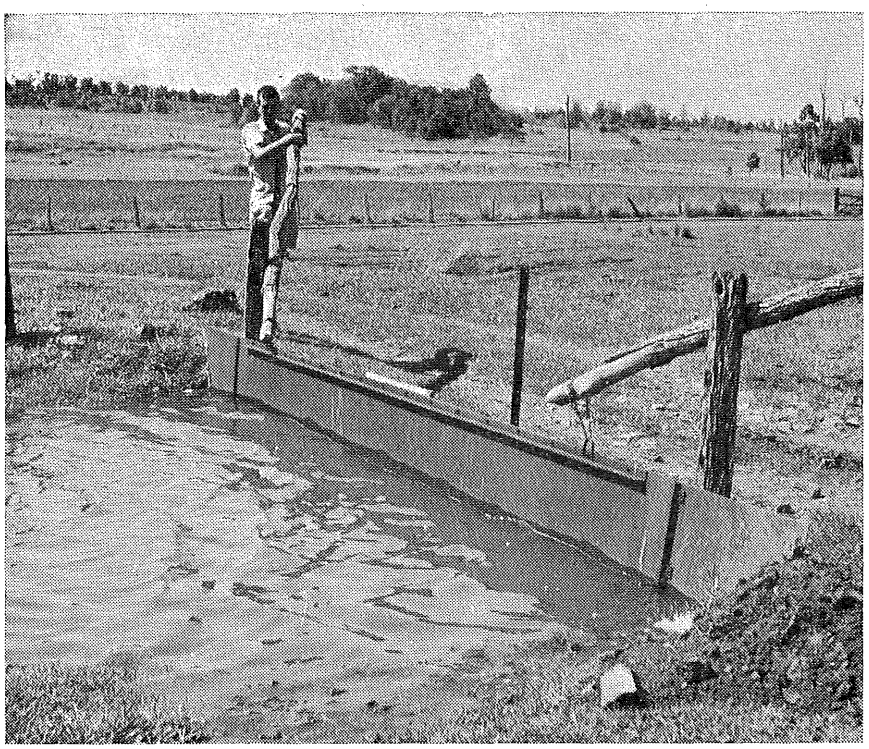

Fig. 1.—Control gate made of steel plate.

In the spillway below the control gate, a 10-ft sharp crested weir with suppressed end contractions (Figure 2) was installed to measure the flow. From the flow-measuring weir the water flowed 15 ft in a grass-lined channel into the kikuyu-lined waterway section under study. This had a flat bottom and sloping sides with a nominal bottom width of 4 ft and with one side slope of 4:1 and the other 2:1. Subsequent cross-section measurements showed the cross-section to be almost parabolic in shape.

As water became available in the dam, the test waterway was subjected to a measured flow for a period long enough to enable water surface measurements to be made, usually 10 or 15 min. As the surface of the dam lowered during a test, the control gate was adjusted to maintain a steady rate of flow. Where successive tests were made, the progression was from low to high flows.

III. MEASUREMENTS AND OBSERVATIONS

(1) Measurement for Discharge Calculations

Measurements were made of head of water flowing over the measuring weir and also of velocity in the test channel itself.

Measurement of head over the measuring weir (Figure 2) was made from a piano wire stretched across the approach channel 6 ft above the weir. Measurement to water surface was made using a piece of $\frac{1}{2}$-in. dowelling to which was attached a sharp metal point. Distance from water surface to the top of the wire was marked with a sharp pencil. A peg was fixed directly below the

Fig. 2.—Measuring weir. Measurement of head of water flowing over the weir is being made from a piano wire stretched across the approach channel 6 ft above the weir.

wire with a nail head in the peg set at weir crest level. A dumpy level was used for this operation, and a plastic measuring scale graduated in 0·001 ft as a staff. Distance from top of the wire to the nail head set at crest level was measured with the same wooden dowel as was used for the water surface reading and again marked with a pencil. Distance between the two pencil marks was measured with the scale and taken as head over the weir. Difference in elevation between the bottom of the level approach channel and the weir crest was measured, using a dumpy level and surveyor's staff.

Velocity in the test channel was measured with a simple pitot tube made from 0·22-in. inside diameter glass tubing bent at right-angles near one end and drawn out to an orifice of 0·1-in. diameter. The tube was fixed with rubber bands to the hollowed thin edge of a piece of 3 ft x 2 in. x $\frac{3}{8}$ in. pine cover strip. In use, the pitot tube was moved to the desired distance beyond the end of the wooden backing and then lowered into the water until the wood just touched the surface. Height of the head of water in the tube was marked with a waterproof pencil on the wood and the position in the channel recorded against the mark. After the flow was over, the distance from each mark to the end of the wooden strip was measured with a plastic measuring scale and this distance recorded as head of water at that point.

Velocity measurements were made at one cross-section only (Station 2). A measuring tape was stretched across at this point so that distance from a point on the right-hand edge of the waterway could be recorded. Velocity measurements were made at 1-ft intervals across the channel at a depth of 0·1 ft below the surface. At the deepest and fastest moving point in the stream, velocities were recorded at 0·1-ft depth, 0·2-ft depth, and so on in 0·1-ft intervals down to the bottom.

(2) Measurement of Cross-Section, Wetted Perimeter and Slope

Measurements were made at four cross-sections at 10-ft intervals in the test channel (Figure 3), designated Stations 1, 2, 3 and 4. At each station a piano wire was stretched across and levelled. The vertical co-ordinates of channel cross-section were measured by measuring down from these wires with a piece of $\frac{1}{2}$-in. wooden dowelling tapered to a $\frac{1}{4}$-in. point. Horizontal co-ordinates were measured with a graduated measuring tape tied across above the piano wire. Measurements were made at 0·5-ft intervals, the procedure followed being to mark the vertical co-ordinate on the dowelling with a sharp pencil and to identify the mark with the horizontal co-ordinate. A separate measuring stick was used for each station and the marks on the sticks were measured in turn, using a plastic rule graduated in 0·001 ft. Measurements were recorded in 0·001 ft but the nature of the surface did not permit an accuracy of more than 0·01 ft.

The procedure followed in making water surface measurements (Figure 4) during the test flows was identical except that pieces of dowelling with sharp metal points attached were used, and measurements were made at 1-ft intervals. This measuring procedure was adopted for the water surface measurements because of the limited availability of water and the necessity to take numerous

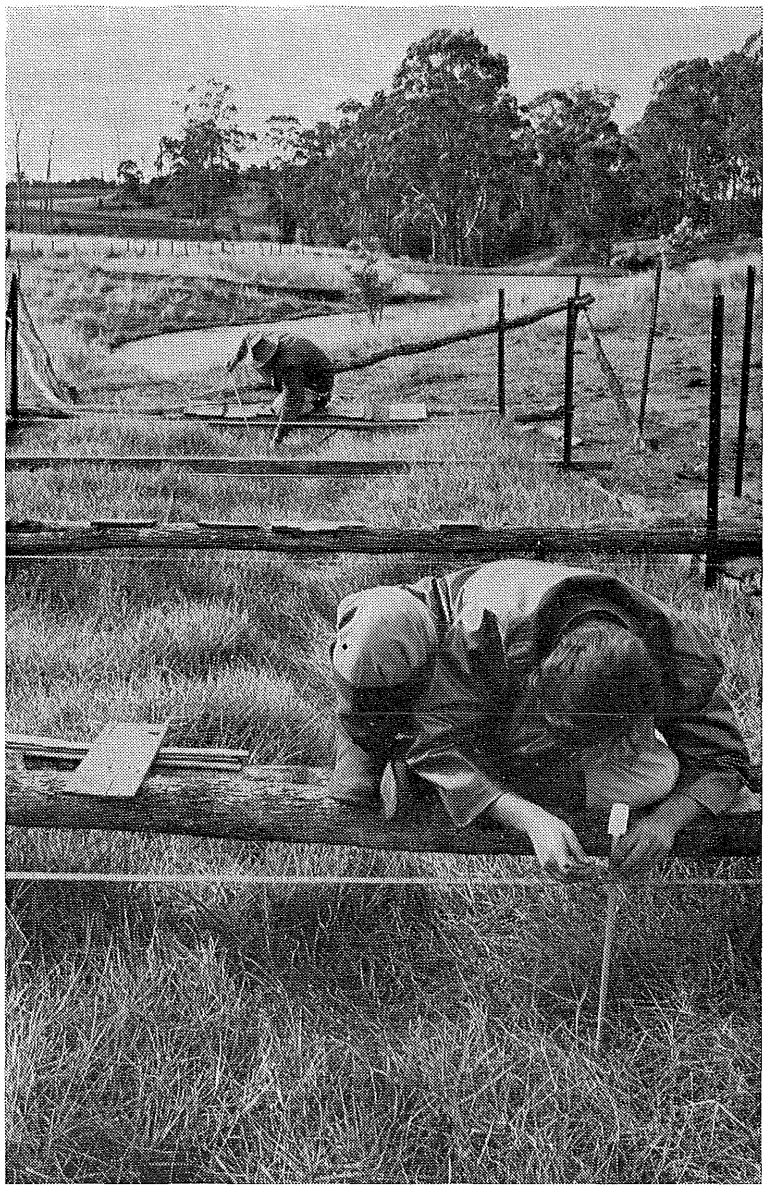

Fig. 3.—Cross-section measurements being made before a test flow (Test 2).

measurements in a short time. It was retained for the channel bottom measurements in an attempt to eliminate the personal error associated with marking the stick in relation to the wire. The marks were made on the top of the wire. Due to the uneven nature of the water surface, it was very difficult to obtain a reliable measurement of this factor. In an effort to reduce errors, Observer 1 changed position with Observer 2 and Observer 3 with Observer 4

Fig. 4.—Measurement of water surface being made at four stations during a test flow (Test 1).

after completing measurements across one section, so that each cross-section was measured twice and by different observers. The two measurements were averaged. Height of wires relative to one another was measured with a dumpy level and surveyor's staff. Distance between wires was measured along the bed slope with a measuring tape.

(3) Vegetative Measurements

Vegetation was measured by a stand count method. The number of stems was counted in six areas 6-in. square chosen at random. Length of stems from ground to leaf tips was also measured.

(4) Scour Measurements

Channel bottom was re-measured after each flow or pair of flows to enable calculation of scour.

(5) Vegetative Submergence

A visual observation of submergence was recorded for each test flow. Submergence was considered to occur only when the plant was completely inundated.

IV. METHODS OF COMPUTATION AND ESTIMATE

(1) Calculation of Discharge

(i) *By Weir Formula.*—Calculation of discharge through the measuring weir was made using Fteley and Stearns' supressed weir formula:

$$Q = 3{\cdot}31\ b\ (H + \alpha h)^{\frac{3}{2}} + 0{\cdot}007\ b\ ,$$

where

Q = flow in cubic feet per second
b = width of weir in feet
H = head
α = a coefficient the value of which varies with head on weir and depth of channel of approach below crest
h = head due to velocity of approach.

(ii) *By Pitot Tube.*—Point velocities in the one fast-moving vertical section were used to calculate the average velocity in the vertical and a relationship established between the average velocity in the vertical and the velocity at 0·1-ft depth which will be referred to as the surface velocity. The ratios are shown in Table 1.

The ratio between average velocity in the vertical and surface velocity was applied to all other surface velocities in the same cross-section.

The cross-section of flow was plotted from the measurements of channel bottom and water surface made from the piano wire stretched across the channel. The cross-section of flow was subdivided by vertical lines at points where surface velocity had been recorded. Areas of each subsection were measured by planimeter and mean velocity computed as the average of the velocities in the vertical on each side of the subsection.

Area was then multiplied by mean velocity to give a flow in cubic feet per second for the subsection and the subsectional flows added together to give a total flow for the whole cross-section.

(iii) *Comparison between Two Methods.*—Both methods left room for errors. The main weaknesses of the weir method were the shortness of the level approach channel, which was only 12 ft with weir crest 1·4 ft above bottom of approach channel, and the difficulty of attaining an even velocity in the approach channel, even with the use of wire netting and rock baffles. Uneven approach velocities were more pronounced in the higher flows and worst in Test 2.

Weaknesses of the pitot tube method lie mainly in the low velocity heads obtained for velocities below 2·5 ft/sec. It was found when calibrating the pitot tubes by testing against a standard pitot tube that velocity could be measured with an accuracy of ±5 per cent. in the velocity range 2·5–6 ft/sec. This meant that the faster flowing water which accounted for the major part of the flow was

measured with this accuracy. The slow-moving water in the unshingled grass at the sides of the waterway and again near the bottom of the waterway could not be measured accurately.

Flows calculated by the weir formula, with flow calculated from pitot tube velocity measurements in brackets, were: Test 3, 3·6 c.f.s. (4·6 c.f.s.); Test 4, 7·5 (9·0); Test 5, 2·7 (3·3); and Test 6, 5·9 (6·5). For these four tests the average value was used in calculations. For Test 1 the flow was not checked by the pitot tube and the weir flow was used. For Test 2 the weir flow (9·7 c.f.s.) was considered totally unreliable and discarded, the calculations being based on flow calculated from pitot tube velocities (15·2 c.f.s).

(2) Hydraulic Computations

Hydraulic computations were based on Manning's formula:—

$$V = \frac{1{\cdot}486}{n} R^{\frac{2}{3}} S^{\frac{1}{2}} \ldots\ldots\ldots (1),$$

where

V = mean velocity in feet per second = Q/A

Q = discharge in cubic feet per second

A = cross-sectional area of flow in square feet

R = hydraulic radius in feet = A/p

p = wetted perimeter in feet

S = hydraulic gradient or slope of the specific energy line in feet per foot

n = coefficient of retardance.

Computations were made for a 20-ft reach between Station 2 and Station 4.

For calculation of cross sectional area (A), measurements made from the piano wire stretched across at each measuring station were plotted. Scale was 2 in. to the foot. Plotting of channel bottom readings and water surface readings and joining of the plotted points gave an outline of the cross-section of water flowing. Measurement of the area was made with a planimeter. Areas at the beginning and end of the reach were averaged to compute average area for the reach.

Mean velocity (V) was computed by dividing discharge by the average of the end cross-sectional areas.

Wetted perimeter (p) was measured from the cross-sectional drawing. Average wetted perimeter for the reach was computed by averaging the values for the beginning and end of the reach.

Hydraulic radius (R) was computed as the quotient of average area of cross-section in square feet and average wetted perimeter in feet.

The slope of the energy line (S) was determined by a method described by Scobey (1939). The formula used was:—

$$S = \frac{(Z_1 + h_1) - (Z_2 + h_2)}{L}$$

$$= \frac{\left\{Z_1 + \frac{v_1^2}{2g}\right\} - \left\{Z_2 + \frac{v_2^2}{2g}\right\}}{L}$$

where

S = slope of the energy line
Z_1 = elevation of the water surface above datum at the beginning of the reach
Z_2 = elevation of the water surface above datum at the end of the reach
v_1 = average velocity in the cross-section at the beginning of the reach
v_2 = average velocity in the cross-section at the end of the reach
g = the gravitational constant = 32·2 in English measures
L = Length of the reach in feet, measured along the bed slope.

With R, v and S determined, these were then substituted in formula (1) to solve for Manning's "n".

Computation of the product of mean velocity and hydraulic radius (VR) was made by multiplying the mean velocity for the reach by the hydraulic radius for the reach.

V. RESULTS AND DISCUSSION

Experimental results set out in Table 1 cover condition of vegetation at each test, the amount of water flowing, cross-sectional area, mean velocity, hydraulic radius, effective slope, calculated Manning's "n", the product VR and Stillwater retardance rating. Additional information covering top width of the flow section, width of complete submergence, centre depth, maximum surface velocity and the ratio of mean velocity in a vertical section to the surface velocities is also given in this table.

In Figure 1 the experimental values of Manning's coefficient "n" are plotted against the product VR for each test flow, against a background of the five n-VR curves developed at Stillwater. It will be noted that for Tests 1, 2, 5 and 6 (long kikuyu grass), the plotted points lie on or near curve B. For Tests 3 and 4 (kikuyu grass grazed short), they lie between curves C and D.

There was no measurable difference in cross-section as a result of any of the test flows. This was borne out by visual observation during tests. The old kikuyu grass lining appeared to give complete protection and the water passing through the channel was quite clear. The complete protection from erosion was expected, but this was not the main object of the study, which was to classify the vegetal retardance of kikuyu grass. The vegetative conditions tested fall into two main categories: long kikuyu grass in Tests 1, 2, 5 and 6 and short-grazed kikuyu grass in Tests 3 and 4. All stands would be regarded as thick.

TABLE 1

EXPERIMENTAL CONDITIONS AND RESULTS FOR CHANNEL LINED WITH KIKUYU GRASS

Test No.	Date of Testing	Duration of Flow (min)	Condition of Lining	No. of Stems per sq. ft.	Average Length of Stems (ft)	Discharge (cu. ft./sec)	Area of Section, A (sq. ft.)	Mean Velocity, V (ft/sec)	Wetted Perimeter, p (ft)	Hydraulic radius, R (ft)	Effective slope, S (ft/ft)	Top Width (ft)	Width of Shingling or Complete Submergence (ft)	Centre Depth (ft)	Maximum Surface Velocity (ft/sec)	Ratio Mean Velocity Vertical/Surface Velocity	Manning's Coefficient of Roughness, n	Value of VR	Stillwater Retardance Rating
1	17.iii.60	15	Green ..	104	1·3	2·55	4·15	0·615	8·76	0·473	0·048	8·5	1·8	0·83	—	—	0·322	0·291	B
2	12.vii.60	20	Leaves frosted	176	1·8	15·18	6·48	2·342	10·49	0·618	0·054	10·1	4·7	1·12	6·0	0·60	0·107	1·447	B
3	26.vii.61	8	Green, grazed short	188	0·3	4·15	2·18	1·906	5·98	0·364	0·047	5·8	3·8	0·57	4·9	0·63	0·086	0·694	C–D
4	26.vii.61	7	Green grazed, short	188	0·3	8·25	2·98	2·769	6·86	0·435	0·048	6·6	4·7	0·74	6·2	0·66	0·067	1·203	C–D
5	25.x.61	10	Green ..	252	2·2	2·99	5·04	0·593	9·11	0·554	0·049	8·9	2·6	0·98	3·9	0·29	0·373	0·329	A–B
6	25.x.61	10	Green ..	252	2·2	6·19	5·97	1·037	9·94	0·601	0·049	9·6	3·7	1·07	6·7	0·34	0·226	0·623	B

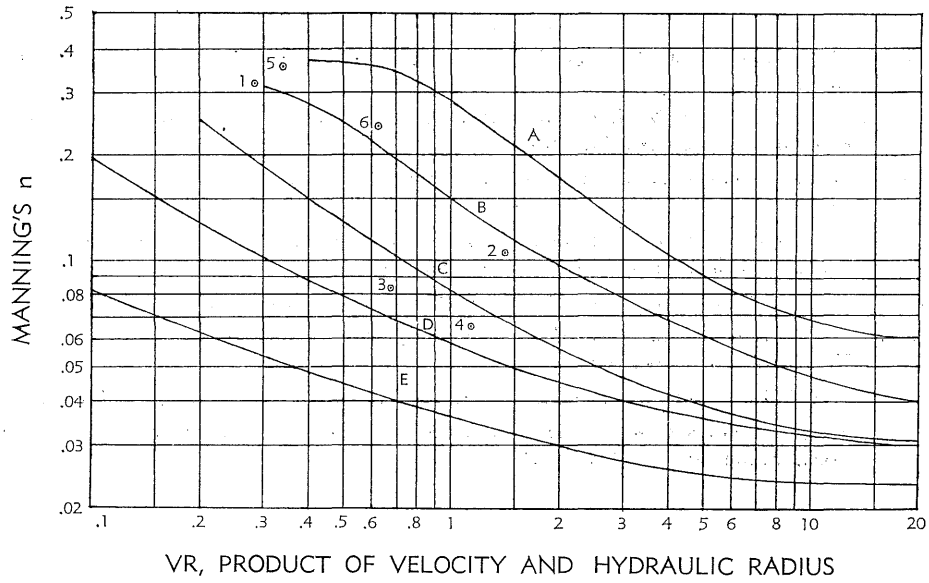

Fig. 5.—Experimental results for kikuyu grass channel in relation to retardance curves developed by Stillwater Outdoor Hydraulic Laboratory. Figures beside plotted points show test numbers for present tests.

Results are generally confined to the lower and mid values of VR and consequent high values of n. The quantity of water available and the size and slope of the test channel prevented the obtaining of high VR values where a bigger percentage of the grass would be shingled and values of n would be lower.

If kikuyu grass does follow the Stillwater retardance curves—and this is quite probable in view of the claimed general applicability of the curves to a wide variety of vegetation—then from the results obtained it is concluded that Retardance C is an appropriate curve for a thick stand of kikuyu grass grazed heavily and Retardance B is appropriate for a thick stand allowed to grow long and rank. Thinner stands would fall on a lower retardance curve in each case.

Further work to check the applicability of these curves over the full range of n-VR relationships is called for, but in the meantime use of the appropriate Stillwater retardance curve as indicated by the tests is justified and will give better results than the use of a fixed value of Manning's "n", which does not take into account the change in retardance as the grass is bent over by high-velocity flows.

VI. ACKNOWLEDGEMENTS

It is desired to acknowledge the assistance of a number of officers of the Agriculture and Soil Conservation Branches of the Department who assisted in taking measurements during test flows, and the co-operation of Mr. D. Newton, of Yarraman, on whose property the test channel was established.

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(Received for publication January 6, 1964)

EFFECT OF TOBACCO SEEDBED TREATMENTS FOR NEMATODE CONTROL ON PLANT GROWTH IN THE FIELD

By J. J. DAVIS, B.Sc.*

SUMMARY

Trials at Millaroo in North Queensland showed that tobacco seedbeds treated with heat or by methyl bromide fumigation provided seedlings free from infestation by the root-knot nematode *Meloidogyne javanica* (Treub).

Early seedling growth was better in the burned beds than in methyl bromide fumigated beds, but an added application of nitrogen fertilizer eliminated this difference by the time of transplanting.

The methyl bromide treatment produced seedlings with markedly higher bromine content than seedlings from fired beds, but this difference was not shown in the cured leaf.

Plants from all the seedbed treatments were similar in field growth and in yield and value of cured leaf.

I. INTRODUCTION

The root-knot nematode *Meloidogyne javanica* (Treub) is a serious pest of tobacco in Queensland. The use of infested seedlings as transplants was proved by Smith (1957) to cause a serious depression in yield of cured leaf even when the seedlings were planted into fumigated fields. Effective seedbed sterilization against nematodes was for a long time achieved by heat produced from burning "antbed" or wood. As both of these materials became scarce in some areas, tobacco growers turned to chemical means of fumigating seedbeds and methyl bromide treatment has become general practice. Investigation by Colbran and Saunders (1957) showed that sterilization by burning produced the larger seedlings. It was necessary therefore to determine whether the kind of seedbed sterilization or the size of transplants influenced yield or quality of cured leaf. Two trials with this object were carried out, one in 1958 and the other in 1960.

II. MATERIALS AND METHODS

General.—Both trials were on a fine sandy loam at Millaroo Research Station on the Burdekin River in North Queensland. In each, the variety Hicks was used and also in each the same three seedbed treatments were made. All

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beds received an overall application of 4:12:8 fertilizer at the rate of 1 lb per 40 sq. ft. at the time of sowing. In both the seedbeds and the field, insect pests and diseases were controlled by standard methods (Smith 1957; Pont 1956).

Seedbed Treatments.—The following treatments were applied in each trial:

A. Methyl bromide under gas-tight covers at the rate of 2·5 lb per 100 sq. ft.

B. Methyl bromide as in A, with application of sodium nitrate at 10 oz per 100 sq. ft. 3 weeks after sowing.

C. Antbed burning, using a 6-in. layer of the central mound structure of *Coptotermes acinaciformis* (Frogg.).

Trial 1.—Each method was applied to a seedbed 40 sq. ft. in area. (See Figure 1.) Sowing was made on July 14, 1958, and the plants lifted for transplanting on September 13. At this time 100 plants taken at random from each bed were assessed for nematode infestation. Seedlings were transplanted into subplots of a 3x3x3 field fertilizer trial, so the seedbed treatments were replicated 27 times. Plots were in a single row and each subplot contained 52 plants. The land had been fumigated on August 15 with EDB, 27·5 per cent. w/v, as a double-row treatment, using 10 gal per ac. The fertilizer treatments, applied on August 28, were (per ac) as follows:

N1, 10 lb N	P0, 0 lb P_2O_5	K0, 0 lb K_2O
N2, 20 lb N	P1, 50 lb P_2O_5	K1, 50 lb K_2O
N3, 30 lb N	P2, 100 lb P_2O_5	K2, 100 lb K_2O

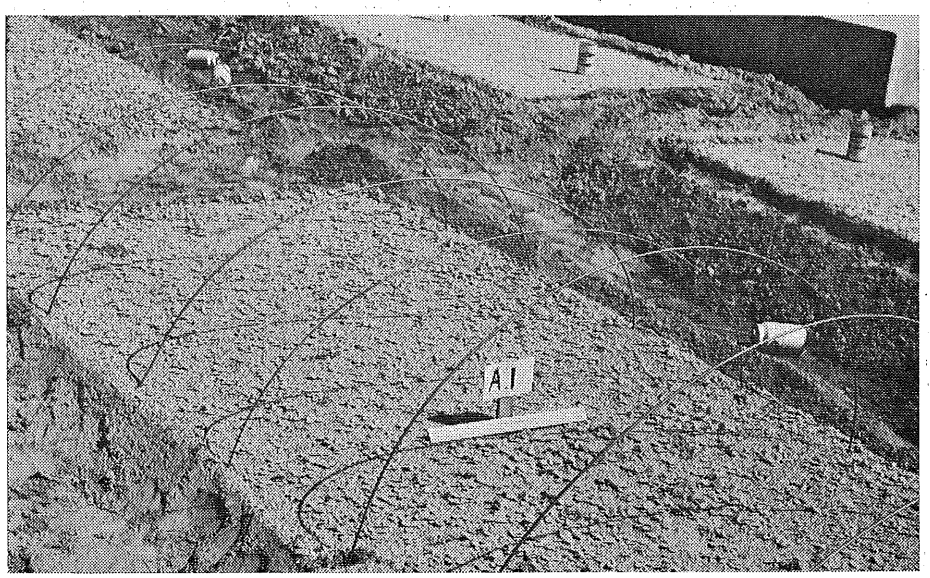

Fig. 1.—One of the seedbeds used in the experiments. Planted June 30, photographed August 10.

The leaf was harvested, cured and graded in the usual way and relative values were assigned to each grade according to leaf quality. After the harvest, plants from each plot were examined for nematode infestation.

Trial 2.—Seedbed sowings were made on June 30, July 4, 7 and 11, 1960, to provide plants of different sizes from each of the three differently sterilized beds. Seedbed plot size for each sowing was 6 ft x 3 ft, with a datum area of 5 ft x 2 ft.

At the time of lifting for transplanting on September 6, the numbers and green weights of washed seedlings were determined from six random 8-in. square quadrats in each bed. After being counted as plantable and unplantable and the green weights of seedlings in both categories determined, the combined plants from each quadrat were dried in a well-ventilated tobacco barn at 65–70°C. Analyses for bromine and chlorine were carried out, using methods given by Colbran and Green (1961). A further 100 plants taken at random from each bed were assessed for nematode infestation.

In the field, each of the seedbed treatment/planting time combinations was replicated 4 times in randomized blocks. Plot size was 4 rows each 35 ft long containing a total of 80 plants. The field had been fumigated on August 18 with EDB, 27·5 per cent. w/v, as an overall treatment, using 20 gal per ac. Harvested leaf was cured and graded as in Trial 1. Yields of cured leaf were determined and samples analysed for bromine and chlorine content. After harvesting, every second plant was assessed for nematode infestation.

III. RESULTS

Trial 1.—Both antbed burning and methyl bromide fumigation effectively controlled root-knot nematode. No infested seedlings were found in any beds at the time of transplanting.

Seedlings in the fired bed made faster growth than those in the methyl bromide treated beds. By the time of transplanting, however, the seedling size was equalized by the additional application of nitrogen fertilizer in one of the methyl bromide treated beds.

Conditions after planting caused some loss of transplants. Death of plants from the fired bed at 9·2 per cent. was higher than that of plants from the methyl bromide treated beds either with or without the additional fertilizer, such deaths being 5·2 and 2·6 per cent. respectively.

Some significant differences were shown between yields and relative values of cured leaf produced by plants from each of the three seedbed treatments (Tables 1 and 2). These differences, however, were in complex relation to the field fertilizer treatments.

TABLE 1

TRIAL 1. YIELDS OF CURED LEAF
(lb/ac)

Fertilizer Treatment	Seedbed Treatment			Mean
	A Methyl Bromide	B Methyl Bromide with Additional Fertilizer	C Burned with Antbed	
N1	1421	1370	1321	1371
N2	1418	1337	1322	1359
N3	1442	1398	1490	1444
P0	1508	1431	1281	1407
P1	1438	1321	1416	1392
P2	1335	1354	1436	1375
K0	1408	1362	1528	1433
K1	1453	1409	1332	1398
K2	1419	1335	1273	1342
Mean ..	1427	1369	1378	1391

At P0 A>>C, B at 1% level

At K0 C>B at 5% level

At K2 A>C at 5% level

No significant differences at other fertilizer rates

TABLE 2

TRIAL 1. RELATIVE VALUES OF CURED LEAF

Fertilizer Treatment	Seedbed Treatment			Mean
	A Methyl Bromide	B Methyl Bromide with Additional Fertilizer	C Burned with Antbed	
N1	90·2	87·2	85·3	87·6
N2	92·6	85·1	87·0	88·2
N3	90·2	87·7	92·7	90·2
P0	96·8	91·2	80·9	89·6
P1	92·9	83·8	92·5	89·7
P2	83·3	85·0	91·6	86·6
K0	89·9	84·8	97·8	90·9
K1	92·0	90·4	85·4	89·3
K2	91·1	84·8	81·7	85·9
Mean ..	91·0	86·7	88·3	88·7

At P0 A>C at 5% level

No significant differences at other fertilizer rates

Only slight nematode infestation occurred on the mature plants after harvesting and this was scattered, without relation to the seedbed treatments.

Trial 2.—Growing conditions in the seedbed were good and differences in seedling size due to differences in sowing time soon disappeared. The various sowing times were not true replicates and these results were not analysed.

Numbers and green weights of seedlings showed only slight variations between the seedbed treatments (Table 3). Mean dry weight of seedlings and percentage of plantable seedlings, however, were greatest in the methyl bromide treated bed to which an additional nitrogen fertilizer application had been made (Table 3).

TABLE 3

TRIAL 2. RESULTS FROM SEEDBEDS

Seedbed Treatment	Type of Seedlings	Mean per Quadrat 8 in. Square				Mean Green Weight per Plant (g)	Mean Bromine Content (p.p.m. Br)	Mean Chlorine Content (% Cl)
		Number	Green Weight (g)	Percentage of Total	Dry Weight (g)			
Methyl bromide ..	Plantable	22	72	36	11·5	3·2	4,488	1·7
	Unplantable	40	46	64		1·2		
Methyl bromide with additional fertilizer	Plantable	36	109	50	14·2	3·1	3,025	1·5
	Unplantable	36	43	50		1·2		
Fired with antbed ..	Plantable	28	86	42	11·1	3·0	110	1·8
	Unplantable	43	50	58		1·2		

The use of methyl bromide greatly increased the bromine content of seedlings (Table 3). The chlorine determinations showed only slight differences.

As in Trial 1, nematodes were effectively controlled in all beds, with only a minor trace of infestation in a few seedlings.

There were no significant differences in yields and relative value of cured leaf from plants coming from the various seedbed treatments. The bromine content of cured leaf was even between treatments and the chlorine content remained uniform (Table 4).

TABLE 4

TRIAL 2. RESULTS FROM CURED LEAF

Seedbed Treatment	Mean Yield (lb/ac)	Relative Leaf Value	Bromine (p.p.m. Br)	Chlorine (% Cl)
Methyl bromide ..	1364	54·5	2803	2·55
Methyl bromide with additional fertilizer	1382	55·8	2689	2·48
Fired with antbed ..	1353	53·8	2727	2·51
	No significant differences		Not analysed	

Nematode infestation in the mature plants was light and scattered.

IV. DISCUSSION AND CONCLUSIONS

These trials again demonstrated that effective seedbed sterilization for root-knot nematode control is achieved either by antbed burning or by methyl bromide fumigation.

Partly by improving water penetration into the beds and partly by the addition of plant nutrients from the ash, firing the beds improved the growth of seedlings in beds so treated compared with those from methyl bromide fumigated seedbeds. By the time of transplanting the response to an additional application of nitrogen fertilizer to one of the methyl bromide treated beds had eliminated the difference in size of transplants.

Under severe conditions after planting out, softer transplants from fired beds, and from methyl bromide fumigated beds, with extra fertilizer, showed higher mortality than those from methyl bromide fumigated beds without additional fertilizer.

Although the methyl bromide treatment greatly increased the amount of bromine in seedlings, this difference in bromine content did not carry through to the cured leaf. Field growth of plants from all seedbeds was normal and the bromine content of cured leaf was comparable with that reported by Colbran and Green (1961) from plants grown on land treated with EDB at 20 gal per ac.

Plants from methyl bromide treated beds did not differ in yield or value of cured leaf produced from those from fired seedbeds.

V. ACKNOWLEDGEMENTS

The manager and staff of the Millaroo Research Station provided facilities for carrying out the work. Statistical analyses were carried out by the Biometrics Branch and chemical analyses were done by the Agricultural Chemical Laboratory Branch.

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(Received for publication October 4, 1963)

STUDIES OF PLANT AND SOIL NEMATODES. 7. QUEENSLAND RECORDS OF THE ORDER TYLENCHIDA AND THE GENERA TRICHODORUS AND XIPHINEMA

By R. C. COLBRAN, M.Agr.Sc., Ph.D.*

SUMMARY

One hundred and five nematode species, including many important plant pathogens, are reported from Queensland.

The records are presented in two sections. In the first the nematode species are arranged in alphabetical order with information on associated plants and localities; in the second the plant species are arranged in similar order with lists of associated nematodes.

I. INTRODUCTION

Nematodes have been important pests of Queensland crops since the beginnings of agriculture in the State. Bancroft (1879) reported that root-knot nematodes, which he referred to as "flask worms", were responsible for colonists abandoning farmlands in south-eastern Queensland. As host plants he mentioned *Solanum nigrum, Sida rhombifolia,* banana, broad bean, grape, most culinary vegetables and many weeds, even those growing in virgin ground. He described and illustrated root-knot of banana, which he considered was responsible for Panama disease (Figure 1).

Tryon (1903) reported that *Heterodera* and *Tylenchus* (now *Meloidogyne* species and *Radopholus similis* (Cobb) respectively) were found in banana roots at Cairns and were responsible for "constitutional derangement involving the fruit".

Colbran (1953, 1954, 1955, 1956, 1960*a,* 1960*b,* 1961, 1962, 1963, and in Colbran and Saunders 1961) described 11 new species of Tylenchida and one new species of *Trichodorus* from Queensland and reported the occurrence of a number of other species. This author (1958) listed Queensland host records of root-knot nematodes (*Meloidogyne* species). As a complementary paper, additional records only of these species have been included.

II. MATERIALS AND METHODS

Nematodes were obtained from plant and soil samples collected by the writer and other officers of the Queensland Department of Primary Industries or submitted by farmers for examination. After extraction, these were relaxed by gentle heat, fixed in F.A.A., and processed by standard lactophenol or glycerine methods before being mounted in martius yellow/cotton blue lactophenol or anhydrous glycerine respectively.

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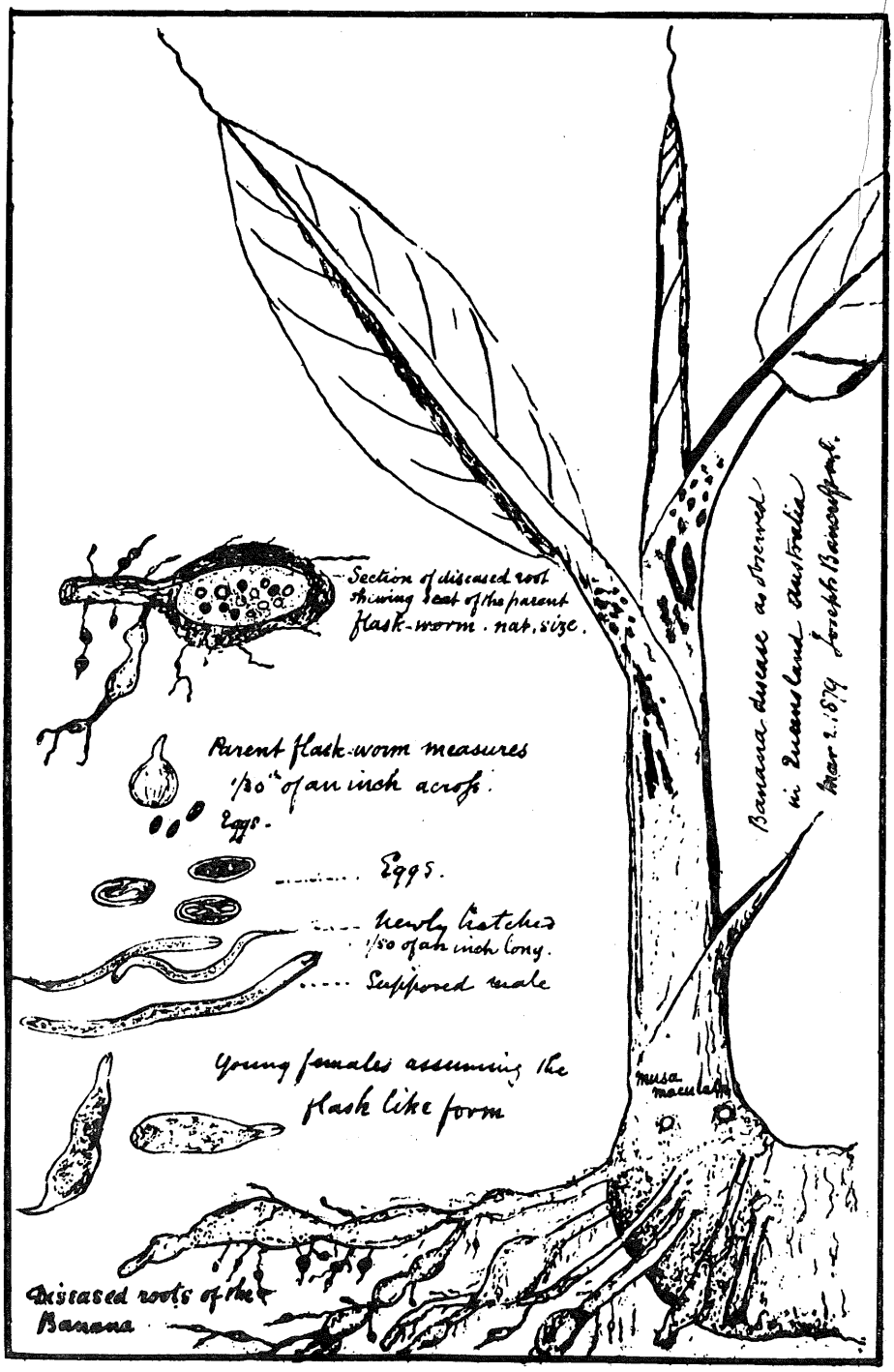

Fig. 1.—Root-knot of banana (After Bancroft 1879).

III. PRESENTATION OF DATA

Records are presented in two sections. In the first, nematode species are arranged in alphabetical order with the lists of the plants with which they were associated, localities and occasional comments; in the second, plant species are arranged in alphabetical order with lists of associated nematode species.

The association of a nematode and plant does not imply that the species is a parasite. When parasitism has been observed it is indicated by x (field conditions) or G (greenhouse conditions). Apart from the parasites of spermaphytes, some species reported in this paper are fungivorous and other are predatory on other nematodes.

Synonymy has been included when considered necessary.

SECTION 1

Nematode/Associated Plant List

ANGUINA Scopoli

(Seed gall nematodes)

Anguina agrostis (Steinbuch)

(Bent grass nematode)

Galls in seed of *Agrostis tenuis* Sibth. (browntop bent grass) from New Zealand. No infestation has been observed in the field.

Anguina microlaenae (Fawcett)*

Galls on the leaves and inflorescences of *Astrebla pectinata* (Lindl.) F. Muell. ex Benth. (barley Mitchell grass), "Tandara", via Morella. (Figure 2.)

Fig. 2.—Galls on barley Mitchell grass caused by *Anguina microlaenae*.

* Or a closely related undescribed species.

APHELENCHOIDES Fisher

Aphelenchoides bicaudatus (Imamura)

Soil. Brisbane, Kennedy, Montville, Stanthorpe, Yeppoon.

A. bicaudatus has been observed to reproduce on fungal cultures.

Aphelenchoides coffeae (Zimmerman)

Soil. Applethorpe, Ayr, Ballandean, Brisbane, Buderim, Coolum, Cooroy, Inglewood, Ipswich, Koongal, Mapleton, Nambour, Rockhampton, Tolga, Yandina, Yeppoon.

A. coffeae has been observed to reproduce on fungal cultures.

Aphelenchoides composticola Franklin

Soil. Brisbane, Ballandean, Biloela, Bouldercombe, Glen Aplin, Redland Bay, Yelarbon, Yeppoon.

Aphelenchoides ritzema-bosi (Schwartz)

(Chrysanthemum foliar nematode)

Chrysanthemum morifolium Ramat. Chrysanthemum. Brisbane, Rockhampton, Toowoomba.

Aphelenchoides saprophilus Franklin

Soil. Brisbane, Inglewood, Thulimbah, Toowoomba, Yelarbon.

Aphelenchoides subtenuis (Cobb)

Portulaca oleracea L. Pigweed. Applethorpe.

APHELENCHUS Bastian

Aphelenchus avenae Bastian

A. avenae is a very common fungivore present in soil throughout Queensland and is frequently observed reproducing on fungal cultures.

BASIRIA Siddiqi

Basiria graminophila Siddiqi

Chrysanthemum morifolium Ramat. Chrysanthemum. Brisbane (Botanic Gardens).

BOLEODORUS Thorne

Boleodorus thylactus Thorne

Panicum maximum Jacq. Guinea grass. Brisbane.

Pinus elliottii Engelm. Slash pine. Beerwah (Coochin logging area).

Vitis vinifera L. Grape. Texas.

Boleodorus volutus Lima and Siddiqi

Melastoma polyanthum Blume. Blue tongue. Coolum.

CRICONEMA Hofmanner and Menzel

(Ring nematodes)

Criconema australe Colbran

Citrus sinensis Osbeck. Sweet orange. Mundubbera.

Digitaria didactyla Willd. Queensland blue couch. Brisbane (Wynnum Bowling Green).

Imperata cylindrica (L.) Beauv. var. *major* (Nees) C. E. Hubbard. Blady grass. Brisbane (Long Pocket).

Medicago sativa L. Lucerne. Brisbane (Moggill).

Soil. Hills overlooking Harwood (New South Wales).

Criconema civellae Steiner

Citrus limon (L.) Burm. f. Rough lemon. Burrum, Mundubbera.

Criconema octangulare (Cobb) Taylor

Hodgkinsonia frutescens C. T. White. Tolga.

Criconema pectinatum Colbran

Ananas comosus (L.) Merr. Pineapple. Elimbah.

Pinus elliottii Engelm. Slash pine. Beerwah.

CRICONEMOIDES Taylor

(Ring nematodes)

Criconemoides demani (Micoletzky)

Soil. Cunningham's Gap.

Criconemoides limitaneum (Luc)

Hodgkinsonia frutescens C. T. White. Tolga.

Criconemoides macrodorum Taylor

Themeda australis (R.Br.) Stapf. Kangaroo grass. Mt. Nebo.

Criconemoides mutabile Taylor

Carica papaya L. Papaw. Brisbane (Sunnybank).

Digitaria didactyla Willd. Queensland blue couch. Brisbane (Rocklea).

Musa sp. Banana variety (cultivar) Williams Hybrid. Brisbane (Rochedale).

Soil. Mt. Glorious.

Criconemoides ornatum Raski

Ananas comosus (L.) Merr. Pineapple. Beerwah, Elimbah, Glasshouse Mountains, Mutanee, Woombye.

Citrus limon (L.) Burm. f. (rough lemon) or *C. sinensis* Osbeck (sweet orange). Brisbane (Mt. Gravatt).

Digitaria didactyla Willd. Queensland blue couch. Brisbane (Sunnybank Bowling Green).

Hornstedtia scottiana (F. Muell.) K. Schum. A wild ginger. Junction of Palmerston Highway and Henderson Drive, North Queensland.

Criconemoides peruense (Steiner)

Soil. Elimbah.

Criconemoides xenoplax Raski

Vitis vinifera L. Grape. Glen Aplin.

DELADENUS Thorne

Deladenus durus (Cobb)

Citrus limon (L.) Burm. f. (rough lemon) or *C. sinensis* Osbeck (sweet orange). Byrnestown, Gayndah.

Musa sp. Banana variety (cultivar) Williams Hybrid. Nambour.

D. durus was observed to reproduce on a culture of *Fusarium* sp. from a gladiolus corm.

DITYLENCHUS Filipjev

Ditylenchus myceliophagus J. B. Goodey

Soil. Clump Point, Dalby, Didillibah, Elimbah.

DOLICHODORUS

(Awl nematodes)

Dolichodorus obtusus Allen

Gahnia clarkei Benl. Burrum.

ECPHYADOPHORA de Man

Ecphyadophora tenuissima de Man

Soil. Applethorpe, Beerwah, Burrum, Cunningham's Gap, Rosemount, Harwood (New South Wales), Windsor (New South Wales).

FERGUSOBIA (Currie)

Fergusobia tumefaciens (Currie)

Associated with the fly *Fergusonina brimblecombei* Tonnoir on:

x *Eucalyptus crebra* F. Muell. Narrow-leaved ironbark. Forest Hill.
x *Eucalyptus hemiphloia* F. Muell. ex Benth. Gum-topped box. Forest Hill.
x *Eucalyptus melanophloia* F. Muell. Silver-leaf ironbark. Forest Hill.

Associated with *Fergusonina tillyardi* Tonnoir on:

x *Eucalyptus tereticornis* Sm. Blue gum. Redland Bay.

Associated with *Fergusonina* sp. on:

x *Angophora costata* (Gaertn.) Domin. Rusty gum. Yarraman. (Figure 3).

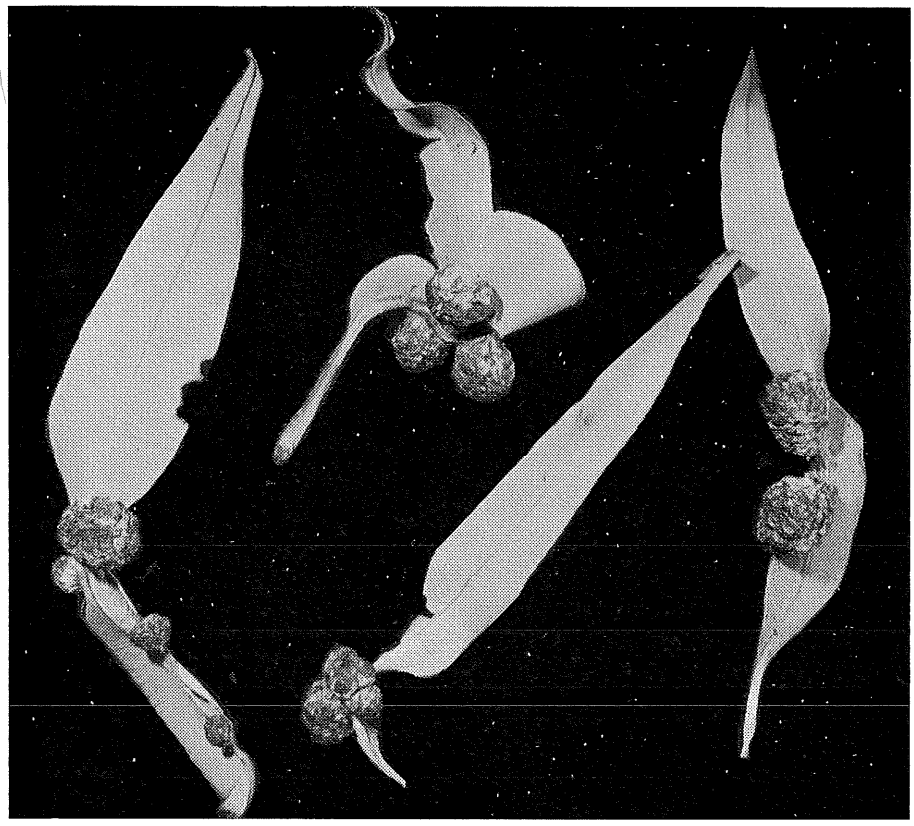

Fig. 3.—Galls on the leaves of *Angophora costata* caused by *Fergusobia tumefaciens* in association with flies of the genus *Fergusonina*.

x *Angophora subvelutina* F. Muell. Broad-leaved apple. Yarraman.
x *Eucalyptus acmenioides* Schau. Yellow stringybark. Brisbane (Toowong).
x *Eucalyptus drepanophylla* F. Muell. ex Benth. Grey ironbark.
x *Eucalyptus intermedia* F. Muell. Pink bloodwood. Redland Bay.
x *Eucalyptus populnea* F. Muell. Poplar box. Barakula.
x *Eucalyptus tessellaris* F. Muell. Moreton Bay ash. Forest Hill.

GRACILIACUS Raski

Graciliacus peperpotti Schoemaker

Pinus elliottii Engelm. Slash pine. Beerwah.

HELICOTYLENCHUS Steiner

(Spiral nematodes)

Helicotylenchus dihystera (Cobb)

Syn. Helicotylenchus nannus Steiner

This is one of the most common tylenchs in Queensland soils.

Ananas comosus (L.) Merr. Pineapple. Amamoor, Bauple, Beerwah, Bowen, Brisbane (Acacia Ridge), Dayboro, Didillibah, Dundowran, Elimbah, Flaxton, Innisfail, Kallangur, Kandanga, Montville, Nikenbah, Palmwoods, Rosemount, Tewantin, Woombye. Although large numbers of *H. dihystera* are a common feature of pineapple soils, unpublished data from field trials indicate that the association has little effect on plant growth.

Annona squamosa (L.). Custard apple. Yeppoon.

Araucaria cunninghamii D. Don. Hoop Pine. Danbulla.

Asclepias fruticosa L. Narrow-leaf cotton bush. Nambour.

x *Avena sativa* L. Oats. Beerwah.

Bauhinia sp. Rockhampton.

Beta vulgaris L. var. *cicla* L. Silver beet. Frenchville.

Beta vulgaris L. var. *vulgaris*. Beetroot. Mt. Isa, Victoria Point, Yeppoon.

Brassica oleracea L. var. *capitata* L. Cabbage. Brisbane (Eight Mile Plains).

Cajanus cajan (L.) Millsp. Pigeon pea. Nambour.

Carica papaya L. Papaw. Yeppoon.

Centrosema pubescens Benth. Centro. Nambour.

Citrus limon (L.) Burm. f. (rough lemon) or *C. sinensis* Osbeck (sweet orange). Brisbane (Mt. Gravatt, Pullenvale), Burrum Chevallum, Clump Point, Gayndah, Kennedy, Koah, Palmwoods, Pennant Hills (New South Wales).

Coleus blumei Benth. Coleus. Koongal.

Cucumis melo L. var. *reticulatus* Naud. Rockmelon. Brisbane (Manly).

Cucumis sativus L. Cucumber. Brisbane.

Cyclamen persicum Mill. Cyclamen. Brisbane (Northgate).

Daucus carota L. Carrot. Mt. Ninderry.

Dianthus caryophyllus L. Carnation. Brisbane (Rochedale), Wallangarra.

x *Digitaria didactyla* Willd. Queensland blue couch. Brisbane (Sunnybank Bowling Green).

x *Dolichos lablab* L. Tonga or hyacinth bean. Beerwah.

Fragaria X ananassa Duchesne. Strawberry. Beerwah, Frenchville, Nambour, Ormiston, Gosford (New South Wales).

Gerbera jamesonii Bolus. Gerbera. Brisbane.

G *Hordeum vulgare* L. Barley.

Hornstedtia sp. A wild ginger. Junction of Palmerston Highway and Henderson Drive (North Queensland).

Imperata cylindrica (L.) Beauv. var. *major* (Nees) C. E. Hubbard. Blady grass. Nambour.

Lactuca sativa L. Lettuce. Biloela, Brisbane (Everton Park).

Lantana camara L. Lantana. Kabra.

x *Lotononis bainesii* Bak. Nambour.

Lupinus angustifolius L. New Zealand blue lupin. Tolga.

Lycopersicon esculentum Mill. Tomato. Ballandean, Gracemere, Kabra, Ormiston, Yeppoon.

Macadamia tetraphylla L.A.S. Johnson. A Macadamia or Queensland nut. Nambour.

Medicago sativa L. Lucerne. Brisbane (Moggill), Jimboomba, Kairi.
Melinis minutiflora Beauv. Molasses grass.
x *Musa* spp. Banana varieties (cultivars) Cavendish, Lady Finger and Williams Hybrid. Brisbane (Rochedale, The Gap), Buderim, Bundaberg, Currumbin, Glasshouse Mountains, Maryborough, Mt. Mee, Nambour, Narangba, Pimpama, Tully, Woongoolba.
H. dihystera produces small surface necroses on banana roots.
Opuntia sp. A prickly pear. Mulambin Beach.
Paspalum dilatatum Poir. Paspalum. Miriam Vale.
Passiflora edulis Sims forma *edulis*. Passionfruit. Bundaberg, Mapleton.
Petunia X hybrida Vilm. Petunia. Rockhampton.
x *Phaseolus atropurpureus* DC. Siratro. Nambour.
Phaseolus vulgaris L. French bean. Frenchville.
Pisum sativum L. Garden Pea. Brisbane, Montville.
Plantago major L. Large plantain. Stanthorpe.
Prunus persica (L.) Batsch. var. *nectarina* Maxim. Nectarine. Penrith (New South Wales).
Saccharum officinarum L. Sugar cane. Bundaberg, Coolum, Mossman.
Secale cereale L. Rye. Beerwah.
Solanum tuberosum L. Potato. Beerwah.
x *Sorghum bicolor* (L.) Moench. Sweet sorghum. Beerwah.
x *Sorghum sudanense* (Piper) Stapf. Sudan grass. Nambour.
x *Stizolobium* spp. Velvet beans. Nambour.
Tapeinocheilos queenslandiae (F.M. Bail.) K. Schum. Junction of Palmerston Highway and Henderson Drive (North Queensland).
x *Trifolium repens* L. White clover. Brisbane (Mt. Gravatt), Cooroy, Kenilworth, Maleny, Pomona, Redbank.
x *Triticum aestivum* L. Wheat. Cobba-da-manna.
Vitis vinifera L. Grape. Coominya, Severnlea, Warwick.
x *Zea mays* L. Maize. Beerwah, Nambour.
Soil. Highvale, Ravenshoe, Samsonvale, Harwood (New South Wales).

Helicotylenchus erythrinae (Zimmerman)

Pilidiostigma tropicum L.S. Smith. Tolga.
Soil. Tully.

Helicotylenchus multicinctus (Cobb)

Ananas comosus (L.) Merr. Pineapple. Kallangur.
Musa banksii F. Muell. A wild banana. Near Bookal-Bookal Creek four miles north of Tully.
Musa hillii F. Muell. A wild banana. Bailey's Creek (North Queensland).
Musa spp. Banana varieties (cultivars) Cavendish, Lady Finger, Williams Hybrid. Brisbane (Brookfield), Bundaberg, Nambour, Pialba, Tully, Victoria Point, Wamuran, Yeppoon.
Soil. Beerwah, Rockhampton.

HEMICRICONEMOIDES Chitwood and Birchfield

Hemicriconemoides brachyurus (Loos)

Casuarina equisetifolia L. Dune sheoak. Mulambin Beach.

Hemicriconemoides chitwoodi Esser

Opuntia sp. A prickly pear. Mulambin Beach.

Hemicriconemoides cocophillus (Loos)

Casuarina equisetifolia L. Dune sheoak. Burrum Heads.
Citrus sinensis Osbeck. Sweet orange. Mundubbera.

Hemicriconemoides mangiferae Siddiqi

Ananas comosus (L.) Merr. Pineapple. Bowen.

Hemicriconemoides obtusus Colbran

Ananas comosus (L.) Merr. Pineapple. Beerwah, Didillibah.
Casuarina equisetifolia L. Dune sheoak. Burrum Heads.
Saccharum officinarum L. Sugar cane. Bundaberg.
Themeda australis (R.Br.) Stapf. Kangaroo grass. Mt. Nebo.

HEMICYCLIOPHORA de Man

(Sheath nematodes)

Hemicycliophora labiata Colbran

Casuarina equisetifolia L. Dune sheoak. Burrum Heads.
Entolasia stricta (R.Br.) Hughes. Brisbane (Oxley).
Medicago sativa L. Lucerne. Brisbane (Moggill).
Themeda australis (R.Br.) Stapf. Kangaroo grass. Mt. Nebo.
Soil. Mulambin Beach, hills overlooking Harwood (New South Wales).

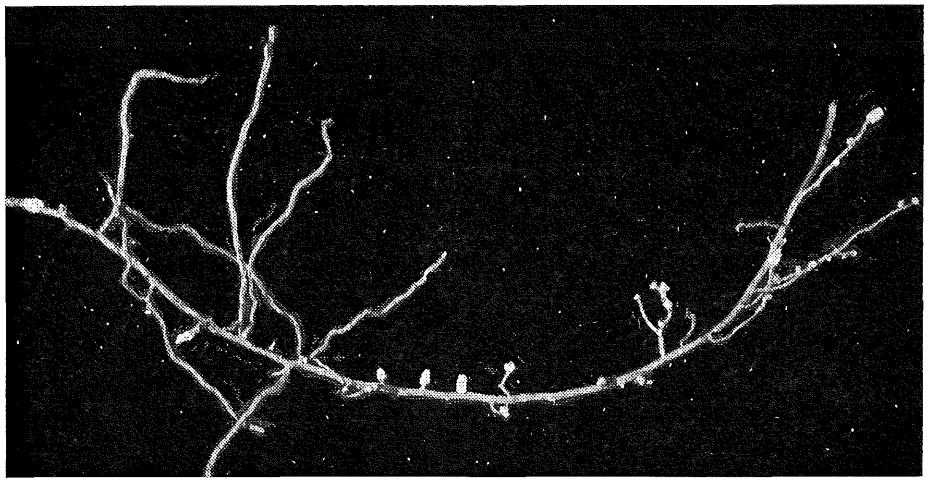

Fig. 4.—Galls on the roots of rough lemon caused by *Hemicycliophora nudata*.

Hemicycliophora nudata Colbran

x *Citrus limon* (L.) Burm. f. Rough lemon. Burrum.
G *Citrus reticulata* Blanco. Mandarin varieties (cultivars) Emperor and Cleopatra.
G *Citrus sinensis* Osbeck. Sweet orange.
Gahnia clarkei Benl. Burrum.
Soil. Hills overlooking Harwood (New South Wales).

H. nudata feeds on the root tips of citrus, producing conspicuous galls (Figure 4). Under glasshouse conditions it has caused severe injury to the root tips of *Capsicum frutescens* L. (capsicum, variety California Wonder) (Figure 5).

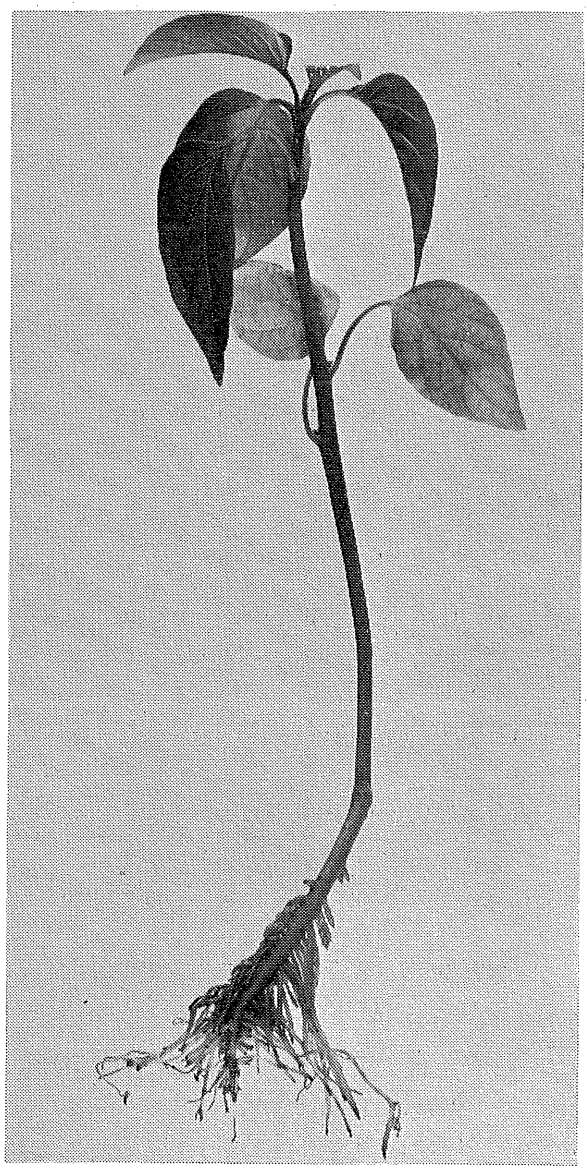

Fig. 5.—Capsicum with roots injured by *Hemicycliophora nudata.*

Hemicycliophora ovata Colbran

Ananas comosus (L.) Merr. Pineapple. Beerwah, Cooroy, Glasshouse Mountains.
Musa sp. Banana variety (cultivar) Williams Hybrid. Tully.
Soil. Harwood (New South Wales).

Hemicycliophora truncata Colbran

Ananas comosus (L.) Merr. Pineapple. Elimbah, Eumundi, Mooloolah, North Arm, Woombye.
Casuarina littoralis Salisb. Black sheoak. Eudlo (Jawarra Park).
Citrus limon (L.) Burm. f. (rough lemon) or *C. sinensis* Osbeck (sweet orange). Brisbane (Mt. Gravatt).
Eucalyptus pilularis Sm. Blackbutt. Fraser Is.
Lycopersicon esculentum Mill. Tomato. Stanthorpe (Bald Mountain).
Prunus persica (L.) Batsch. Peach. Ballandean.
Themeda australis (R.Br.) Stapf. Kangaroo grass. Mt. Nebo.
Vitis vinifera L. Grape. Glen Aplin.
Soil. Brisbane (Rochedale), hills overlooking Harwood and Maclean (New South Wales).

HETERODERA Schmidt

(Cyst nematodes)

Heterodera schachtii Schmidt

(Sugar-beet nematode)

x *Beta vulgaris* L. var. *vulgaris*. Beetroot. Lawes.
x *Brassica napobrassica* (L.) Mill. Swede turnip. Lawes.
x *Brassica oleracea* L. var. *capitata* L. Cabbage. Lawes.

Heterodera trifolii Goffart

(Clover cyst nematode)

x *Trifolium repens* (L.) White clover. Maleny.

Light infestations of *H. trifolii* have been found on two farms.

MELOIDOGYNE Goeldi

(Root-knot nematodes)

Additions to Queensland host records of *Meloidogyne* species (Colbran 1958).

Meloidogyne arenaria thamesi Chitwood

x *Brassica oleracea* L. var. *capitata* L. Cabbage. Moderate. Brisbane (Eight Mile Plains).
x *Calopogonium mucunoides* Desv. Calopo. Heavy. South Johnstone.
x *Nicotiana tabacum* L. Tobacco. Heavy. Bundaberg.
x *Phaseolus vulgaris* L. French bean. Heavy. Boulia.

Meloidogyne hapla Chitwood

x *Arachis hypogaea* L. Peanut. Moderate. Tingoora.
G *Crotalaria goreensis* Guill. & Perr. Gambia pea. Light.
x *Dolichos lablab* L. Tonga or hyacinth bean. Heavy. Thornlands.
x *Lotononis bainesii* Bak. Heavy (nodules); light (roots). Mt. Mee.
x *Persea americana* Mill. Avocado. Light. Nambour, Palmwoods, Redland Bay.
x *Plantago major* L. Large plantain. Light. Brisbane (Newmarket).
x *Rosa multiflora* Thunb. Heavy. Brisbane (Rochedale), Lawnton.
G *Stylosanthes gracilis* H.B.K. Stylo. Moderate.
G *Trifolium baccarinii* Chiov. Light.
G *Trifolium burchellianum* Ser. ex DC. Cape clover. Light.
G *Trifolium glomeratum* L. Clustered clover. Light.
G *Trifolium hybridum* L. Alsike clover. Light.
x *Trifolium repens* L. White clover. Moderate. Lawes, Maleny, Pomona.
G *Trifolium rueppellianum* Fresen. Heavy.
G *Trifolium semipilosum* Fresen. Light.

Meloidogyne incognita (Kofoid and White)

Syn. *Meloidogyne incognita* var. *acrita* Chitwood
G *Centella asiatica* (L.) Urb. Pennyweed. Moderate.
x *Chenopodium album* L. Fat hen. Light. Lawes.
x *Clerodendrum splendens* G. Don. A clerodendrum. Heavy. Brisbane (Toowong).
x *Dianthus caryophyllus* L. Carnation. Heavy. Brisbane.
x *Dolichos lablab* L. Tonga or hyacinth bean. Heavy. Kairi.
x *Hibiscus schizopetalus* Hook. f. Hibiscus. Heavy. Palm Beach.
x *Lantana montevidensis* (Spreng.) Briq. Creeping lantana. Moderate. Brisbane (Long Pocket).
x *Musa* spp. Banana varieties (cultivars) Cavendish, Lady Finger and Williams Hybrid. Heavy. Narangba.
x *Pisum sativum* L. Garden pea. Heavy. Millaroo, Palmwoods.
x *Reinwardtia indica* Dum. Heavy. Brisbane.
x *Spergula arvensis* L. Corn spurry. Heavy. Glen Aplin.
x *Streptosolen jamesonii* (Benth.) Meirs. Browallia. Heavy. Brisbane (Toowong).
G *Trifolium baccarinii* Chiov. Heavy.
G *Trifolium burchelliamum* Ser. ex DC. Cape clover. Heavy.
G *Trifolium glomeratum* L. Clustered clover. Heavy.
x *Trifolium repens* L. White clover. Heavy. Cooroy, East Barron, Kenilworth (Figure 6).

Fig. 6.—White clover infested with *Meloidogyne incognita*.

G *Trifolium rueppellianum* Fresen. Heavy.
G. *Trifolium semipilosum* Fresen. Heavy.
G *Trifolium subterraneum* L. Subterranean clover. Heavy.
x *Vigna sinensis* (L.) Endl. ex Hassk. Cowpea, variety Phoenix. Heavy. Bli Bli.

Meloidogyne javanica javanica (Treub)

(Javanese root-knot nematode)

x *Avena sativa* L. Oats. Light. Beerwah.
x *Centrosema pubescens* Benth. Centro. Light. Nambour.
Crassocephalum crepidioides Benth. Thickhead. Moderate. Beerwah.
x *Cyamopsis tetragonoloba* (L.) Taub. Guar bean. Moderate. Nambour.
x *Desmodium uncinatum* DC. A tick trefoil. Moderate. Brookfield.
G *Dolichos lablab* L. Tonga or hyacinth bean. Heavy. (Figure 7.)
x *Echinochloa crus-galli* var. *frumentacea* (Roxb.) Wight. White panicum. Moderate. Nambour.
x *Ficus macrophylla* Desf. Moreton Bay fig. Moderate. Bribie Island.
x *Glycine max* (L.) Merr. Soybean. Heavy. Nambour.
G *Melinis minutiflora* Beauv. Molasses grass. Light.
x *Pisum arvense* L. Field pea. Moderate. Beerwah.
x *Samanea saman* (Jacq.) Merr. Rain-tree. Heavy. Ayr.
x *Secale cereale* L. Rye. Light. Glasshouse Mountains.
G *Solanum pseudocapsicum* L. Madeira winter cherry. Heavy.
x *Sorghum bicolor* (L.) Moench. Sweet sorghum. Moderate. Beerwah, Nambour.
x *Sorghum sudanense* (Piper) Stapf. Sweet Sudan grass. Light. Nambour.
Spergula arvensis L. Corn spurry. Heavy. Glen Aplin.
x *Stylosanthes humilis* H.B.K. Townsville lucerne. Light. Nambour.
G *Trifolium glomeratum* L. Clustered clover. Heavy.
G *Trifolium hybridum* L. Alsike clover. Moderate.
G *Trifolium rueppellianum* Fresen. Heavy.
G *Trifolium semipilosum* Fresen. Heavy.
G *Trifolium subterraneum* L. Subterranean clover. Moderate
G *Trifolium tembense* Fresen. Light.
G *Trifolium usambarense* Taub. Light.
x *Triticum aestivum* L. Wheat. Light. Beerwah. (Figure 8.)
x *Vicia sativa* L. Common vetch. Moderate. Beerwah.
x *Zingiber officinale* Rosc. Ginger. Heavy. Palmwoods.

PARAPHELENCHUS (Micoletzky)

Paraphelenchus pseudoparietinus (Micoletzky)

Cucumis sativus L. Cucumber. Yeppoon.

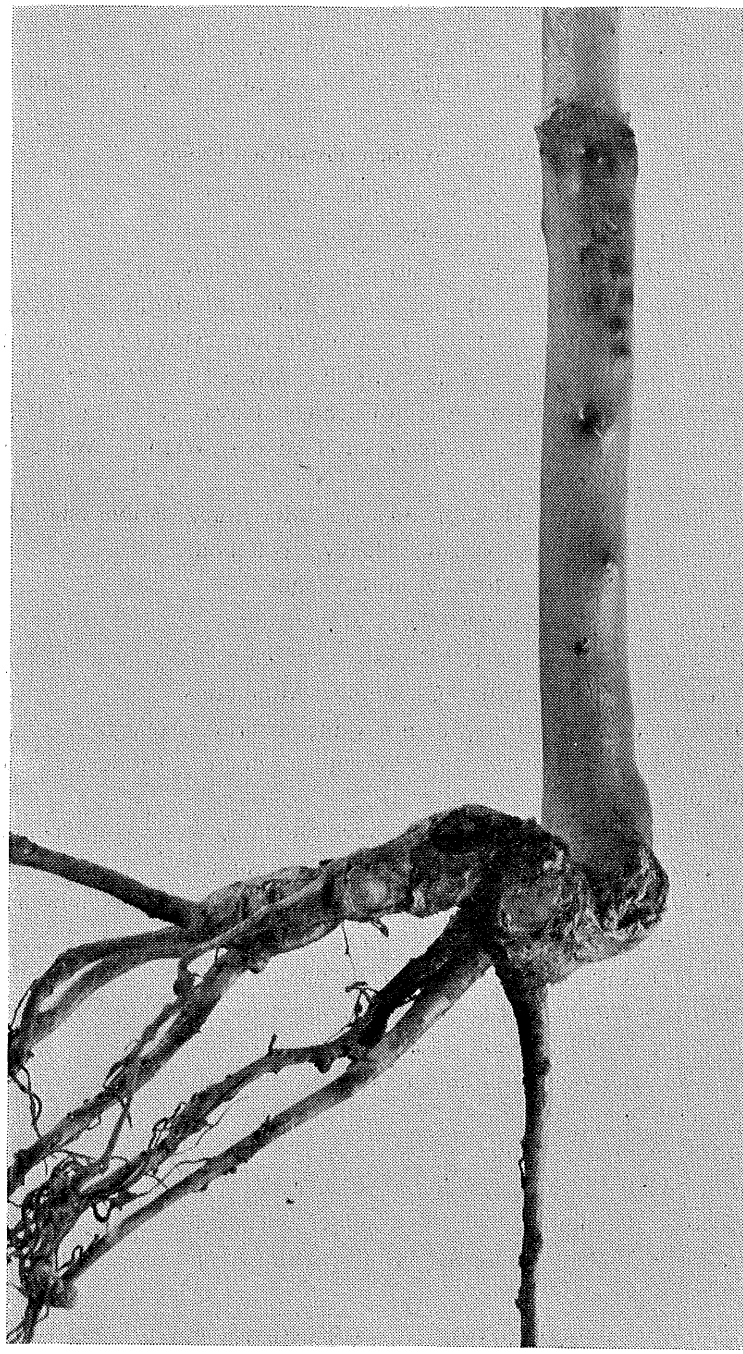

Fig. 7.—Galls on the aboveground portion of the stem of *Dolichos lablab* caused by *Meloidogyne javanica javanica.*

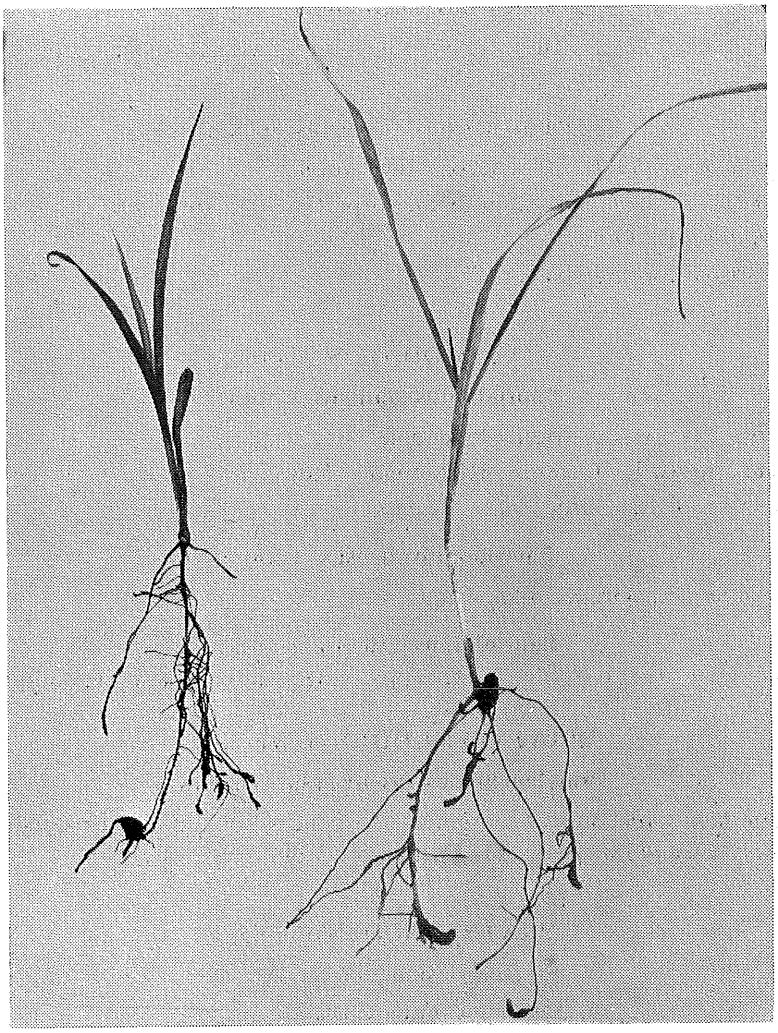

Fig. 8.—Seedlings of sweet sorghum (left) and wheat (right) infested with *Meloidogyne javanica javanica*.

PARATYLENCHUS Micoletzky

(Pin nematodes)

Paratylenchus curvitatus van der Linde

Syn. Paratylenchus dianthus Jenkins and Taylor

Chrysanthemum morifolium Ramat. Chrysanthemum. Brisbane (Botanic Gardens).

Paratylenchus elachistus Steiner

Syn. Paratylenchus minutus Linford

Vitis vinifera L. Grape. Lyra, Severnlea.

Paratylenchus microdorus Andrassy

Themeda australis (R.Br.) Stapf. Kangaroo grass. Brisbane (Long Pocket), Cunningham's Gap, Mt. Nebo.

Paratylenchus projectus Jenkins

Nicotiana tabacum L. Tobacco. Inglewood.

Vicia sativa L. Common vetch. Stanthorpe (C.O.D. Nursery).

Vitis vinifera L. Grape. Severnlea.

Soil. Brisbane (Sunnybank).

PAURODONTUS Thorne

Paurodontus apiticus Thorne

Ananas comosus (L.) Merr. Pineapple. Beerwah.

Medicago sativa L. Lucerne. Richmond ("Rowena").

Pisum sativum L. Garden pea. Yeppoon.

Paurodontus densus Thorne

Vitis vinifera L. Grape. Inglewood.

Paurodontus gracilis Thorne

Ananas comosus (L.) Merr. Pineapple. Beerwah, Cooroy, Tewantin.

PRATYLENCHOIDES Winslow

Pratylenchoides crenicauda Winslow

Gerbera jamesonii Bolus. Gerbera. Brisbane (Botanic Gardens, Nashville). *P. crenicauda* was associated with a root-rot of gerberas.

PRATYLENCHUS Filipjev

(Root-lesion nematodes)

Pratylenchus brachyurus (Godfrey)

x *Ananas comosus* (L.) Merr. Pineapple. Beerwah, Brisbane (Brackenridge, Moggill), Cooroy, Didillibah, Elimbah, Innisfail, Kallangur, Kandanga, Mackay, Mutanee, Palmwoods, Woombye, Yeppoon, Yorkey's Knob. Root-rot of pineapples due to *P. brachyurus* is common in Queensland plantations.

x *Arachis hypogaea* L. Peanut. Gayndah, Gordonbrook, Memerambi, Tingoora, Wondai. (Figure 9). *P. brachyurus* is an important pathogen of peanuts in the South Burnett, causing lesions on the roots, hypocotyls, pegs and shells.

x *Araucaria cunninghamii* D. Don. Hoop pine. Benarkin, Gympie.

x *Citrullus vulgaris* Schrad. Watermelon. Coominya.

Citrus limon (L.) Burm. f. (rough lemon) or *C. sinensis* Osbeck (sweet orange). Brisbane (Rochedale), Burrum, Murray Upper, Palmwoods.

Cucurbita maxima Duchesne. Pumpkin. Lawnton.
x *Cyperus rotundus* L. Nut grass. Brisbane.
G *Dolichos lablab* L. Tonga or hyacinth bean.
Eucalyptus maculata Hook. Spotted gum. Brisbane (Long Pocket).
x *Fragaria X ananassa* Duchesne. Strawberry. Parada, Woombye, Woondum.
x *Helianthus annuus* L. Sunflower. Wondai.
Hornstedtia scottiana (F. Muell.) K. Schum. A wild ginger. Junction of Palmerston Highway and Henderson Drive (North Queensland).
Lupinus angustifolius L. New Zealand blue lupin. Tolga.
x *Lycopersicon esculentum* Mill. Tomato. Charters Towers.
Medicago denticulata Willd. Burr medic. Slacks Creek.
x *Medicago sativa* L. Lucerne. Slacks Creek.
Musa sp. Banana variety (cultivar) Williams Hybrid. Narangba.
x *Nicotiana tabacum* L. Tobacco. Dimbulah, Glasshouse Mountains.
G *Paspalum scrobiculatum* L. Scrobic. *P. brachyurus* has caused severe stunting of *P. scrobiculatum* under glasshouse conditions.
x *Persea americana* Mill. Avocado. Ormiston.
x *Phaseolus mungo* L. Mung bean. Ormiston.
Prunus persica (L.) Batsch. Peach. Imbil.
Saccharum officinarum L. Sugar cane. Bundaberg.
x *Solanum tuberosum* L. Potato. East Barron. (Figure 10). *P. brachyurus* produces a disease known as potato 'pimple'. Fourteen acres at East Barron were affected in the 1962 season when potatoes followed a paspalum/Rhodes grass pasture.
G *Stizolobium* spp. Velvet beans.
Trifolium pratense L. Red clover. Southbrook.
x *Trifolium repens* L. White clover. Conondale, Cooroy, Kybong, Maleny, Wolvi.
x *Vigna sinensis* (L.) Endl. ex Hassk. Cowpea. Charters Towers, Nambour, Ormiston.
x *Zea mays* L. Maize. Kingaroy, Wondai.
Soil. Corumba, Ravenshoe (in a pasture of Rhodes grass, green panic and glycine).

Pratylenchus coffeae (Zimmerman)

Syn. Pratylenchus musicola (Cobb)

P. coffeae has been found in lesions on the roots of the following plants in the Stanthorpe-Wallangarra-Dalveen area:
x *Brassica oleracea* L. var. *boytrytis* L. Cauliflower.
x *Brassica oleracea* L. var. *capitata* L. Cabbage.
x *Brassica oleracea* L. var. *italica* Plenck. Broccoli.
x *Bromus unioloides* H.B.K. Prairie grass.
x *Coronopus didymus* (L.) Sm. Bittercress.
x *Cydonia oblonga* Mill. Quince.
x *Cynodon dactylon* (L.) Pers. Couch.
x *Digitaria adscendens* (H.B.K.) Henrard. Summer grass.

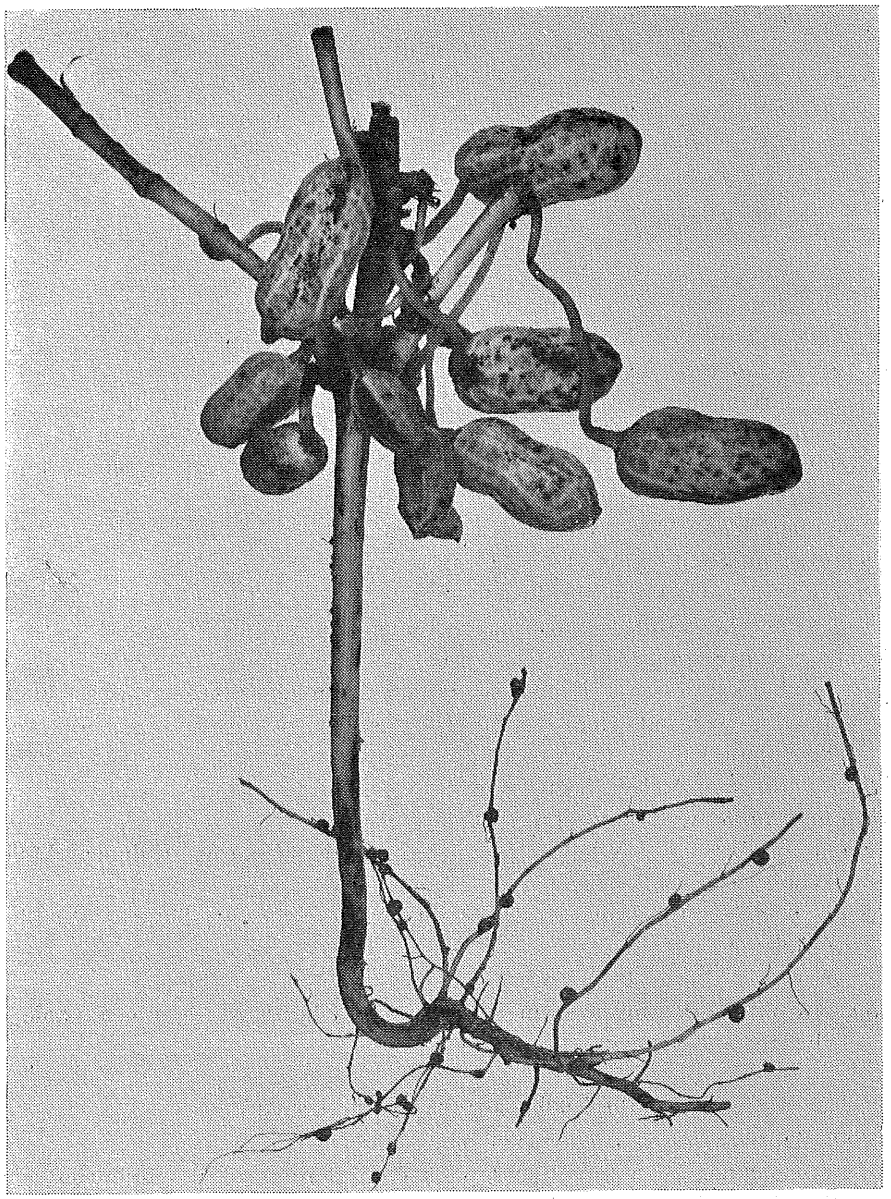

Fig. 9.—Peanut plant with lesions on the pods, pegs and hypocotyl caused by *Pratylenchus brachyurus*.

x *Hypochaeris radicata* L. Flatweed.
x *Juglans regia* L. English walnut.
G *Linum usitatissimum* L. Linseed.
x *Lolium rigidum* Gaud. Wimmera ryegrass.
x *Lupinus angustifolius* L. New Zealand blue lupin.
x *Lycopersicon esculentum* Mill. Tomato.
x *Malus sylvestris* (L.) Mill. Apple.
x *Olea europaea* L. Olive.

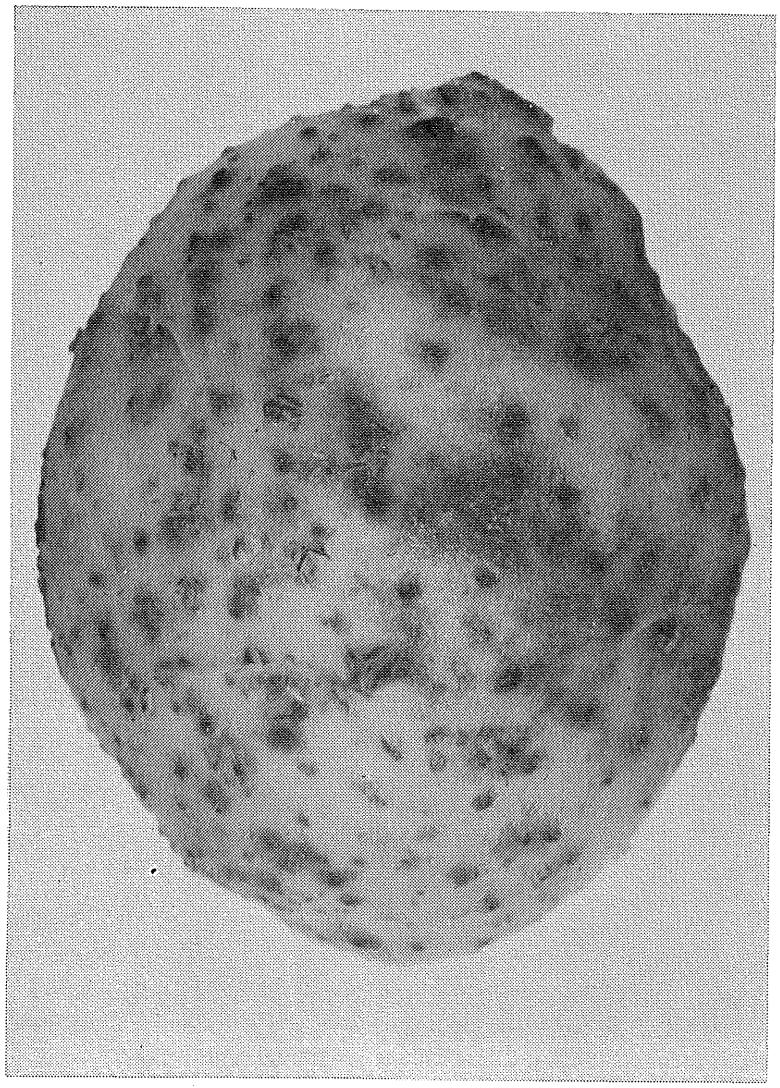

Fig. 10.—Potato tuber infested with *Pratylenchus brachyurus.*

x *Phaseolus vulgaris* L. French bean.
x *Pisum sativum* L. Garden pea.
x *Prunus armeniaca* L. Apricot.
x *Prunus persica* (L.) Batsch. Peach.
x *Prunus salicina* Lindl. Japanese plum.
x *Pyrus communis* L. Pear.
x *Rumex acetosella* L. Sorrel.
x *Solanum tuberosum* L. Potato.
x *Sonchus oleraceus* L. Common sowthistle.
x *Spergula arvensis* L. Corn spurry.
x *Trifolium pratense* L. Red clover.

x *Verbena bonariensis* L. Purple-top.
x *Vigna sinensis* (L.) Endl. ex Hassk. Cowpea.
x *Vitis vinifera* L. Grape.

P. coffeae has also been found in lesions on the roots of the banana variety Williams Hybrid at Japoonvale and Mooloolah.

Pratylenchus neglectus (Rensch)

Syn. Pratylenchus minyus Sher and Allen
Prunus armeniaca L. Apricot. Warwick.

Pratylenchus vulnus Allen and Jensen

x *Chrysanthemum morifolium* Ramat. Chrysanthemum. Brisbane (Botanic Gardens).
x *Fragaria X ananassa* Duchesne. Strawberry. Frenchville, Home Hill, Mt. Cotton, Ormiston. *P. vulnus* is associated with a root-rot of strawberries in Queensland.

Pratylenchus zeae Graham

Ananas comosus (L.) Merr. Pineapple. Beerwah, Kallangur, Nambour.
Asclepias fruticosa L. Narrow-leaf cotton bush. Beerwah.
Citrus limon (L.) (Burm.) f. (rough lemon) or *C. sinensis* Osbeck (sweet orange). Clump Point, Kennedy, Mundubbera.
Digitaria didactyla Willd. Queensland blue couch. Booval, Brisbane (Darra).
Fragaria X ananassa Duchesne. Strawberry. Nambour.
Lactuca sativa L. Lettuce. Ipswich, Maleny.
Lolium perenne L. Perennial ryegrass. Slacks Creek.
Lupinus angustifolius L. New Zealand blue lupin. Tolga.
Mangifera indica L. Mango. Ayr.
x *Medicago sativa* L. Lucerne. Samford.
x *Nicotiana tabacum* L. Tobacco. Dimbulah.
Panicum maximum Jacq. Guinea grass. Brisbane.
x *Phaseolus vulgaris* L. French bean. Cooroy.
x *Prunus persica* (L.) Batsch. Peach. Brisbane (Pinkenba).
x *Saccharum officinarum* L. Sugar cane. Bundaberg, Imbil, Mossman.
Sechium edule Sw. Choko. Montville.
x *Sorghum bicolor* (L.) Moench. Sweet sorghum. Brisbane (Moggill), Nambour. Extensive root-rot of sorghum due to *P. zeae* was noted at Moggill in 1956 (Figure 11).
Trifolium subterraneum L. Subterranean clover. Lawes.
Vitis vinifera L. Grape. Coominya.
Pasture of green panic, Rhodes grass and glycine, Ravenshoe.
Soil. Coominya, Elimbah, Glasshouse Mountains, Highvale, Maleny, Tewantin, Maclean (New South Wales).

PSEUDHALENCHUS Tarjan

Pseudhalenchus anchilisposomus Tarjan

Ananas comosus (L.) Merr. Pineapple. Bauple, Beerwah, Bowen, Brisbane (Moggill), Didillibah, Elimbah, Glasshouse Mountains, Innisfail, Nambour, Tewantin, Woombye.

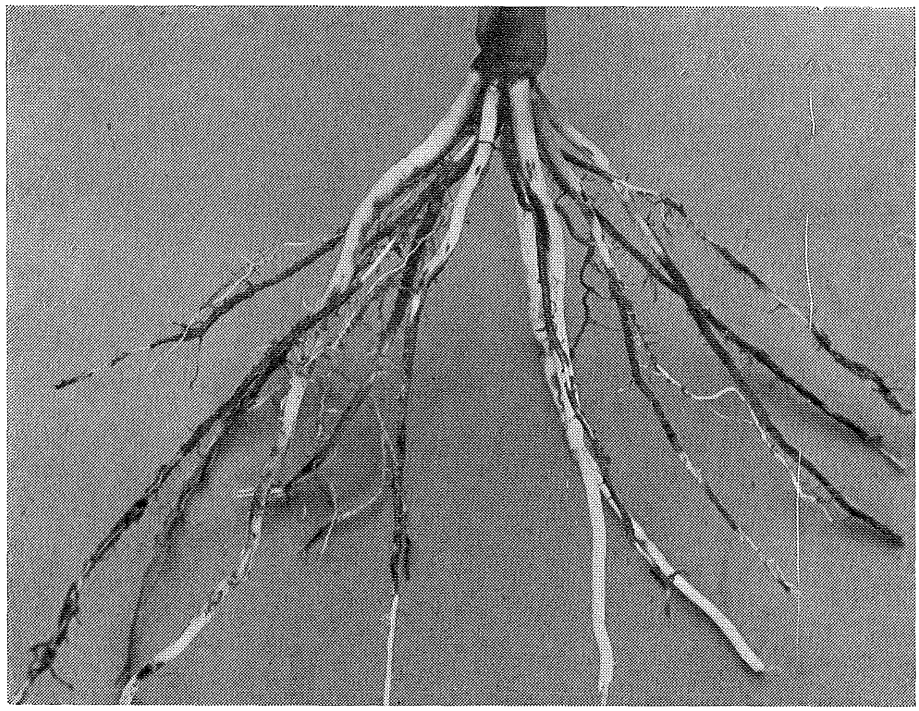

Fig. 11.—Lesions on sorghum roots infested with *Pratylenchus zeae.*

Citrus limon (L.) Burm. f. (rough lemon) or *C. sinensis* Osbeck (sweet orange). Bundaberg, Burrum, Kennedy.
Cupaniopsis anacardioides (A. Rich.) Radlk. Cupania tree. Mulambin Beach.
Gerbera jamesonii Bolus. Gerbera. Brisbane (Nashville).
Lycopersicon esculentum Mill. Tomato. Yeppoon.
Pandanus sp. Pandanus or screw pine. Mulambin Beach.
Prunus persica (L.) Batsch. Peach. Bacchus.
Trifolium repens L. White clover. Cooroy, Currumbin.
Vitis vinifera L. Grape. Glen Aplin, Inglewood.

Pseudhalenchus minutus Tarjan

Ananas comosus (L.) Merr. Pineapple. Beerwah, Brisbane (Moggill), Glasshouse Mountains, Nambour, Woombye.
Citrus limon (L.) Burm. f. (rough lemon) or *C. sinensis* Osbeck (sweet orange). Brisbane (Mt. Gravatt).
Digitaria didactyla Willd. Queensland blue couch. Brisbane (Sunnybank).
Fragaria X ananassa Duchesne. Strawberry. Brisbane (Salisbury).
Opuntia sp. A prickly pear. Mulambin Beach.
Pinus elliotti Engelm. Slash pine. Kilkivan (Jimmy's Scrub logging area).
Vitis vinifera L. Grape. Severnlea.
Soil. Near Utchee Creek (North Queensland).

PSILENCHUS de Man

Psilenchus duplexus (Hagemeyer and Allen)

Ananas comosus (L.) Merr. Pineapple. Didillibah.
Trifolium repens L. White clover. Pomona.

Psilenchus gracilis Thorne

Citrus limon (L.) Burm. f. (rough lemon) or *C. sinensis* Osbeck (sweet orange). Rockhampton.

Psilenchus hilarulus de Man

Themeda australis (R.Br.) Stapf. Kangaroo grass. Mt. Nebo.
Trifolium repens L. White clover. Crawley Vale.

Psilenchus magnidens Thorne

Ananas comosus (L.) Merr. Pineapple. Beerwah, Elimbah, Glasshouse Mountains, Pomona, Tewantin.
Citrus limon (L.) Burm. f. (rough lemon) or *C. sinensis* Osbeck (sweet orange). Brisbane (Rochedale).
Malus sylvestris (L.) Mill. Apple. Lyra.
Saccharum officinarum L. Sugar cane. Harwood (New South Wales).
Trifolium repens L. White clover. Cooroy, Maleny.
Zea mays L. Maize. Coolum.
Soil. Lyra.

Psilenchus tumidus Colbran

Ananas comosus (L.) Merr. Pineapple. Beerwah, Elimbah, Palmwoods, Pomona.
Citrus limon (L.) Burm. f. (rough lemon) or *C. sinensis* Osbeck (sweet orange). Brisbane (Mt. Gravatt).
Digitaria didactyla Willd. Queensland blue couch. Brisbane (Rocklea).

RADOPHOLUS Thorne

(Burrowing nematodes)

Radopholus inequalis Sauer

Casuarina littoralis Salisb. Black sheoak. Eudlo (Jawarra Park), Burrum.
Citrus sinensis Osbeck. Sweet orange. Mundubbera.
Eucalyptus micrantha DC. Scribbly gum. Eudlo.

Radopholus neosimilis Sauer

Casuarina littoralis Salisb. Black sheoak. Burrum.

Radopholus similis (Cobb)

(Burrowing nematode)

x *Annona squamosa* L. Custard apple var. Island Gem. Redland Bay.
x *Desmodium uncinatum* DC. A tick trefoil. Nambour.
x *Musa* spp. Banana varieties (cultivars) Cavendish, Lady Finger and Williams Hybrid. Brisbane (Brookfield, Moggill), Bundaberg, Byfield, Caboolture, Cairns, Currumbin, Eudlo, Gilston, Kallangur, Mt. Mee, Nambour, Pialba, Redland Bay, Samford, Victoria Point, Wamuran, Woongoolba. Root-rot of bananas due to *R. similis* is very common in Queensland and northern New South Wales.

x *Musa banksii* F. Muell. A wild banana. Rain-forest on a slope below a banana plantation infested with *R. similis* at Tully.
G *Phaseolus mungo* L. Mung bean.
x *Sorghum sudanense* (Piper) Stapf. Sweet Sudan grass. Nambour.
x *Stizolobium* spp. Velvet beans. Nambour.
x *Stylosanthes humilis* H.B.K. Townsville lucerne. Nambour.
x *Zea mays* L. Maize. Nambour.

ROTYLENCHULUS Linford and Oliveira
(Reniform nematodes)

Rotylenchulus parvus (Williams)

x *Zea mays* L. Maize. Wooroolin. Females of *R. parvus* were plentiful on maize roots on a farm at Wooroolin in the 1962-63 season (Figure 12).

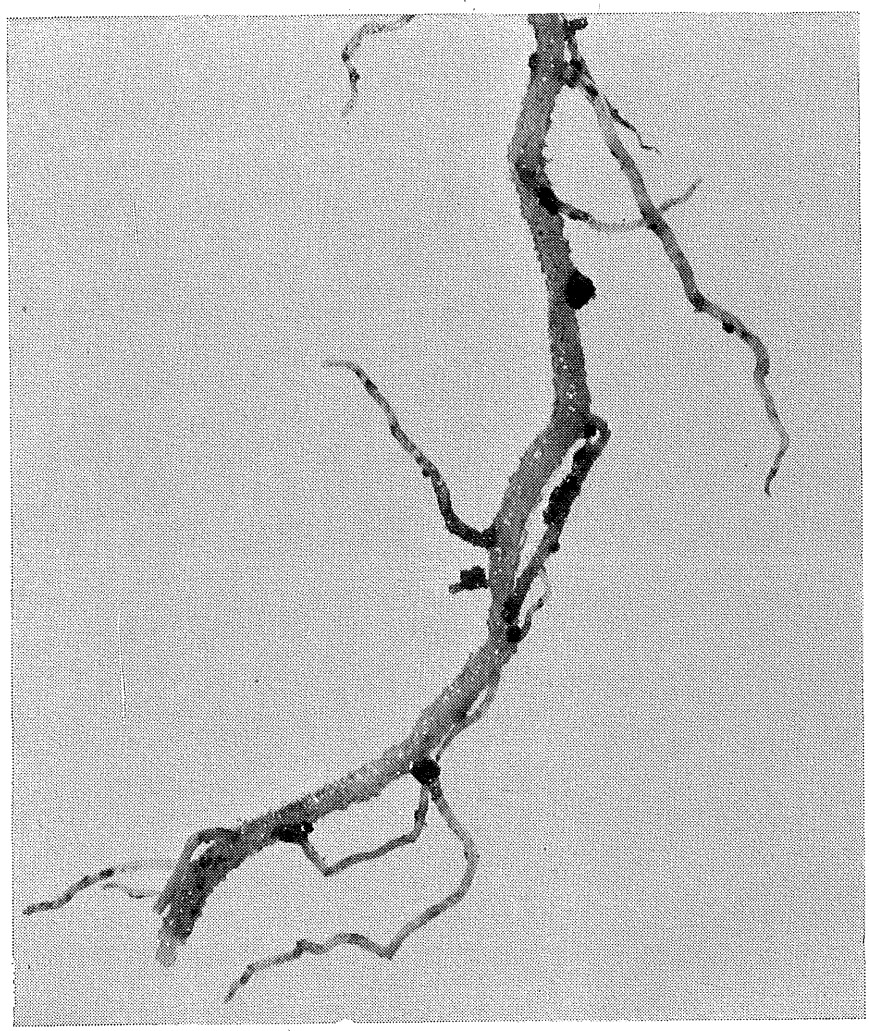

Fig. 12.—Maize root infested with *Rotylenchulus parvus.*

Rotylenchulus reniformis Linford and Oliveira

(Reniform nematode)

x *Calopogonium mucunoides* Desv. Calopo. South Johnstone.

x *Carica payaya* L. Papaw. Yeppoon.

Chrysanthemum morifolium Ramat. Chrysanthemum. Brisbane (Botanic Gardens).

Lycopersicon esculentum Mill. Tomato. Bowen, Rita Is., Yeppoon (Figure 13).

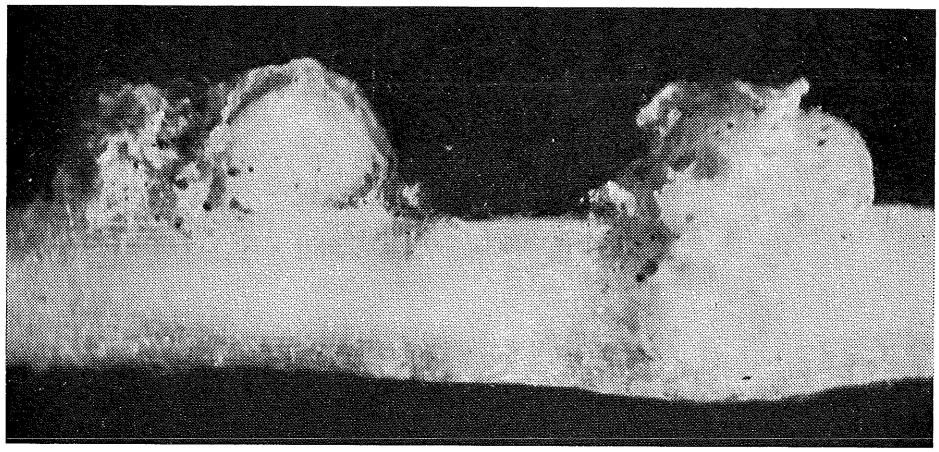

Fig. 13.—Egg masses of *Rotylenchulus reniformis* on a tomato root.

Heavy infestations of *R. reniformis* have been found in tomato plantings at Bowen.

Mangifera indica L. Mango. Ayr.

ROTYLENCHUS Filipjev

(Spiral nematodes)

Rotylenchus brevicaudatus Colbran

Ananas comosus (L.) Merr. Pineapple. Beerwah, Innisfail.

Carica papaya L. Papaw. Brisbane (Sunnybank).

Citrus limon (L.) Burm. f. (rough lemon) or *C. sinensis* Osbeck (sweet orange). Brisbane (Mt. Gravatt, Pullenvale, Rochedale), Emerald Creek (North Queensland), Gayndah, Lawnton.

Cucurbita pepo L. var. *medullosa* Alef. Marrow. Rockhampton.

x *Cyperus rotundus* L. Nut grass. Lawes.

Digitaria didactyla Willd. Queensland blue couch. Brisbane (Wynnum).

Fragaria X ananassa Duchesne. Strawberry. Ormiston.

Lycopersicon esculentum Mill. Tomato. Yeppoon.

Medicago sativa L. Lucerne. Brisbane (Moggill), Jimboomba (Maclean's Bridge).

Panicum maximum Jacq. Guinea grass. Brisbane.

Saccharum officinarum L. Sugar cane. Imbil.

Trifolium subterraneum L. Subterranean clover. Lawes.

Vitis vinifera L. Grape. Inglewood, Roma. Severnlea.

Soil. Dalby, Ipswich, Stanthorpe, hills overlooking Harwood (New South Wales).

SCUTELLONEMA Andrassy

(Spiral nematodes)

Scutellonema brachyurum (Steiner)

Ananas comosus (L.) Merr. Pineapple. Montville, North Arm.

Beta vulgaris L. var. *vulgaris*. Beetroot. Victoria Point.

Carica papaya L. Papaw. Ormiston.

x *Cyamopsis tetragonoloba* (L.) Taub. Guar bean. Nambour.

Dianthus caryophyllus L. Carnation. Wallangarra.

Fragaria X ananassa Duchesne. Strawberry. Brisbane (Salisbury), Mt. Cotton.

Lactuca sativa L. Lettuce. Ipswich.

Lycopersicon esculentum Mill. Tomato. Ipswich.

Musa spp. Banana varieties (cultivars) Cavendish, Williams Hybrid. Nambour, Redland Bay.

Passiflora edulis Sims forma *edulis*. Passionfruit. Yeppoon.

Prunus armeniaca L. Apricot. Warwick.

Prunus persica (L.) Batsch. Peach. Bacchus, Wyberba.

Trifolium repens L. White clover. Maleny.

Vitis vinifera L. Grape. Severnlea.

Soil. Brisbane (Eight Mile Plains), Rockhampton.

Scutellonema minutum Sher

Soil. Fraser Is., Miriam Vale.

Scutellonema truncatum Sher

Soil. Miriam Vale.

SEINURA Fuchs

Seinura oswegoensis (van der Linde)

Soil. Brisbane (Eight Miles Plains).

Seinura christiei J. B. Goodey

Syn. Aphelenchoides tenuicaudatus Christie

Soil. Applethorpe, Brisbane (Acacia Ridge), Elimbah, Gilston, Glasshouse Mountains, Maryborough, Mt. Mee, Wamuran.

S. christiei is a predatory species which has been observed in the process of feeding on *Pratylenchus coffeae*. It is also common in banana plantations infested with *Radopholus similis*.

STICTYLUS Thorne

Stictylus asymmetricus Thorne

Kochia tomentosa (Moq.) F. Muell. Inglewood.

TELOTYLENCHUS Siddiqi

Telotylenchus hastulatus (Colbran) Fisher*

Syn. Belonolaimus hastulatus Colbran

Citrus sinensis Osbeck. Sweet orange. Mundubbera.

Cupaniopsis anacardioides (A. Rich) Radlk. Cupania tree. Mulambin Beach.

Telotylenchus whitei Fisher*

Eucalyptus resinifera Sm. Red stringybark. Burrum.

Kochia tomentosa (Moq.) F. Muell. Inglewood.

Pinus elliottii Engelm. Slash pine. Beerwah (Coochin logging area).

TRICHODORUS Cobb

(Stubby-root nematodes)

Trichodorus minor Colbran

Ananas comosus (L.) Merr. Pineapple. Beerwah, Caboolture, Cooroy, Glasshouse Mountains, Kallangur.

Camellia sinensis (L.) O. Kuntze. Tea. Innisfail.

Chloris gayana Kunth. Rhodes grass. Upper Barron.

Citrus limon (L.) Burm. f. (rough lemon) or *C. sinensis* Osbeck (sweet orange). Beerwah, Brisbane, Burrum, Gayndah, Lawnton, Mundubbera.

Digitaria didactyla (Willd.). Queensland blue couch. Booval, Brisbane (Rocklea, Sunnybank, Wynnum), Woombye.

Fragaria X ananassa Duchesne. Strawberry. Nambour.

Lycopersicon esculentum Mill. Tomato. Brisbane (Moggill), Redcliffe. (Figure 14).

Malus sylvestris (L.) Mill. Apple. Applethorpe.

Medicago sativa L. Lucerne. Brisbane (Moggill).

Musa banskii F. Muell. A wild banana. Tully.

Musa sp. Banana variety (cultivar) Williams Hybrid. Brisbane (Rochedale), Nambour.

Nicotiana tabacum L. Tobacco. Biboohra.

Ornithopus sativus Brot. Serradella. Slacks Creek.

Pinus elliottii Engelm. Slash pine. Beerwah.

Pisum sativum L. Garden pea. Millaroo.

Saccharum officinarum L. Sugar cane. Bli Bli.

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* In press.

Fig. 14.—Tomato seedlings. Left, normal; right, damaged by *Trichodorus minor*.

Secale cereale L. Rye. Calavos.
Sesbania aculeata Pers. Sesbania pea. Coolum.
Trifolium repens L. White clover. Maleny.
Vitis vinifera L. Grape. Ballandean, Coominya, Severnlea, Warwick.
Pasture containing green panic, Rhodes grass and glycine, Ravenshoe.
Soil. Hills overlooking Harwood and Maclean (New South Wales).

Trichodorus porosus Allen

Carica papaya L. Papaw. Sunnybank.
Cupressus sempervirens L. var. *stricta* Ait. Pencil pine. Brisbane (Park Ridge).
Persea americana Mill. Avocado. Beerwah, Glasshouse Mountains, Mapleton, Montville, Palmwoods, Woombye.
Saccharum officinarum L. Sugar cane. Ayr, Childers, Home Hill, Isis, Mackay.
Vitis vinifera L. Grape. Glen Aplin.

TYLENCHORHYNCHUS Cobb

(Stunt nematodes)

Tylenchorhynchus brevidens Allen

Soil. Texas.

Tylenchorhynchus capitatus Allen

Cucurbita maxima Duchesne. Pumpkin. Toowoomba.
Dianthus caryophyllus L. Carnation. Wallangarra.
Fragaria X ananassa Duchesne. Strawberry. Nambour.
Lycopersicon esculentum Mill. Tomato. Ballandean.
Plantago major L. Larger plantain. Stanthorpe.
Solanum tuberosum L. Potato. Toowoomba.

Tylenchorhynchus ewingi Hopper

Soil. Brisbane (Botanic Gardens).

Tylenchorhynchus martini Fielding

Citrus limon (L.) Burm. f. (rough lemon) or *C. sinensis* Osbeck (sweet orange). Murray Upper (North Queensland).
Saccharum officinarum L. Sugar cane. Ayr, Bundaberg, Home Hill, Ingham, Mackay, Mossman, Harwood (New South Wales).

Tylenchorhynchus striatus Allen

Trifolium repens L. White clover. Currumbin.

TYLENCHULUS Cobb

Tylenchulus obscurus Colbran

Hodgkinsonia frutescens C. T. White. Tolga. (Figure 15).
Soil. East Palmerston, Tully.

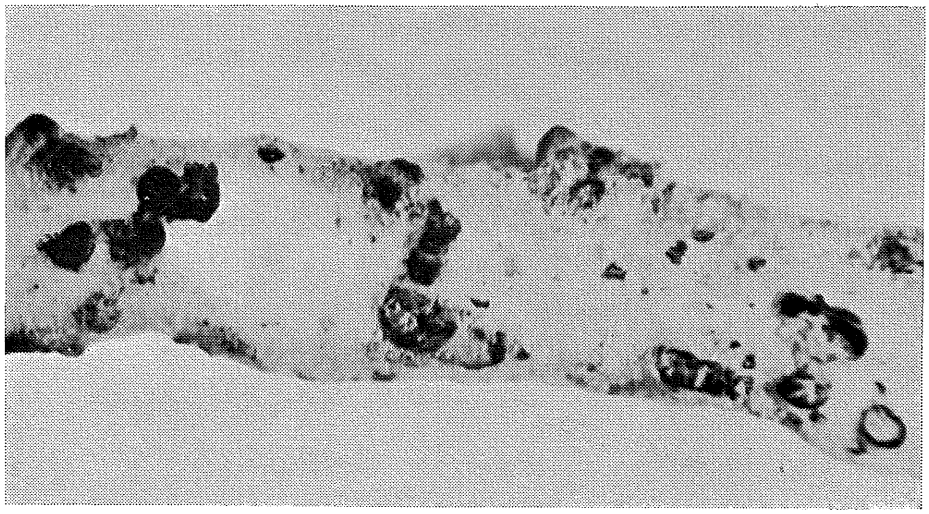

Fig. 15.—A root of *Hodgkinsonia frutescens* covered with the scales of *Tylenchulus obscurus.*

Tylenchulus semipenetrans Cobb

(Citrus nematode)

x *Citrus limon* (L.) Burm. f. (rough lemon) or *C. sinensis* Osbeck (sweet orange). Ayr, Beerwah, Brisbane, Burrum, Charters Towers, Clump Point, Condamine, Elimbah, Gayndah, Grantham, Howard, Jimboomba, Koah, Lawes, Mundubbera, Rockhampton, Yeppoon. (Figure 16).

x *Olea europaea* L. Olive. Glen Aplin, Lawes.

x *Vitis vinifera* L. Grape. Charters Towers, Glen Aplin, Warwick, Yelarbon.

TYLENCHUS Bastian

Tylenchus baloghi Andrassy

Digitaria didactyla Willd. Queensland blue couch. Brisbane (Kalinga).

Nicotiana tabacum L. Tobacco. Ayr.

Soil. Montville.

Tylenchus bryophilus Steiner

Moss. Cunningham's Gap.

Tylenchus costatus de Man

Ananas comosus (L.) Merr. Pineapple. Dayboro.

Citrus limon (L.) Burm. f. (rough lemon) or *C. sinensis* Osbeck (sweet orange). Koah.

Ficus carica (L.) Cultivated fig. Biloela.

x *Lupinus angustifolius* L. New Zealand blue lupin. Applethorpe.

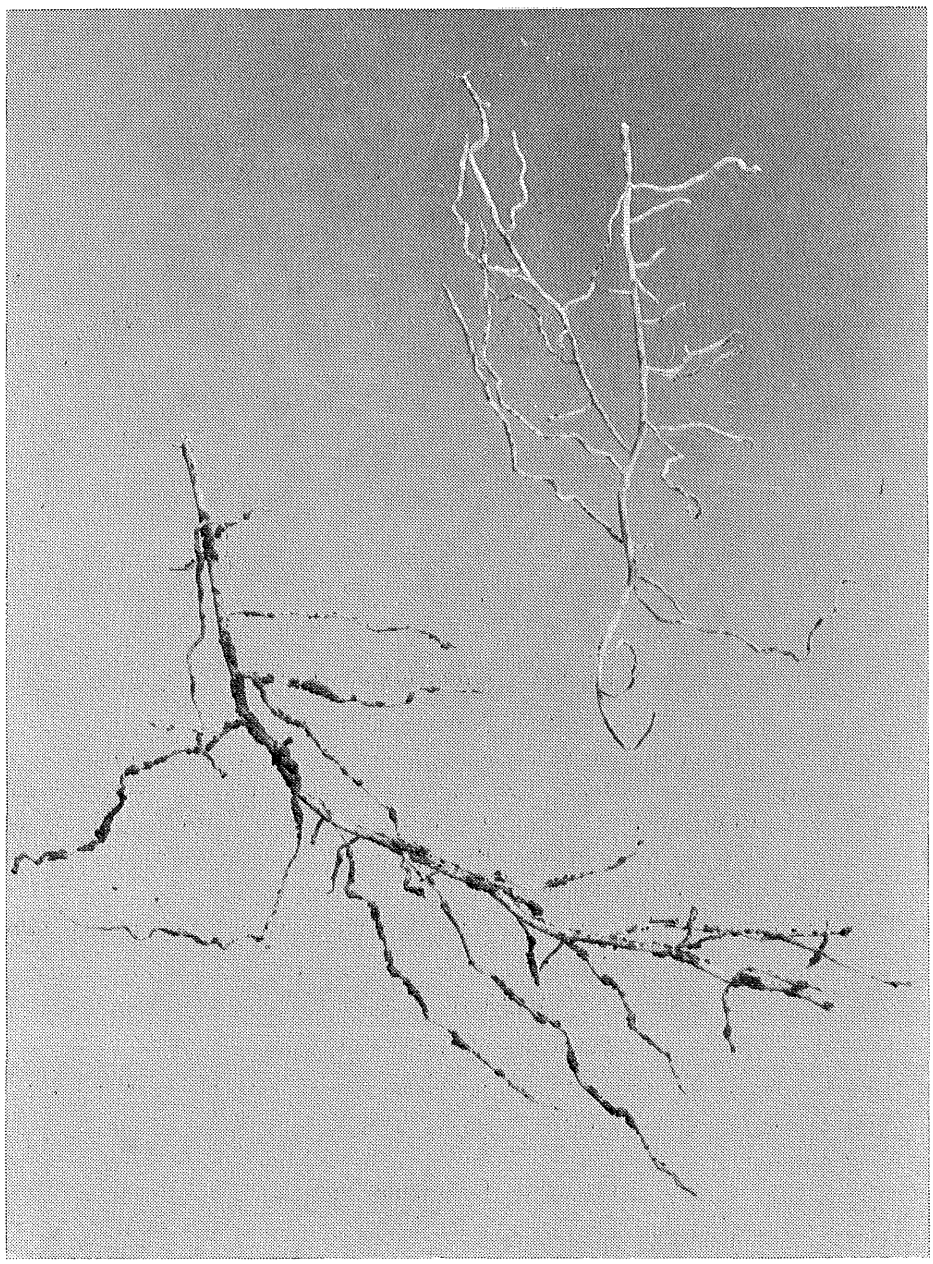

Fig. 16.—Citrus roots. Upper, healthy; lower, infested with *Tylenchulus semipenetrans.*

Malus sylvestris (L.) Mill. Apple. Lyra.
Prunus persica (L.) Batsch. Peach. Lyra.
Vitis vinifera L. Grape. Ballandean, Glen Aplin, Young (New South Wales). Soil. Tully.

Tylenchus discrepans Andrassy

Acronychia acidula F. Muell. Tolga.
Digitaria didactyla Willd. Queensland blue couch. Brisbane (Keperra).
Musa sp. Banana variety (cultivar) Williams Hybrid. Brisbane (The Gap).
Portulaca oleracea L. Pigweed. Applethorpe.
Trifolium repens L. White clover. Cooroy, Maleny.
Soil. Tully.

Tylenchus emarginatus Cobb

Syn. (new). *T. hexalineatus* Gaerart
T. (*Cephalenchus*) *megacephalus* J. B. Goodey
Ficus opposita Miq. A sandpaper fig. Mulambin Beach.
Medicago sativa L. Lucerne. Brisbane (Moggill).
Opuntia sp. A prickly pear. Mulambin Beach.
Pinus elliottii Engelm. Slash pine. Beerwah.
Themeda australis (R. Br.) Stapf. Kangaroo grass. Mt. Nebo.
Vitis vinifera L. Grape. Lyra.
Soil. Hills overlooking Harwood (New South Wales).

Tylenchus exiguus de Man

Acronychia acidula F. Muell. Tolga.
Ananas comosus (L.) Merr. Pineapple. Bowen, Coolum, Elimbah, Glasshouse Mountains, Nambour.
Beta vulgaris L. var. *vulgaris*. Beetroot. Yeppoon.
Bromus unioloides H.B.K. Prairie grass. Thulimbah.
Citrus limon (L.) Burm. f. (rough lemon) or *C. sinensis* Osbeck (sweet orange). Brisbane (Mt. Gravatt, Pullenvale, Rochedale), Bundaberg, Burrum, Gatton, Gayndah, Lawnton, Tinana.
Dianthus caryophyllus L. Carnation. Wallangarra.
Digitaria didactyla Willd. Queensland blue couch. Brisbane (Sunnybank).
Ficus carica L. Cultivated fig. Biloela.
Hodgkinsonia frutescens C. T. White. Tolga.
Lactuca sativa L. Lettuce. Mt. Ninderry.
Lycopersicon esculentum Mill. Tomato. Yeppoon.
Musa banksii F. Muell. A wild banana. Tully.
Musa sp. Banana variety (cultivar) Williams Hybrid. Bundaberg, Mt. Ninderry, Woombye.
Paspalum dilatatum Poir. Paspalum. Miriam Vale.
Phaseolus vulgaris L. French bean. Brisbane (Manly), Gracemere, Ipswich, Rockhampton.
Pisum sativum L. Garden pea. Yeppoon.
Trifolium repens L. White clover. Cooroy.
Triticum aestivum L. Wheat. Thulimbah.
Vitis vinifera L. Grape. Inglewood, Roma, Severnlea, Stanthorpe.
Zea mays L. Maize. Beerwah.
Soil. Cunningham's Gap, Highvale, Miriam Vale.

Tylenchus filiformis Butschlii

Ananas comosus (L.) Merr. Pineapple. Didillibah.

Araucaria cunninghamii D. Don. Hoop pine. Dunballa.

Soil. Cunningham's Gap, Tully.

Tylenchus leptosoma de Man

Mangifera indica L. Mango. Buderim.

Musa sp. Banana variety (cultivar) Williams Hybrid. Bundaberg.

Trifolium repens L. White clover. Wolvi.

Soil. Beerwah, Burrum, Cooroy, Glasshouse Mountains, Maleny, Harwood (New South Wales).

Tylenchus polyhypnus Steiner and Albin

Ananas comosus (L.) Merr. Pineapple. Innisfail, Kandanga, Rosemount.

Citrus limon (L.) Burm. f. (rough lemon) or *C. sinensis* Osbeck (sweet orange). Bouldercombe.

Fragaria X ananassa Duchesne. Strawberry. Brisbane (Salisbury).

Lycopersicon esculentum Mill. Tomato. Bowen, Yeppoon.

Passiflora edulis Sims forma *edulis*. Passionfruit. Yeppoon.

Portulaca oleracea L. Pigweed. Applethorpe.

Vitis vinifera L. Grape. Roma.

Tylenchus thornei Andrassy

Trifolium repens L. White clover. Wolvi.

XIPHINEMA Cobb

Xiphinema americanum Cobb

Annona squamosa L. Custard apple. Redland Bay.

Citrus limon (L.) Burm. f. Rough lemon. Burrum.

Macadamia tetraphylla L.A.S. Johnson. A Macadamia or Queensland nut. Brisbane (Sunnybank).

Persea americana Mill. Avocado. Brisbane (Sunnybank), Mapleton, Monvtille, Mt. Tamborine, Palmwoods.

Prunus persica (L.) Batsch. Peach. Severnlea.

Vitis vinifera L. Grape. Severnlea.

Soil. Miriam Vale, Harwood (New South Wales).

Xiphinema diversicaudatum Micoletzky

Casuarina equisetifolia L. Dune sheoak. Mulambin Beach.

Citrus limon (L.) Burm. f. Rough lemon. Burrum.

Opuntia sp. A prickly pear. Mulambin Beach.

Xiphinema elongatum Schuurmans Stekhoven and Teunissen
Syn. X. campinense Lordello
X. pratense Loos
Chloris gayana Kunth. Rhodes grass. Brisbane (Archerfield, Moggill), Elimbah.
Citrus limon (L.) Burm. f. (rough lemon) or *C. sinensis* Osbeck (sweet orange). Brisbane (Long Pocket).
Digitaria didactyla Willd. Blue couch grass. Brisbane (Banyo).
Fragaria X ananassa Duchesne. Strawberry. Victoria Point.
Medicago sativa L. Lucerne. Brisbane (Moggill).
Persea americana Mill. Avocado. Ormiston.
Saccharum officinarum L. Sugar cane. Bundaberg.
Soil. Elimbah, Ormiston.

Xiphinema radicicola Goodey

Melastoma polyanthum Plume. Blue tongue. Coolum.
Paspalum dilatatum Poir. Paspalum. Brisbane (Archerfield), Byfield, Maleny.
Trifolium repens L. White clover. Maleny.
Soil. Burrum.

SECTION II

Host/Nematode List

Acronychia acidula F. Muell.
- *Tylenchus discrepans*
- *Tylenchus exiguus*

Agrostis tenuis Sibth. Browntop bent grass.
- *Anguina agrostis*

Ananas comosus (L.) Merr. Pineapple.
- *Criconema pectinatum*
- *Criconemoides ornatum*
- *Helicotylenchus dihystera*
- *Helicotylenchus multicinctus*
- *Hemicriconemoides mangiferae*
- *Hemicriconemoides obtusus*
- *Hemicycliophora ovata*
- *Hemicycliophora truncata*
- *Paurodontus apiticus*
- *Paurodontus gracilis*
- *Pratylenchus brachyurus*
- *Pratylenchus zeae*
- *Pseudhalenchus anchilisposomus*
- *Pseudhalenchus minutus*
- *Psilenchus duplexus*
- *Psilenchus magnidens*
- *Psilenchus tumidus*
- *Rotylenchus brevicaudatus*
- *Scutellonema brachyurum*
- *Trichodorus minor*
- *Tylenchus costatus*
- *Tylenchus exiguus*
- *Tylenchus filiformis*
- *Tylenchus polyhypnus*

Angophora costata (Gaertn.) Domin. Rusty gum.
- *Fergusobia tumefaciens*

Angophora subvelutina F. Muell. Broad-leaved apple.
- *Fergusobia tumefaciens*

Annona squamosa L. Custard apple.
- *Heliocotylenchus dihystera*
- *Radopholus similis*
- *Xiphinema americanum*

Arachis hypogaea L. Peanut.
Meloidogyne hapla — *Pratylenchus brachyurus*

Araucaria cunninghamii D. Don. Hoop pine.
Helicotylenchus dihystera — *Tylenchus filiformis*
Pratylenchus brachyurus

Asclepias fruticosa L. Narrow-leaf cotton bush.
Helicotylenchus dihystera — *Pratylenchus zeae*

Astrebla pectinata (Lindl.) F. Muell. ex Benth. Barley Mitchell grass.
Anguina microlaenae

Avena sativa L. Oats.
Helicotylenchus dihystera — *Meloidogyne javanica javanica*

Bauhinia sp.
Helicotylenchus dihystera

Beta vulgaris L. var. *cicla* L. Silver beet.
Helicotylenchus dihystera

Beta vulgaris L. var. *vulgaris*. Beetroot.
Helicotylenchus dihystera — *Scutellonema brachyurum*
Heterodera schachtii — *Tylenchus exiguus*

Brassica napobrassica (L.) Mill. Swede turnip.
Heterodera schachtii

Brassica oleracea L. var. *botrytis* L. Cauliflower.
Pratylenchus coffeae

Brassica oleracea L. var. *capitata* L. Cabbage.
Helicotylenchus dihystera — *Meloidogyne arenaria thamesi*
Heterodera schachtii — *Pratylenchus coffeae*

Brassica oleracea L. var. *italica* Plenck. Broccoli.
Pratylenchus coffeae

Bromus unioloides H.B.K. Prairie grass.
Pratylenchus coffeae — *Tylenchus exiguus*

Cajanus cajan (L.) Millsp. Pigeon pea.
Helicotylenchus dihystera

Calopogonium mucunoides Desv. Calopo.
Meloidogyne arenaria thamesi — *Rotylenchulus reniformis*

Camellia sinensis (L.) O. Kuntze. Tea.
Trichodorus minor

Capsicum frutescens L. Capsicum, variety California Wonder.
Hemicycliophora nudata

Carica papaya L. Papaw.
Criconemoides mutabile — *Rotylenchus brevicaudatus*
Helicotylenchus dihystera — *Scutellonema brachyurum*
Rotylenchulus reniformis — *Trichodorus porosus*

Casuarina equisetifolia L. Dune sheoak.
Hemicriconemoides brachyurus
Hemicriconemoides cocophillus
Hemicriconemoides obtusus
Hemicycliophora labiata
Xiphinema diversicaudatum

Casuarina littoralis Salisb. Black sheoak.
Hemicycliophora truncata
Radopholus inequalis
Radopholus neosimilis

Centella asiatica (L.) Urb. Pennyweed.
Meloidogyne incognita

Centrosema pubescens Benth. Centro.
Helicotylenchus dihystera
Meloidogyne javanica javanica

Chenopodium album L. Fat hen.
Meloidogyne incognita

Chloris gayana Kunth. Rhodes grass.
Trichodorus minor
Xiphinema elongatum

Chrysanthemum morifolium Ramat. Chrysanthemum.
Aphelenchoides ritzema-bosi
Basiria graminophila
Paratylenchus curvitatus
Pratylenchus vulnus
Rotylenchulus reniformis

Citrullus vulgaris Schrad. Watermelon.
Pratylenchus brachyurus

Citrus limon (L.) Burm. f. (rough lemon) and/or *C. sinensis* Osbeck (sweet orange).
Criconema australe
Criconema civellae
Criconemoides ornatum
Deladenus durus
Helicotylenchus dihystera
Hemicriconemoides cocophillus
Hemicycliophora nudata
Hemicycliophora truncata
Pratylenchus brachyurus
Pratylenchus zeae
Pseudhalenchus anchilisposomus
Pseudhalenchus minutus
Psilenchus gracilis
Psilenchus magnidens
Psilenchus tumidus
Radopholus inequalis
Rotylenchus brevicaudatus
Telotylenchus hastulatus
Trichodorus minor
Tylenchorhynchus martini
Tylenchulus semipenetrans
Tylenchus costatus
Tylenchus exiguus
Tylenchus polyhypnus
Xiphinema americanum
Xiphinema diversicaudatum
Xiphinema elongatum

Citrus reticulata Blanco. Mandarin varieties (cultivars) Emperor and Cleopatra.
Hemicycliophora nudata

Clerodendrum splendens G. Don. A clerodendrum.
Meloidogyne incognita

Coleus blumei Benth. Coleus.
Helicotylenchus dihystera

Coronopus didymus (L.) Sm. Bittercress.
Pratylenchus coffeae

Crassocephalum crepidioides Benth. Thickhead.
Meloidogyne javanica javanica

Crotalaria goreensis Guill. & Perr. Gambia pea
Meloidogyne hapla

Cucumis melo L. var. *reticulatus* Naud. Rockmelon.
Helicotylenchus dihystera

Cucumis sativus L. Cucumber.
Helicotylenchus dihystera *Paraphelenchus pseudoparietinus*

Cucurbita maxima Duchesne. Pumpkin.
Pratylenchus brachyurus *Tylenchorhynchus capitatus*

Cucurbita pepo L. var. *medullosa* Alef. Marrow.
Rotylenchus brevicaudatus

Cupaniopsis anacardioides (A. Rich.) Radlk. Cupania tree.
Pseudhalenchus anchilisposomus *Telotylenchus hastulatus*

Cupressus sempervirens L. var. *stricta* Ait. Pencil pine.
Trichodorus porosus

Cyamopsis tetragonoloba (L.) Taub. Guar bean.
Meloidogyne javanica javanica *Scutellonema brachyurum*

Cyclamen persicum Mill. Cyclamen.
Helicotylenchus dihystera

Cydonia oblonga Mill. Quince.
Pratylenchus coffeae

Cynodon dactylon (L.) Pers. Couch.
Pratylenchus coffeae

Cyperus rotundus L. Nut grass.
Pratylenchus brachyurus *Rotylenchus brevicaudatus*

Daucus carota L. Carrot.
Helicotylenchus dihystera

Desmodium uncinatum DC. A tick trefoil.
Meloidogyne javanica javanica *Radopholus similis*

Dianthus caryophyllus L. Carnation.
Helicotylenchus dihystera *Tylenchorhynchus capitatus*
Meloidogyne incognita *Tylenchus exiguus*
Scutellonema brachyurum

Digitaria adscendens (H.B.K.) Henrard. Summer grass.
Pratylenchus coffeae *Tylenchus discrepans*
Tylenchus baloghi

Digitaria didactyla Willd. Queensland blue couch.

Criconema australe
Criconemoides mutabile
Criconemoides ornatum
Helicotylenchus dihystera
Hemicriconemoides obtusus
Pratylenchus zeae
Pseudhalenchus minutus
Psilenchus tumidus
Rotylenchus brevicaudatus
Trichodorus minor
Tylenchus baloghi
Tylenchus discrepans
Tylenchus exiguus
Xiphinema elongatum

Dolichos lablab L. Tonga or hyacinth bean.

Helicotylenchus dihystera
Meloidogyne hapla
Meloidogyne incognita
Meloidogyne javanica javanica
Pratylenchus brachyurus

Echinochloa crus-galli var. *frumentacea* (Roxb.) Wight. White panicum.
Meloidogyne javanica javanica

Entolasia stricta (R. Br.) Hughes
Hemicycliophora labiata

x *Eucalyptus acmenioides* Schau. Yellow stringybark.
Fergusobia tumefaciens

Eucalyptus crebra F. Muell. Narrow-leaved ironbark.
Fergusobia tumefaciens

Eucalyptus drepanophylla F. Muell. ex Benth. Grey ironbark.
Fergusobia tumefaciens

Eucalyptus hemiphloia F. Muell. ex Benth. Gum-topped box.
Fergusobia tumefaciens

Eucalyptus intermedia F. Muell. Pink bloodwood.
Fergusobia tumefaciens

Eucalyptus maculata Hook. Spotted gum.
Pratylenchus brachyurus

Eucalyptus melanophloia F. Muell. Silver-leaf ironbark.
Fergusobia tumefaciens

Eucalyptus micrantha DC. Scribbly gum.
Radopholus inequalis

Eucalyptus pilularis Sm. Blackbutt.
Hemicycliophora truncata

Eucalyptus populnea F. Muell. Poplar box.
Fergusobia tumefaciens

Eucalyptus resinifera Sm. Red stringybark.
Telotylenchus whitei

Eucalyptus tereticornis Sm. Blue gum.
Fergusobia tumefaciens

Eucalyptus tessellaris F. Muell. Moreton Bay ash.
Fergusobia tumefaciens

Ficus carica L. Cultivated fig.
Tylenchus costatus — *Tylenchus exiguus*

Ficus macrophylla Desf. Moreton Bay fig.
Meloidogyne javanica javanica

Ficus opposita Miq. A sandpaper fig.
Tylenchus emarginatus

Fragaria X ananassa Duchesne. Strawberry.
Helicotylenchus dihystera — *Scutellonema brachyurum*
Pratylenchus brachyurus — *Trichodorus minor*
Pratylenchus vulnus — *Tylenchorhynchus capitatus*
Pratylenchus zeae — *Tylenchus polyhypnus*
Pseudhalenchus minutus — *Xiphinema elongatum*
Rotylenchus brevicaudatus

Gahnia clarkei Benl
Dolichodorus obtusus — *Hemicycliophora nudata*

Gerbera jamesonii Bolus. Gerbera.
Helicotylenchus dihystera — *Pseudhalenchus anchilisposomus*
Pratylenchoides crenicauda

Glycine max (L.) Merr. Soybean.
Meliodogyne javanica javanica

Helianthus annuus L. Sunflower.
Pratylenchus brachyurus

Hibiscus schizopetalus Hook. f. Hibiscus.
Meliodogyne incognita

Hodgkinsonia frutescens C. T. White
Criconema octangulare — *Tylenchulus obscurus*
Criconemoides limitaneum — *Tylenchus exiguus*

Hordeum vulgare L. Barley.
Helicotylenchus dihystera

Hornstedtia scottiana (F. Muell.) K. Schum. A wild ginger.
Criconemoides ornatum — *Pratylenchus brachyurus*

Hornstedtia sp. A wild ginger.
Helicotylenchus dihystera

Hypochaeris radicata L. Flatweed.
Pratylenchus coffeae

Imperata cylindrica (L.) Beauv. var. *major* (Nees) C. E. Hubbard. Blady grass.
Criconema australe — *Helicotylenchus dihystera*

Juglans regia L. English walnut.
Pratylenchus coffeae

Kochia tomentosa (Moq.) F. Muell.
Stictylus asymmetricus — *Telotylenchus whitei*

Lactuca sativa L. Lettuce.
Helicotylenchus dihystera — *Scutellonema brachyurum*
Pratylenchus zeae — *Tylenchus exiguus*

Lantana camara L. Lantana.
Helicotylenchus dihystera

Lantana montevidensis (Spreng.) Briq. Creeping lantana.
Meloidogyne incognita

Linum usitatissimum L. Linseed.
Pratylenchus coffeae

Lolium perenne L. Perennial ryegrass.
Pratylenchus zeae

Lolium rigidum Gaud. Wimmera ryegrass.
Pratylenchus coffeae

Lotononis bainesii Bak.
Helicotylenchus dihystera — *Meliodogyne hapla*

Lupinus angustifolius L. New Zealand blue lupin.
Helicotylenchus dihystera — *Pratylenchus zeae*
Pratylenchus brachyurus — *Tylenchus costatus*
Pratylenchus coffeae

Lycopersicon esculentum Mill. Tomato.
Helicotylenchus dihystera — *Rotylenchus brevicaudatus*
Hemicycliophora truncata — *Scutellonema brachyurum*
Pratylenchus brachyurus — *Trichodorus minor*
Pratylenchus coffeae — *Tylenchorhynchus capitatus*
Pseudhalenchus anchilisposomus — *Tylenchus exiguus*
Rotylenchulus reniformis — *Tylenchus polyhypnus*

Macadamia tetraphylla L. A. S. Johnson. A Macadamia or Queensland nut.
Helicotylenchus dihystera — *Xiphinema americanum*

Malus sylvestris (L.) Mill. Apple.
Pratylenchus coffeae — *Trichodorus minor*
Psilenchus magnidens — *Tylenchus costatus*

Mangifera indica L. Mango.
Pratylenchus zeae — *Tylenchus leptosoma*
Rotylenchulus reniformis

Medicago denticulata Willd. Burr medic.
Pratylenchus brachyurus

Medicago sativa L. Lucerne.
Criconema australe — *Pratylenchus zeae*
Helicotylenchus dihystera — *Rotylenchus bravicaudatus*
Hemicycliophora labiata — *Trichodorus minor*
Paurodontus apiticus — *Tylenchus emarginatus*
Pratylenchus brachyurus — *Xiphinema elongatum*

Melastoma polyanthum Blume. Blue tongue.
Boleodorus volutus
Xiphinema radicicola

Melinis minutiflora Beauv. Molasses grass.
Helicotylenchus dihystera
Meloidogyne javanica javanica

Musa banksii F. Muell. A wild banana.
Helicotylenchus multicinctus
Radopholus similis
Trichodorus minor
Tylenchus exiguus

Musa hillii F. Muell. A wild banana.
Helicotylenchus multicinctus

Musa spp. Banana varieties (cultivars) Cavendish, Lady Finger and Williams Hybrid.
Criconemoides mutabile
Deladenus durus
Helicotylenchus dihystera
Helicotylenchus multicinctus
Hemicycliophora ovata
Meloidogyne incognita
Pratylenchus brachyurus
Pratylenchus coffeae
Radopholus similis
Scutellonema brachyurum
Trichodorus minor
Tylenchus discrepans
Tylenchus exiguus
Tylenchus leptosoma

Nicotiana tabacum L. Tobacco.
Meloidogyne arenaria thamesi
Paratylenchus projectus
Pratylenchus brachyurus
Pratylenchus zeae
Trichodorus minor
Tylenchus baloghi

Olea europaea L. Olive.
Pratylenchus coffeae
Tylenchulus semipenetrans

Opuntia sp. A prickly pear.
Helicotylenchus dihystera
Hemicriconemoides chitwoodi
Pseudhalenchus minutus
Tylenchus emarginatus
Xiphinema diversicaudatum

Ornithopus sativus Brot. Serradella.
Trichodorus minor

Pandanus sp. Pandanus or screw pine.
Pseudhalenchus anchilisposomus

Panicum maximum Jacq. Guinea grass.
Boleodorus thylactus
Pratylenchus zeae
Rotylenchus brevicaudatus

Paspalum dilatatum Poir. Paspalum.
Helicotylenchus dihystera
Tylenchus exiguus
Xiphinema radicicola

Paspalum scrobiculatum L. Scrobic.
Pratylenchus brachyurus

Passiflora edulis Sims forma *edulis*. Passionfruit.
Helicotylenchus dihystera
Scutellonema brachyurum
Tylenchus polyhypnus

Persea americana Mill. Avocado.
- *Meloidogyne hapla*
- *Pratylenchus brachyurus*
- *Trichodorus porosus*
- *Xiphinema americanum*
- *Xiphinema elongatum*

Petunia × *hybrida* Vilm. Petunia.
- *Helicotylenchus dihystera*

Phaseolus atropurpureus DC. Sirato.
- *Helicotylenchus dihystera*

Phaseolus mungo L. Mung bean.
- *Pratylenchus brachyurus*
- *Radopholus similis*

Phaseolus vulgaris L. French bean.
- *Helicotylenchus dihystera*
- *Meloidogyne arenaria thamesi*
- *Pratylenchus coffeae*
- *Pratylenchus zeae*
- *Tylenchus exiguus*

Pilidiostigma tropicum L.S.Smith
- *Helicotylenchus erythrinae*

Pinus elliottii Engelm. Slash pine.
- *Boleodorus thylactus*
- *Criconema pectinatum*
- *Graciliacus peperpotti*
- *Pseudhalenchus minutus*
- *Telotylenchus whitei*
- *Trichodorus minor*
- *Tylenchus emarginatus*

Pisum arvense L. Field pea.
- *Meloidogyne javanica javanica*

Pisum sativum L. Garden pea.
- *Helicotylenchus dihystera*
- *Meloidogyne incognita*
- *Paurodontus apiticus*
- *Pratylenchus coffeae*
- *Trichodorus minor*
- *Tylenchus exiguus*

Plantago major L. Larger plantain.
- *Helicotylenchus dihystera*
- *Meloidogyne hapla*
- *Tylenchorhynchus capitatus*

Portulaca oleracea L. Pigweed.
- *Aphelenchoides subtenuis*
- *Tylenchus discrepans*
- *Tylenchus polyhypnus*

Prunus armeniaca L. Apricot.
- *Pratylenchus coffeae*
- *Pratylenchus neglectus*
- *Scutellonema brachyurum*

Prunus persica (L.) Batsch. Peach.
- *Hemicycliophora truncata*
- *Pratylenchus brachyurus*
- *Pratylenchus coffeae*
- *Pratylenchus zeae*
- *Pseudhalenchus anchilisposomus*
- *Scutellonema brachyurum*
- *Tylenchus costatus*
- *Xiphinema americanum*

Prunus persica (L.) Batsch. var. *nectarina* Maxim. Nectarine.
Helicotylenchus dihystera

Prunus salicina Lindl. Japanese plum.
Pratylenchus coffeae

Pyrus communis L. Pear.
Pratylenchus coffeae

Reinwardtia indica Dum.
Meloidogyne incognita

Rosa multiflora Thunb.
Meloidogyne hapla

Rumex acetosella L. Sorrel.
Pratylenchus coffeae

Saccharum officinarum L. Sugar cane.
Helicotylenchus dihystera
Hemicriconemoides obtusus
Pratylenchus brachyurus
Pratylenchus zeae
Psilenchus magnidens
Rotylenchus brevicaudatus
Trichodorus minor
Trichodorus porosus
Tylenchorhynchus martini
Xiphinema elongatum

Samanea saman (Jacq.) Merr. Rain-tree.
Meloidogyne javanica javanica

Secale cereale L. Rye.
Helicotylenchus dihystera
Meloidogyne javanica javanica
Trichodorus minor

Sechium edule Sw. Choko.
Pratylenchus zeae

Sesbania aculeata Pers. Sesbania pea.
Trichodorus minor

Solanum pseudocapsicum L. Madeira winter cherry.
Meloidgyne javanica javanica
Trichodorus minor

Solanum tuberosum L. Potato.
Helicotylenchus dihystera
Pratylenchus brachyurus
Pratylenchus coffeae
Tylenchorhynchus capitatus

Sonchus oleraceus L. Common sowthistle.
Pratylenchus coffeae

Sorghum bicolor (L.) Moench. Sweet sorghum.
Helicotylenchus dihystera
Meloidogyne javanica javanica
Pratylenchus zeae

Sorghum sudanense (Piper) Stapf. Sudan grass.
Helicotylenchus dihystera
Meliodogyne javanica javanica
Radopholus similis

Spergula arvensis L. Corn spurry.
Meloidogyne incognita — *Pratylenchus coffeae*
Meloidogyne javanica javanica

Stizolobium spp. Velvet beans.
Helicotylenchus dihystera — *Radopholus similis*
Pratylenchus brachyurus

Streptosolen jamesonii (Benth.) Miers. Browallia.
Meloidogyne incognita

Stylosanthes gracilis H.B.K. Stylo.
Meloidogyne hapla

Stylosanthes humilis H.B.K. Townsville lucerne.
Meloidogyne javanica javanica — *Radopholus similis*

Tapeinocheilos queenslandiae (F. M. Bail.) K. Schum.
Helicotylenchus dihystera

Themeda australis (R. Br.) Stapf. Kangaroo grass.
Criconemoides macrodorum — *Paratylenchus microdorus*
Hemicriconemoides obtusus — *Psilenchus hilarulus*
Hemicycliophora labiata — *Tylenchus emarginatus*
Hemicycliophora truncata

Trifolium baccarinii Chiov.
Meloidogyne hapla — *Meloidogyne incognita*

Trifolium burchellianum Ser. ex DC. Cape clover.
Meloidogyne hapla — *Meloidogyne javanica javanica*
Meloidogyne incognita

Trifolium fragiferum L. Strawberry clover.
Meloidogyne incognita — *Meloidogyne javanica javanica*

Trifolium glomeratum L. Clustered clover.
Meloidogyne hapla — *Meloidogyne javanica javanica*
Meloidogyne incognita

Trifolium hybridum L. Alsike clover.
Meloidogyne hapla — *Meloidogyne javanica javanica*

Trifolium pratense L. Red clover.
Meloidogyne incognita — *Pratylenchus brachyurus*
Meloidogyne javanica javanica — *Pratylenchus coffeae*

Trifolium repens L. White clover
Helicotylenchus dihystera — *Psilenchus magnidens*
Heterodera trifolii — *Scutellonema brachyurum*
Meloidogyne hapla — *Trichodorus minor*
Meloidogyne incognita — *Tylenchorhynchus striatus*
Meloidogyne javanica javanica — *Tylenchus discrepans*
Pratylenchus brachyurus — *Tylenchus exiguus*
Pseudhalenchus anchilisposomus — *Tylenchus leptosoma*
Psilenchus duplexus — *Tylenchus thornei*
Psilenchus hilarulus — *Xiphinema radicicola*

Trifolium rueppellianum Fresen.
Meloidogyne hapla — *Meloidogyne javanica javanica*
Meloidogyne incognita

Trifolium semipilosum Fresen.
Meloidogyne hapla — *Meloidogyne javanica javanica*
Meloidogyne incognita

Trifolium subterraneum L. Subterranean clover.
Meloidogyne incognita — *Pratylenchus zeae*
Meloidogyne javanica javanica — *Rotylenchus brevicaudatus*

Trifolium tembense Fresen.
Meloidogyne incognita — *Meloidogyne javanica javanica*

Trifolium usambarense Taub.
Meloidogyne incognita — *Meloidogyne javanica javanica*

Triticum aestivum L. Wheat.
Helicotylenchus dihystera — *Tylenchus exiguus*
Meloidogyne javanica javanica

Verbena bonariensis L. Purple-top.
Pratylenchus coffeae

Vicia sativa L. Common vetch.
Meloidogyne javanica javanica — *Paratylenchus projectus*

Vigna sinensis (L.) Endl. ex Hassk. Cowpea.
Meloidogyne incognita — *Pratylenchus coffeae*
Pratylenchus brachyurus

Vitis vinifera L. Grape.
Boleodorus thylactus — *Rotylenchus brevicaudatus*
Criconemoides xenoplax — *Scutellonema brachyurum*
Helicotylenchus dihystera — *Trichodorus minor*
Hemicycliophora truncata — *Trichodorus porosus*
Paratylenchus elachistus — *Tylenchulus semipenetrans*
Paratylenchus projectus — *Tylenchus costatus*
Paurodontus densus — *Tylenchus emarginatus*
Pratylenchus coffeae — *Tylenchus exiguus*
Pratylenchus zeae — *Tylenchus polyhypnus*
Pseudhalenchus anchilisposomus — *Xiphinema americanum*
Pseudhalenchus minutus

Zea mays L. Maize.
Helicotylenchus dihystera — *Radopholus similis*
Pratylenchus brachyurus — *Rotylenchulus parvus*
Psilenchus magnidens — *Tylenchus exiguus*

Zingiber officinale Rosc. Ginger.
Meloidogyne iavanica javanica

IV. DISCUSSION

There are still many undescribed species in the Departmental Nematology Collection. The data in this paper, however, demonstrate that plant-parasitic nematodes are well represented in Queensland soils and there are many associations meriting investigation.

Galling of citrus roots at Burrum by *Hemicycliophora nudata* suggests that other native species may become pests of cultivated crop plants.

V. ACKNOWLEDGEMENTS

Officers of the Queensland Department of Primary Industries assisted in the collection of soil samples, extraction of nematodes, preparation of photographs and identification of plant species. This assistance is gratefully acknowledged.

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(Received for publication November 5, 1963)

EFFECT OF 2,4-D ON SOME TROPICAL PASTURE LEGUMES

The use of 2,4-D to control broad-leaved weeds during the establishment of improved tropical pastures has been regarded as unsatisfactory because of the damaging effect of 2,4-D on tropical pasture legumes. In view of this, an indicator pot experiment was established in December 1962 to obtain quantitative information on the effect of 2,4-D on five tropical pasture legume species, which are listed in Table 1.

The herbicide used was a concentrated emulsifiable solution containing 50 per cent. w/v 2,4-dichlorophenoxyacetic acid present as the dimethylamine salt.

The design was a randomized block layout of five replications. There was an untreated control and a sprayed plot of each legume. Free-draining 2¾-in. dia. wax paper cups of approximately 220-ml capacity were used. One plot consisted of two separate cups. Each cup was filled with screened red-brown sandy clay loam of basaltic origin.

All seed was pregerminated and inoculated with the appropriate Rhizobium strain prior to planting on December 14, 1962. Two plants were grown in each cup. In some instances there were fewer than four established plants in each plot at the time of spraying (see Table 1).

TABLE 1

RESULTS OF INDICATOR POT EXPERIMENT, 1962-63

Species	Mean No. of Trifoliate Leaves/ Plant at Time of Spraying	Untreated. Mean No. of Living Plants/Plot		2,4–D. Mean No. of Living Plants/Plot		Untreated. Mean D.M. Yield/Plot (g)	2,4–D. Mean D.M. Yield/Plot (g)
		At Time of Spraying	At Time of Harvest	At Time of Spraying	At Time of Harvest		
Centrosema pubescens Benth. (commercial)	1·0	4·0	4·0	4·0	3·4	0·42	0·26
Centrosema pubescens Benth. (mixed strains, C.P.I. 23086 and C.P.I. 25355)	1·1	3·6	3·6	4·0	3·4	0·36	0·27
Pueraria phaseoloides Benth. (Q 367)	1·5	4·0	4·0	3·8	2·8	0·30	0·14
Stylosanthes gracilis H.B.K. (Q 2289)	1·9	3·8	3·8	3·8	3·8	0·04	0·03
Calopogonium mucunoides Desv. (Q 5647)	1·7	4·0	4·0	4·0	4·0	0·30	0·19
Phaseolus atropurpureus (cross of two strains—siratro) ..	2·0	4·0	4·0	4·0	0·0	0·28	0·00
Necessary differences for significance { 5%						0·06	
1%						0·07	

Differences within species: untreated dry-matter yield significantly greater than 2,4-D dry-matter yield at 1% level for all species except *Stylosanthes gracilis*

The herbicide was applied on January 3, 1963, 20 days after planting, at 4 oz of acid equivalent per ac. The herbicide concentrate was mixed with water and applied with an Oxford Precision Sprayer at 20 gal per ac and 30 lb per sq. in. through flat fan nozzles. All cups which were to be sprayed were stood on the ground at random in a 30 ft x 5 ft 8 in. plot and sprayed simultaneously with one traverse of the sprayer boom. Spraying was carried out in the shelter of a building to minimize spray drift.

The cups were kept under cover in a lath-house and watered daily by sprinkling with tap-water. Surviving plants were harvested (roots and tops) on January 16, 1963, oven-dried at approximately 95°C and weighed. The results are presented in Table 1.

Under the trial conditions, *Stylosanthes gracilis* (stylo) and *Calopogonium mucunoides* (calopo) survived 2-4-D, whereas *Phaseolus atropurpureus* (siratro) was completely killed. *Centrosema pubescens* (centro) and *Pueraria phaseoloides* (puero) partially survived the spray treatment. Apart from stylo, all species were significantly reduced in dry-matter yield as a result of spraying with 2,4-D.

It was decided to test the findings from the pot experiment further in a small plot field trial in February 1963 at the Queensland Department of Primary Industries Sub-station at Utchee Creek, near South Johnstone.

The four tropical pasture legume species tested are shown in Table 2. The herbicide used was the same as that used in the indicator pot experiment. The field layout was a randomized block of four replications. There was an untreated control and sprayed plot of each legume. The plot size was 12 ft x 7 ft with 1-ft guard strip between plots. Three rows were planted at approximately 2 ft. 4 in. centres down the length of each plot. The plots were planted on February 13, 1963, with seed which had been inoculated with the appropriate Rhizobium strain and were thinned on March 15, 1963, to give a plant stand

Fig. 1.—*Centrosema pubescens.* Left, untreated; right, sprayed with 2,4-D.

as near as possible to 147 plants per plot. The trial was sited on a red-brown sandy clay loam of basaltic origin which is deficient in available phosphate. No fertilizer was applied to the trial.

The herbicide was applied to the sprayed plots on April 4, 1963, 50 days after planting, at 1 lb of acid equivalent per ac. The herbicide concentrate was mixed with water and applied with an Oxford Precision Sprayer at 20 gal per ac and 30 lb per sq. in. through flat fan nozzles.

Sufficient rain fell throughout the course of the trial for normal plant growth. All plots were kept as free as possible of broad-leaved weeds by periodic hand weeding to minimize weed competition in the untreated control plots.

The surviving legume in a 9 ft x 7 ft quadrat in each plot was cut to ground level and weighed on May 30, 56 days after spraying. Legume samples of 300 g were taken at harvest time from each treatment and dried at approximately 95°C. The dry-matter yields are shown in Table 2.

TABLE 2

RESULTS OF FIELD TRIAL, UTCHEE CREEK, 1963

Species	No. of Trifoliate Leaves/Plant at Time of Spraying	Untreated. Mean D.M. Yield/Plot (lb/7 sq. yd.)	2,4-D. Mean D.M. Yield/Plot (lb/7 sq. yd.)
Centrosema pubescens Benth. (commercial)	5–11	1·38	0·25
Stylosanthes gracilis H.B.K. (Q 2289)	17–23	0·66	0·67
Calopogonium mucunoides Desv. (Q 5646)	7–16	2·45	0·80
Phaseolus atropurpureus (cross of two strains—siratro)	5–20	1·44	0·09
Necessary differences for significance 5%		0·77	
1%		1·05	

Differences within species: untreated dry-matter yield significantly greater than 2,4-D dry-matter yield at 1% level for all species except *Stylosanthes gracilis*

Fig. 2.—*Stylosanthes gracilis.* Left, untreated; right, sprayed with 2,4-D.

Fig. 3.—*Calopogonium mucunoides.* Left, untreated; right, sprayed with 2,4-D.

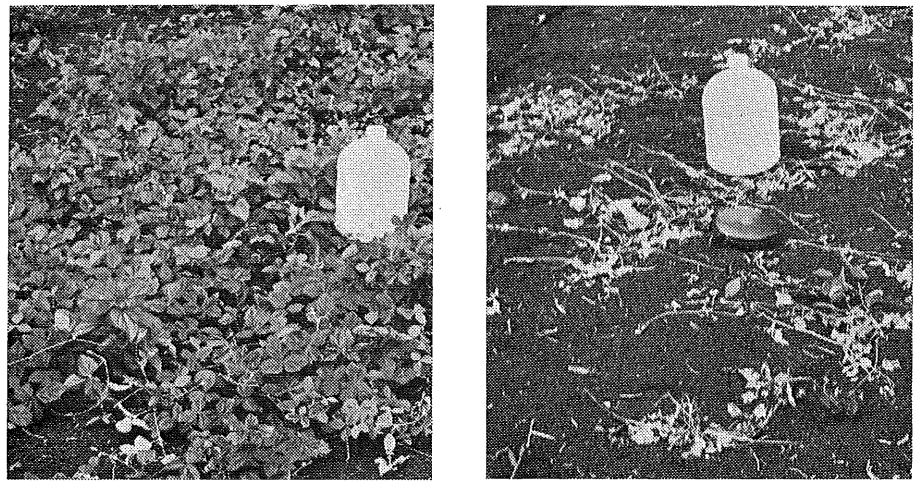

Fig. 4.—Siratro. Left, untreated; right, sprayed with 2,4-D.

Apart from stylo, all species were significantly reduced in dry-matter yield as a result of spraying with 2,4-D. Inspection of the plots the day after spraying showed that all sprayed plots had drooped. Fifteen days after spraying, Siratro appeared almost completely dead, centro and calopo were severely damaged, but stylo had recovered and was little affected.

Figures 1–8 illustrate the effect of 2,4-D on the four tropical legume species tested in the field trial. All photographs were taken on May 1, 77 days after planting and 27 days after applying the spray treatments. The polythene bottle is standing in each plot.

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(Received for publication December 12, 1963)

EFFECT OF 2,4-D PREPLANT SOIL SPRAYING ON THE ESTABLISHMENT OF CENTROSEMA PUBESCENS

A field trial in the 1962-63 season at the Queensland Department of Primary Industries Utchee Creek Sub-Station in North Queensland showed that under wet tropical conditions the last cleaning cultivation for broad-leaved weed control prior to the establishment of a mixed pasture of guinea grass (*Panicum maximum* Jacq.) and centro (*Centrosema pubescens* Benth.) could be safely replaced by various preplant herbicide treatments. 2,4-D was one of the herbicides which gave satisfactory results in this trial.

The pot experiment reported in this note was established to determine whether or not it is necessary for rain to fall before it is safe to plant centro in soil which has been sprayed with 2,4-D.

The herbicide used was a concentrated emulsifiable solution containing 50 per cent. w/v 2,4-dichlorophenoxyacetic acid present as the dimethylamine salt.

The soil was a red-brown sandy clay loam of basaltic origin. Analysis of a composite sample to 6 in. from the area in which the soil had been taken gave the following results:—

pH	5·5
Available P_2O_5	18 p.p.m.
Replaceable K	0·12 m–equiv. per 100 g
Morgan Test NO_3 Nitrogen	Low
Morgan Test NH_3 Nitrogen	Low

A 2 x 2 x 2 factorial design arranged in a randomized block of three replicates was used. The factors tested were:

S0—No spray
S1—2,4-D at 2 lb of acid equivalent per ac
I0—No surface irrigation immediately after spraying
I1—Surface irrigation with 1 in. of water immediately after spraying
T0—Planted immediately after spraying and irrigating
T1—Planted 7 days after spraying and irrigating

Free-draining 8½-in. dia. plastic pots were filled to a depth of 2½ in. with crushed metal. Each pot was then filled with 8 lb of air-dried soil which had passed through a ⅜-in. sieve. All pots were surface-watered with an excess of tap-water the day prior to spraying and allowed to free drain.

The herbicide concentrate was mixed with water and applied to the surface of the sprayed pots on March 14, 1963, with an Oxford Precision Sprayer at 20 gal per ac and 25 lb per sq. in. through flat fan nozzles. Rain immediately after spraying was simulated by rapid surface irrigation of the irrigated pots with tap-water.

To avoid surface watering during the first weeks of the experiment the pots were sub-irrigated by burying the lower 5 in. of each pot in two wooden boxes (3 ft x 3 ft x 1 ft) which had been partially filled first with a 3-in. layer of river sand, followed by a 3-in. layer of crushed metal, and finally a 3-in. layer of sand (Figure 1). The sand and metal in the boxes was kept in a near-saturated condition by frequent watering with tap-water. The boxes containing the pots were sheltered from the weather in a lath-house.

The T0 planting was made on March 14, 1963. Thirty-five scarified seeds of commercial centro which had been inoculated with the appropriate Rhizobium strain were planted at 1 in. x 1 in. spacings at a depth of approximately ¾ in. A hole for each individual seed was made with the unsharpened end of a lead pencil and the soil returned over each seed by pressing with a stick. Care was taken to ensure that 2,4-D was not transferred from sprayed to unsprayed pots at planting time. The T1 group was planted in the same way on March 21, 1963.

Fig. 1.—The experiment in progress. The pots were sub-irrigated through sand-boxes.

Four plants were retained in each pot by thinning at random one week after each planting. Seedlings which emerged after the initial thinnings were clipped off at the soil surface. The original plants remained alive in all treatments except in T0S1T1, where one died, and T0S1I0, where three died (totals of the three replicates).

Supplementary surface waterings were made to each pot on four occasions, namely April 23 (1·72 in.), April 26 (0·86 in.), May 7 (0·43 in.) and May 10 (0·43 in.). The top growth of living plants was harvested on May 21, 68 days after applying the spray treatments, dried at approximately 95°C and weighed.

Table 1 shows that planting centro seed immediately after spraying suppressed the dry-matter yield of top growth by 74 per cent. over a growing period of 68 days. However, by delaying planting for 7 days after spraying the suppression in yield was reduced to 36 per cent. over a growing period of 61 days. There was no significant difference in dry-matter yield between sprayed treatments which received surface irrigation after spraying and those which did not.

TABLE 1

EFFECT OF TREATMENTS ON DRY-MATTER YIELDS
(g/pot)

Factor	S0	S1	Means
T0	1·197	0·310	0·753
T1	1·033	0·658	0·846
Factor	**I0**	**I1**	
T0	0·615	0·892	
T1	0·823	0·868	
S0	1·025	1·205	1·115
S1	0·413	0·555	0·484
Means	0·719	0·880	0·800
	Marginal	Individual	
Necessary differences for significance 5%	0·183	0·259	
Necessary differences for significance 1%	0·254	0·359	

S0 significantly greater than S1 at 1 per cent. level. The interaction term T×S is highly significant due to the fact that the difference between S0 and S1 is more marked with T0 than T1

The effect of treatments on trifoliate leaf numbers is given in Table 2. Planting centro seed immediately after spraying reduced the number of expanded trifoliate leaves by 53 per cent. However, seed planted 7 days after spraying showed a reduction of only 1 per cent. Again, there was no significant difference between sprayed treatments which received surface irrigation after spraying and those which did not.

TABLE 2

EFFECT OF TREATMENTS ON TRIFOLIATE LEAF NUMBERS
(No. of expanded trifoliate leaves per pot at harvest*)

Factor	Transformed Means			Equivalent Means		
	S0	S1	Means	S0	S1	Means
T0	5·170	3·542	4·356	26·7	12·6	19·0
T1	4·502	4·471	4·487	20·3	20·0	20·1
Factor	I0	I1		I0	I1	
T0	4·122	4·590		17·0	21·1	
T1	4·397	4·577		19·3	21·0	
S0	4·789	4·884	4·836	22·9	23·8	23·4
S1	3·730	4·283	4·007	13·9	18·4	16·0
Means	4·259	4·584	4·421	18·1	21·0	
	Marginal	Individual				
Necessary differences for significance 5%	0·505	0·715				
Necessary differences for significance 1%	0·701	0·992				

* Only living plants at harvest counted

S0 significantly greater than S1 at 1 per cent. level. The T × S interaction is highly significant the S effect occurring only with T0

Analysis of variance showed that there was no significant difference between treatments in the total number of plants which had emerged up to the time of harvest.

These results indicate the need for delaying the planting of centro seed in red basaltic soil which has been treated with 2,4-D. Further field trials are necessary to determine the optimum period of delay under wet tropical conditions. It can be expected that rainfall of 1 in. or less following spraying would have little or no effect in reducing the toxicity of 2,4-D on centro planted in red basaltic soil which had been treated with 2,4-D.

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(Received for publication January 9, 1964)

USE OF DICHLORAN AGAINST SCLEROTINIA ROT OF TOMATOES

Sclerotinia rot, caused by the fungus *Sclerotinia sclerotiorum* (Lib.) de Bary, is a serious winter disease of many vegetable crops in Queensland. Beans and tomatoes are most affected, while losses in Brussels sprouts, carrots, capsicums, lettuce, parsnips and marrows are serious at times. The possibility of chemical control had not been thought likely in view of the general ineffectiveness of spray materials tested here and elsewhere in the past. However, recent experiments on the Near North Coast have shown that dichloran (2,6-dichloro-4-nitroaniline) sprays provide good control of the disease in beans (Pegg 1962). The work described here was undertaken to determine whether or not the same control is possible in the case of tomato stem rot.

Two properties were chosen where the disease was known to be regularly present, and portion of an existing planting on each was selected as a trial area. In Trial A, at Victoria Point, 12 adjacent plots of trellised Grosse Lisse tomatoes each 1⅓ chains long were marked out and each alternate one sprayed weekly with dichloran at the rate of 2 lb per 100 gal of water, using a knapsack sprayer. The remaining six plots received no dichloran. In Trial B, at Slacks Creek, 12 adjacent plots each consisting of 1 chain of trellised Q5 tomatoes were similarly used.

Spraying was commenced on April 24, 1963, and continued until August 7, 1963, so altogether 15 applications were made. Dichloran was applied separately and in addition to the routine pest and disease control spray schedule.

A moderate amount of infection occurred on both trial areas (Figure 1) and allowed a satisfactory assessment to be made. In Trial A, the first serious outbreak occurred in early June, 5-6 weeks after spraying was commenced. In Trial B, the first outbreak occurred in early July. Yield figures and stem-rot counts were taken at weekly intervals. Only conspicuous stem-rot infections were counted. In most cases this type of infection led to wilting and death of the part of the plant above the affected area. Minor infections of leaflets and fruits were not included. From the graph in Figure 2 it can be seen that dichloran gave a considerable degree of protection from stem rot in both trials.

Fig. 1.—Portion of an untreated plot, Trial B.

In Trial B, uneven cropping over the area obscured the beneficial effect which these sprays may have had on total yields, but in Trial A this effect was clearly shown (Table 1).

TABLE 1

Total Yield, Trial A (lb per 1½ chain plot)

Replicate No.	1	2	3	4	5	6	Mean
Dichloran 1/100 ..	324·3	261·0	338·5	273·7	237·5	298·8	289·0
Control	150·5	238·7	203·5	151·2	197·6	216·8	193·0

The difference between the mean yields of treated and untreated plots was 96 lb, which represents a mean increase yield per chain length of row of 72 lb. The total weight of dichloran (50% W.P.) applied to this length of row was calculated to be 5 oz.

Apart from one occasion when the spray was applied during cold showery weather, no plant injury was observed. In this one instance there was a slight burning of the most exposed foliage.

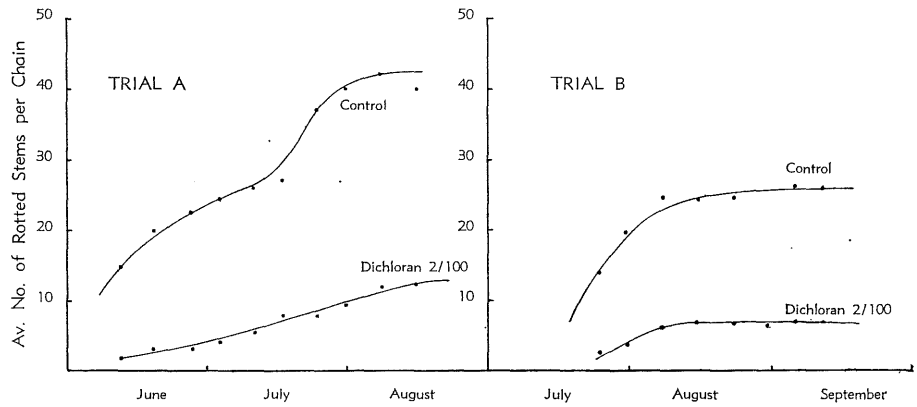

Fig. 2.—Results of the two trials.

The number of spray applications in both trials was in excess of requirements, this being particularly so in the case of Trial B. Although control is highly dependent upon preventive spraying, it would not be desirable in practice to apply sprays so far outside the susceptible period.

The period of susceptibility is strongly influenced by two factors, the season and the age of the crop. In south-eastern Queensland serious losses occur only within the period April to September inclusive, when temperatures are low enough to suit the fungus. Further, it is unusual for the disease to seriously affect the planting before a considerable amount of fruit has set. It may be said, therefore, that spraying needs to be undertaken only within the April-September period, when the plants have reached a susceptible age.

Increasing susceptibility with age appears to be associated with various forms of injury. The commonest infection sites are leaf or stem injuries caused by rubbing against trellis wires and lower leaves weakened by foliage diseases such as leaf mould and Alternaria and Stemphylium leaf spots. Protection from wind injury and foliage diseases therefore may also contribute significantly towards the prevention of this disease.

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(Received for publication October 10, 1963)

EFFECTS OF STUBBLE TREATMENTS ON SUBSEQUENT WHEAT CROPS

Following the harvest of the wheat crop on the Darling Downs in south-eastern Queensland, there are two immediate choices available to the farmer: he may either burn the stubble, a practice which is widely adopted, or retain it so that it is eventually mixed with the surface soil. One view of the latter practice is that it leads to reduction in yield due to plants having a restricted supply of available nitrogen. It is, of course, an established fact that straw or other undecomposed organic matter present in quantity at the time of sowing will adversely affect crop growth. With regard to wheat, Peterson (1950) drew particular attention to this fact, finding that it was necessary in Utah to apply chemical nitrogen to mulched wheat to compensate for the reduction in soil nitrates resulting from the use of straw. This paper is concerned with some aspects of this subject.

Materials and Methods

Field investigations were carried out on an area located on plain country in the Jondaryan district. The natural vegetation comprised native grasses, mainly Queensland blue grass (*Dichanthium sericeum*) and pitted blue grass (*Bothriochloa decipiens*), with some tall oat grass (*Themeda avenacea*) and species of *Panicum*.

The land was first ploughed in August 1945 and the first crop, setaria, planted in September 1946. From then onwards up to the time the investigation commenced in 1956, a programme of winter cropping was carried out. The soil was a dark grey brown clay of the Waco Association, with a chemical analysis as set out in Table 1.

TABLE 1

CHEMICAL ANALYSES OF SOIL FROM TRIAL AREA

—	Depth (in.)				
	0–4	4–8	8–12	24	36
pH	7·6	7·6	7·9	8·4	8·8
Av. P_2O_5 (p.p.m.)	>400	>400	>400	>400	>400
Total N (%)	·08	·08	·08	·06	—
Repl. K (m-equiv. %)	2·25	1·63	·79	—	—
K as % tot. repl. bases	3·51	2·44	1·62	—	—
Tot. repl. bases (m-equiv. %)	64	66·8	48·8	—	—

The investigation comprised two field trials, both of which were set down as randomized blocks—7 x 4 for the first trial in 1956-57 and 8 x 4 for the second trial in 1957-58. For both experiments, the stubble to which treatments were applied was estimated by quadrat sampling to yield 2000 lb of air-dry material per ac. The plot size was 1/20 ac.

Treatments employed were as follows:

(*a*) Control (stubble retained; no fertilizer applied)

(*b*) Urea at 40 lb per ac, broadcast on stubble

(*c*) Urea at 80 lb per ac, broadcast on stubble

(*d*) Ammonium sulphate at 87·6 lb per ac, broadcast on stubble

(*e*) Ammonium sulphate at 175·2 lb per ac, broadcast on stubble

(*f*) Urea at 40 lb per ac as a spray on stubble

(*g*) Urea at 80 lb per ac as a spray on stubble

(*h*) Stubble burnt, no fertilizer applied (Second trial only).

Treatments were applied to the stubble on December 19, 1956, and December 17, 1957, respectively. The broadcast applications of urea and ammonium sulphate were evenly distributed by hand. Spray applications were made with a 1 h.p. Marino spray unit mounted on a truck, the spray being applied at 160 gal per ac to the standing stubble at a pressure of 250 lb/sq. in. A wetting agent was incorporated in the spray at the rate of 4 oz per 100 gal. The burning of the stubble in the particular plots of the second experiment was facilitated by the ploughing of border furrows.

Following the application of treatments, land preparation for the wheat crop proceeded along normal lines, using scarifier and combine. Plots were sown to the variety Festival on July 15, 1957, and July 2, 1958, respectively, at 55 lb per ac. In both 1957 and 1958, composite soil samples were taken for the estimation of available soil nitrogen.

The trials were harvested on November 16, 1957, and December 5, 1958, using standard farming equipment. Plot yields were recorded for both seasons. Composite 12-lb grain samples were obtained for quality assessment in 1957, while in 1958 2-lb grain samples were taken for protein testing. Some samples were also checked for mottling.

The rainfall received during 1957 was below normal and the crop suffered from the dry conditions. The following year, however, the growing season was better and the crop did not suffer any undue stress due to lack of moisture.

Results

Observations made at regular intervals during the course of the experiment revealed that fragmentation of the stubble was a progressive feature and there were differences in this regard between the various treatments.

Data relating to the availability of nitrogen in the soil are given in Table 2.

TABLE 2

AVAILABLE SOIL NITROGEN
(p.p.m. nitrate nitrogen)

Stage	Treatment	Depth (in.)				
		0–4	4–12	12–24	24–36	36–48
		1957				
Planting (22.vii.57)	Control	4·9	7·0	3·9	1·9	0·4
	80 lb urea spray	7·0	8·4	4·9	2·2	0·9
Flowering (15.x.57)	Control	1·5	1·2	1·1	1·3	1·7
Harvest (15.xi.57)	Control	4·3	2·4	1·2	1·1	1·5
		1958				
		0–4	4–12	12–18	18–24	24–36	36–48
Planting (4.vi.58)	Control	17·0	14·4	17·6	16·5	17·3	14·3
	80 lb urea spray	17·7	16·2	20·8	21·1	17·5	14·1
	Stubble burnt	27·4	22·8	15·1	13·6	14·6	13·9
Flowering (13.x.58)	Control	3·1	3·4	—	—	—	—
	80 lb urea spray	2·9	7·8	—	—	—	—
	Stubble burnt	3·8	5·7	—	—	—	—

The mean treatment yields of wheat grain for both trials for both seasons are recorded in Table 3. No significant differences in yield were obtained.

TABLE 3

MEAN TREATMENT YIELDS
(bus per ac)

Treatment	Year	
	1957	1958
Control	11·8	25·9
40 lb urea broadcast	12·2	25·8
80 lb urea broadcast	12·0	25·1
87·6 lb ammonium sulphate broadcast ..	12·4	26·3
175·2 lb ammonium sulphate broadcast ..	12·3	25·0
40 lb urea spray	12·0	26·1
80 lb urea spray	12·0	25·7
Stubble burnt	—	27·1

The 12-lb composite grain samples from the 1957 trial were tested for quality. The following results were obtained:—

Control.—Protein 12·1 per cent. Strong, well-balanced, good extensibility, poor mixing quality. Baking quality poor.

40 *lb urea broadcast.*—Protein 12·8 per cent. Medium strong, well-balanced, fair extensibility, poor mixing quality. Baking quality poor.

80 *lb urea broadcast.*—Protein 13·0 per cent. Strong, well-balanced, good extensibility, poor mixing quality. Fairly good baking quality.

87·6 *lb. ammonium sulphate broadcast.*—Protein 12·7 per cent. Strong, overstable, fair extensibility, poor mixing quality. Fairly good baking quality.

175·2 *lb. ammonium sulphate broadcast.*—Protein 13·2 per cent. Medium strong, well-balanced, fair extensibility, poor mixing quality. Fairly good baking quality.

40 *lb. urea spray.*—Protein 12·9 per cent. Strong, well-balanced, good extensibility, poor mixing quality. Fairly good baking quality.

80 *lb urea spray.*—Protein 11·3 per cent. Medium strong, slightly overstable, fair extensibility, poor mixing quality. Good baking quality.

The above results indicate a general improvement in baking quality following the use of nitrogen fertilizers.

Grain protein content of samples from the various treatments in the 1958 trial is shown in Table 4.

TABLE 4

MEAN PROTEIN PERCENTAGE OF GRAIN (13·5% MOISTURE CONTENT)

Treatment	Mean Protein
Control	13·35
40 lb urea broadcast	14·08
80 lb urea broadcast	14·35
87·6 lb ammonium sulphate broadcast ..	13·65
175·2 lb ammonium sulphate broadcast ..	14·20
40 lb urea spray	13·85
80 lb urea spray	14·10
Stubble burnt	13·60

Necessary differences for significance: 5%, ·49; 1%, ·66.

It will be seen that all but one of the nitrogen treatments (87·6 lb ammonium sulphate broadcast) were significantly ($P < \cdot 05$) better than control. The stubble-burnt plots were not significantly better than the control (stubble retained).

It has been noted by several workers (e.g. Callaghan and Millington 1956) that mottling in wheat is associated with a depletion in soil fertility. As the present experiments were concerned with soil nitrogen it was considered relevant to investigate mottling, even if on a restricted scale. Accordingly, samples of 100 grains were taken at random from each of the protein samples of Block III. Each grain was examined individually and classified as to mottling.

The results of this visual examination of the grains are shown in Table 5.

TABLE 5

GRAIN MOTTLING PERCENTAGES

Class	Treatment							
	Control	Urea 40 lb b'cast	Urea 80 lb b'cast	Amm. Sulp. 87·6 lb	Amm. Sulph. 175·2 lb	Urea 40 lb spray	Urea 80 lb spray	Stubble burnt
NM ..	49	56	73	63	69	58	72	55
SM ..	7	7	8	9	6	7	3	12
M ..	14	19	12	13	10	8	12	10
WM ..	12	15	3	8	6	8	8	7
BM ..	18	3	4	7	9	19	5	16
CM ..	—	—	—	—	—	—	—	—

NM, free from mottling; SM, slightly mottled; M, up to one-third of the grain mottled; WM, one-third to half of the grain mottled; BM, greater than half of the grain mottled; CM, completely mottled.

While there is one discrepancy (40 lb urea spray), the figures in general support the viewpoint that soil fertility and mottling in wheat are inversely associated.

The data presented indicate that continuous grain cropping results in a reduction in the protein content of grain. Similar data have been obtained by McKay and Moss (1949). The trials also show that this reduction in grain protein content is not arrested by stubble burning as opposed to stubble retention.

Evidence is adduced that addition of nitrogen to the stubble is effective in increasing grain protein content. At this stage of cultivation history (13 years), however, no increase in yield resulted from the addition of up to 40 lb of nitrogen per ac. Seasonal conditions, however, especially in 1957, resulted in some moisture stress.

Baking quality was improved in general by the addition of nitrogen either to the stubble or to the soil surface.

The incidence of mottling was not increased by stubble retention as opposed to stubble burning. The addition of nitrogen, however, reduced mottling incidence.

Acknowledgements

Facilities for conducting the experiments were provided by Mr. K. McIntyre, of Jondaryan. The statistical analyses were carried out by the Biometrics Branch of the Department. Analyses of grain and soil were performed by the Agricultural Chemical Laboratory Branch. This assistance is gratefully acknowledged.

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(Received for publication October 14, 1963)

APPLICATION OF THE UDY PROTEIN TEST TO QUEENSLAND WHEATS AND FLOURS

Udy (1956, 1957) developed dye ion-binding for the estimation of protein in wheat and flour. This method was applied to Queensland wheat and flours, and during these tests the following procedure was developed, using standard laboratory apparatus.

A 500-mg sample of flour or a 600-mg sample of wheat (ground to pass 0·022-in. sieve) was shaken vigorously with 25 ml of Orange G dye solution (containing 2 mg dye per ml acetic acid pH 2·2)—flour for 3 min, wheat for 30 min. The solutions were centrifuged till clear and an aliquot of each test solution was diluted 20 times with acetic acid pH 2·2.

The optical densities of the diluted test solutions were read against a blank containing 0·04 mg dye per ml. From these densities and a concentration curve, the amount of dye (in mg) bound by the sample was estimated and then plotted against the Kjeldahl protein content (per cent.) of the sample.

Fifty-two flour samples representing nine different varieties were tested and the regression line obtained was

$$(N \times 5{\cdot}7)\% = 1{\cdot}464\,B - 3{\cdot}624,$$

where B = mg dye bound per 500 mg flour.

$$r = 0{\cdot}991$$

$$\text{s.e. of estimate} = 0{\cdot}28 \text{ protein}$$

Thirty-six wheats of six different varieties were used to produce the line

$$(N \times 5{\cdot}7)\% = 2{\cdot}503\,B - 9{\cdot}975,$$

where B = mg dye bound per 600 mg wheat.

$$r = 0{\cdot}983$$

$$\text{s.e. of estimate} = 0{\cdot}35\% \text{ protein}$$

These results are in reasonably good agreement with those obtained by Udy (1956, 1957), who showed that protein analyses using this method correlate fairly well with those of the slower Kjeldahl method.

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(Received for publication January 7, 1964)

EFFECT OF PREVIOUS CROPPING WITH SOYBEANS ON THE NODULATION OF SOYBEANS ON THE DARLING DOWNS, QUEENSLAND

Bowen (1956) placed soybean (*Glycine max* (L.) Merr.) in the group of introduced legumes of the cowpea miscellany nodulating naturally in various parts of Queensland.

Persistent reports have been made of nodulation difficulty with soybeans when grown on the black soils of the Darling Downs. Several crops on a dark-brown clay loam at Hermitage Research Station on the south-eastern Darling Downs have been observed to show no nodulation after being sown with inoculated seed. However, volunteer plants in ground previously under soybeans have been observed to be well nodulated.

A pot experiment was set out using inoculated and uninoculated seed of the soybean variety Nanda. The soil used was a dark-brown clay loam from two locations at Hermitage. The first location was, as far as can be ascertained, virgin for soybeans. The second location was under soybeans in 1960-61: this crop had been inoculated at planting, but failed to nodulate.

Pot treatments applied on February 26, 1962, were:

(A) First-year soil; seed inoculated
(B) First-year soil; seed uninoculated
(C) Second-year soil; seed inoculated
(D) Second-year soil; seed uninoculated.

Each treatment was replicated four times. Six-inch plastic pots, undrained, were used for the experiment and watering was arranged so that each pot was periodically adjusted to approximate field capacity.

On May 14, 1962, roots were examined for nodulation, which was assessed by counting the nodules per plant root system; fresh weights of nodules and plant roots were also recorded. Results are shown in Table 1.

TABLE 1

RESULTS OF NODULATION TRIAL

Treatment	A	B	C	D
Number of plants ..	11	15	13	19
Number of nodules ..	15	nil	144	133
Weight of roots (g) ..	40·3	57·1	46·0	45·3
Weight of nodules (g)	0·67	—	8·18	8·60

On the first-year soil, poor nodulation was recorded with inoculated seed (1·3 nodules per plant), whereas nodulation was much improved on second-year soil using both uninoculated and inoculated seed (7-10 nodules per plant). Uninoculated seed planted in first-year ground failed to nodulate.

No differences in plant growth were observed between treatments during the seedling stage. At the time of root inspection, plants in Treatment B showed a definite yellowing in colour and slight stunting in growth indicative of a probable effect of no nodulation. Plants in Treatments C and D generally were of a greener colour than those in Treatments A and B, but as the soils were collected from different locations it would be unwise to ascribe this colour effect to differences in nodulation.

Results of the pot experiment indicated that the failure of soybeans to nodulate in new ground is a probable cause of the difficulty in obtaining satisfactory nodulation in the crop on the dark-brown clay soils of the Darling Downs. The strain of inoculum used appeared to be effective, but not in the first year.

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(Received for publication January 9, 1964)

FURTHER INVESTIGATIONS INTO THE BEHAVIOUR OF DELICIOUS APPLES STORED IN CONTROLLED ATMOSPHERES

Stevenson and Carroll (1963) obtained good results by holding Delicious apples at 32°F in an atmosphere containing 5 per cent. oxygen plus 2·5 per cent. carbon dioxide. In order to determine whether these results were due to seasonal effects, a further experiment was carried out during the 1963 apple season to confirm these earlier results, to study the effect of other atmospheres and to determine whether superficial scald could be more effectively controlled by diphenylamine in place of ethoxyquin, which had not proved completely successful in the earlier work.

Methods and Materials

The experimental fruit was obtained from the same six orchards in the Granite Belt as were used in the 1961 and 1962 investigations. Picking date was March 3, 1963, which falls into the period found by Stevenson (1959) to be the most satisfactory for optimum storage behaviour of this variety. The fruit was stored in 12 wide-mouthed, 44-gal gas-tight drums, each drum containing one ½-bus case of fruit from each of the six orchards. Storage temperatures used were 32°, 34° and 36°F and four drums were held at each temperature. Controls consisting of one ½-bus case from each of the six orchards were also stored at each of the three temperatures. The five storage atmospheres used in the experiment were—

(1) Normal air storage
(2) 2·5 per cent. oxygen plus 2·5 per cent. carbon dioxide
(3) 2·5 per cent. oxygen plus 5·0 per cent. carbon dioxide
(4) 5·0 per cent. oxygen plus 2·5 per cent. carbon dioxide
(5) 5·0 per cent. oxygen plus 5·0 per cent. carbon dioxide

Prior to storage the fruit was dipped in a solution containing 2000 p.p.m. diphenylamine (DPA) in 35 per cent. ethyl alcohol to control superficial scald. The required storage atmospheres were obtained and maintained by passing air diluted with calculated amounts of nitrogen through manometric flowmeters into the drums. Regular analyses were made for oxygen and carbon dioxide during the storage period and necessary corrections made by adjustment of the air dilution and flow rates. The fruit was removed from store on September 4, 1963, held at 70° for seven days and inspected for disorders. Firmness of the fruit was measured on five fruit selected at random from each case, using a Magness penetrometer fitted with the $\frac{7}{16}$-in. plunger.

Results

The results are summarized in Tables 1 and 2. The incidence of mould, breakdown, withering and superficial scald was slight irrespective of storage

temperature or atmosphere in which the fruit was held. For this reason analyses of variance were not carried out on the percentages of these disorders present. There is a trend which suggests that controlled atmosphere storage reduces the incidence of internal breakdown. A similar trend has been reported in earlier work by Stevenson and Carroll (1963). The incidence of bitter pit was significantly reduced by the use of controlled atmospheres, all atmospheres having less pit than normal air storage, but there were no significant differences between the controlled atmospheres used in the experiment. Total disorders present, comprising the sum of fruit affected by mould, scald breakdown, withering and bitter pit, were significantly affected by storage atmosphere. There were significantly fewer disorders in all the controlled atmospheres than in normal air storage but there were no significant differences between any of the controlled atmospheres used. Firmness was significantly affected by atmosphere and storage temperature. The firmest fruit resulted from storage at 34°F. An atmosphere containing 2·5 per cent. oxygen plus 2·5 per cent. carbon dioxide yielded significantly firmer fruit than atmospheres of 2·5 per cent. oxygen plus 5·0 per cent. carbon dioxide and 5·0 per cent. oxygen plus 5·0 per cent. carbon dioxide. Fruit held in these atmospheres was significantly firmer than that held in 5·0 per cent. oxygen plus 2·5 per cent. carbon dioxide, which was in turn significantly firmer than that held in normal storage atmospheres.

TABLE 1

MEAN PERCENTAGES OF MOULD, SCALD, BREAKDOWN AND WITHERED FRUIT AFTER REMOVAL FROM COOL STORE

Treatment	Mould (%)	Scald (%)	Breakdown (%)	Withered Fruit (%)
Normal air storage				
32°F	2·01	0·70	0·66	0
34°F	3·64	0·70	2·71	0
36°F	3·25	0	2·71	0
2·5% O_2 + 2·5% CO_2				
32°F	3·27	0	0·31	0
34°F	2·27	0·40	0·74	1·32
36°F	0·90	0	0	0·51
2·5% O_2 + 5·0% CO_2				
32°F	2·73	0	0·28	0
34°F	2·65	0	0	1·65
36°F	1·29	0·31	0	0·59
5·0% O_2 + 2·5% CO_2				
32°F	1·98	2·08	0·59	0·63
34°F	2·50	0	0·56	0·53
36°F	3·85	0	0	2·31
5·0% O_2 + 5·0% CO_2				
32°F	2·38	0·28	0·40	0
34°F	1·94	0	0	0·25
36°F	2·20	0	0·71	1·01

No analyses of variance carried out

TABLE 2

EQUIVALENT MEAN PERCENTAGES* OF BITTER PIT, TOTAL DISORDERS AND FIRMNESS AFTER REMOVAL FROM COOL STORE

Treatment	Bitter Pit (%)	Total Disorders (%)	Firmness (lb)
Normal air storage			
32°F	6·76	11·31	10·68
34°F	8·29	16·53	10·38
36°F	5·28	12·23	9·75
2·5% O_2 + 2·5% CO_2			
32°F	2·21	6·42	14·59
34°F	0·87	5·79	14·05
36°F	0·14	1·47	14·34
2·5% O_2 + 5·0% CO_2			
32°F	1·23	4·54	13·73
34°F	1·50	6·62	13·78
36°F	0·20	1·65	13·01
5·0% O_2 + 2·5% CO_2			
32°F	0·83	6·34	12·35
34°F	2·58	7·17	14·01
36°F	0·58	5·30	10·94
5·0% O_2 + 5·0% CO_2			
32°F	1·98	5·73	12·86
34°F	0·45	1·73	14·43
36°F	1·53	5·41	13·57

* Inverse sine transformation used for analysis

Bitter Pit—2·5 per cent. oxygen plus 2·5 per cent. carbon dioxide; 2·5 per cent. oxygen plus 5·0 per cent. carbon dioxide; 5·0 per cent. oxygen plus 2·5 per cent. carbon dioxide; 5·0 per cent. oxygen plus 5·0 per cent. carbon dioxide significantly less than normal air storage (1 per cent. level)

Total Disorders—2·5 per cent. oxygen plus 2·5 per cent. carbon dioxide; 2·5 per cent. oxygen plus 5·0 per cent. carbon dioxide; 5·0 per cent. oxygen plus 2·5 per cent. carbon dioxide; 5·0 per cent. oxygen plus 5·0 per cent. carbon dioxide significantly less than normal air storage (1 per cent. level)

Firmness—34°F significantly firmer than 36°F (1 per cent. level); 32°F significantly firmer than 36°F (5 per cent. level); 2·5 per cent. oxygen plus 2·5 per cent. carbon dioxide significantly firmer than 2·5 per cent. oxygen plus 5·0 per cent. carbon dioxide; 5·0 per cent. oxygen plus 5·0 per cent. carbon dioxide (1 per cent. level); 2·5 per cent. oxygen plus 5·0 per cent. carbon dioxide; 5·0 per cent. oxygen plus 5·0 per cent. carbon dioxide significantly firmer than 5·0 per cent. oxygen plus 2·5 per cent. carbon dioxide (1 per cent. level); 5·0 per cent. oxygen plus 2·5 per cent. carbon dioxide significantly firmer than normal air storage (1 per cent. level)

The interaction temperatures × atmospheres is highly significant; temperature differences vary at the different atmospheres:—

Normal air storage: 32°F significantly firmer than 36°F (5 per cent. level)

2·5 per cent. oxygen plus 2·5 per cent. carbon dioxide: no significant differences

2·5 per cent. oxygen plus 5·0 per cent. carbon dioxide: no significant differences

5·0 per cent. oxygen plus 2·5 per cent. carbon dioxide:
32°F significantly firmer than 36°F (1 per cent. level)
34°F significantly firmer than 32°F (1 per cent. level)

5·0 per cent. oxygen plus 5·0 per cent. carbon dioxide: 34°F significantly firmer than 32°F (1 per cent. level)

Discussion

A number of important facts emerge from the results. It has been clearly demonstrated that superficial scald can be effectively controlled by the use of dips containing 2000 p.p.m. diphenylamine. This compound has been shown by Hall, Scott, and Coote (1961) to be approximately twice as effective as ethoxyquin, and failure by Stevenson and Carroll (1963) to eliminate this disorder with ethoxyquin dips containing 2000 p.p.m. is no doubt due to this fact. This indicates that ethoxyquin dips of the order of 4000 p.p.m. are necessary to effectively control scald in this variety. The results indicate that controlled atmosphere storage will reduce bitter pit during storage of this variety, but whether this is due to the choice of atmosphere or the retarding of senescence by the process is not known; the matter is worthy of further study. Fruit held in the controlled atmospheres was up to 4 lb firmer than the air-stored controls and this was reflected by the green ground colour of the controlled atmosphere fruit compared with the yellow ground colour of the controls. Inspection of the fruit indicated at least a further six weeks' storage life of the controlled atmosphere stored fruit.

The results clearly indicated that this variety can be successfully held in controlled atmospheres of oxygen and carbon dioxide provided adequate control of superficial scald can be effected. As ethoxyquin will not be marketed commercially in Australia and diphenylamine has not yet been approved for commercial use in this country, the controlled atmosphere storage of this variety cannot be recommended at present. The small differences in storage behaviour obtained between the atmospheres tested confirms the results of American workers, who have obtained good results with atmospheres containing from 2·0 to 5·0 per cent. oxygen plus 0 to 5·0 per cent. carbon dioxide (Van Doren 1952; Smock 1958; Eaves 1953, 1954, 1955).

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(Received for publication October 7, 1963)

INACTIVATION OF ALPHA-AMYLASE USED AS AN INDICATION OF EFFECTIVE HEAT TREATMENT OF QUEENSLAND WHOLE EGG PULP

Investigations in the U.S.A. on thermal death times for Salmonella in liquid whole egg (Osborn, Straka, and Lineweaver 1954) and also on the narrow heat range in which pasteurization of whole-egg pulp can be carried out, showed that at temperatures below 135°F Salmonellae are not killed, whereas above 145°F the pulp tends to coagulate. Coagulation is almost instantaneous above 163°F (Payawal 1946; Brooks and Taylor 1955).

A laboratory method for the determination of the destruction of alpha-amylase and Salmonellae in whole egg pulp by heat pasteurization was published by Shrimpton *et al.* (1962), who correlated the inactivation of alpha-amylase with the Salmonellae "kill". It was established that no viable organisms of the more resistant Salmonella strain *S. seftenberg* N.C.T.T. 9959 were recovered after pasteurization at 64·4°C (148°F) for 2½ min, in a continuous flow plant. Complete inactivation of alpha-amylase was accomplished under these conditions.

The applicability of the method was investigated in regard to Queensland factory pasteurized whole-egg pulp as well as the suitability of the pulp following pasteurization at 148°F for commercial use.

Methods

Factory Pasteurization.—The liquid-egg pasteurization plant used by the South Queensland Egg Marketing Board is a continuous automatic system of high-temperature short-time pasteurization. It consists of the stainless-steel plate exchanger now universally used for the heating and cooling of all potable liquids.

A positive displacement pump feeds the egg pulp, at a constant rate, to the plate heat exchanger, where it is heated, firstly by regeneration and then by hot water, to the required pasteurizing temperature. It is held for 2½ min in the series of stainless-steel holding plates, and then rapidly cooled firstly by regeneration and then by chilled water to 38°F before being filled into cans for subsequent freezing. During the trials, the pump was set at 200 r.p.m. to deliver 600 gal of pulp per hr.

Alpha-amylase Test.—The alpha-amylase present in the whole egg, when incubated with standard starch solution, will degrade the starch and prevent the formation of a blue starch/iodine complex on the subsequent addition to iodine.

The intensity of the blue colour formed varies inversely as the residual alpha-amylase activity. It was measured with a Bausch and Lomb Spectronic 20 as percentage transmission at 585 mμ. A Lovibond comparator disc (4·26) has since been placed on the market by Tintometer Ltd., Salisbury, England.

Bacteriological Tests.—The tests for the presence of Salmonellae involved enrichment with double-strength tetrathionate broth and incubation at 37°C. However, McCoy (1962) showed no difference between single and double strengths of tetrathionate broths in the number of resulting positive Salmonellae isolations.

The selective medium was Brilliant Green MacConkey's agar. Harvey (1956) compared bile salt lactose media using bismuth sulphite agar and Brilliant Green MacConkey's agar and recommended the latter. However, as some strains of Proteus also produce red colonies on this medium, biochemical and serological tests were necessary. Polyvalent "O" and polyvalent "H" specific and non-specific Salmonella sera were used in the slide agglutination tests.

Experimental

Egg pulp was pasteurized at the factory at 148°F for 2½ min. Samples were collected and subjected to the alpha-amylase test.

Bacteriological tests were performed on the samples for possible detection of Salmonella strains.

The effect of freezing the pulp on the test was investigated. Pulps were examined after pasteurization and again after being frozen to 0°F.

The combination of freezing and storage of samples in relation to the results of the test was also examined. Samples were tested prior to storage and again after 4 months at 0°F.

Baking trials were conducted by the Central Technical College, Brisbane, to determine the influence of heat treatments on the baking quality of cake mixtures containing whole-egg pulp. Sponges were made from unpasteurized egg pulp and pulps pasteurized at 145°F and at 148°F for 2½ min. The pulps were originally frozen but were defrosted before commencement of the trials.

Results

All factory-pasteurized samples revealed effective heat treatment by the alpha-amylase test, which regards as effectively pasteurized all pulps with a final light transmission reading of not greater than 70 per cent.

Bacteriological tests detected no strains of surviving Salmonellae in the heat-treated pulps, and consistently plate counts of less than 10,000 bacteria per g with absence of coliforms were obtained.

Neither freezing nor storage at 0°F for a period of 4 months affected the negative results of the alpha-amylase test on samples after pasteurization. The number of samples tested was limited, but it would appear that the possibility of reactivation of the enzyme is remote.

The baking trials revealed that slightly longer beating times were required for the egg pulp pasteurized at 145°F and 148°F as compared with the unpasteurized pulp. Nevertheless, with slight modifications, sponges were produced comparable in volume, texture and quality (Figure 1).

All of the 336 pasteurized samples subjected to the test showed effective pasteurization.

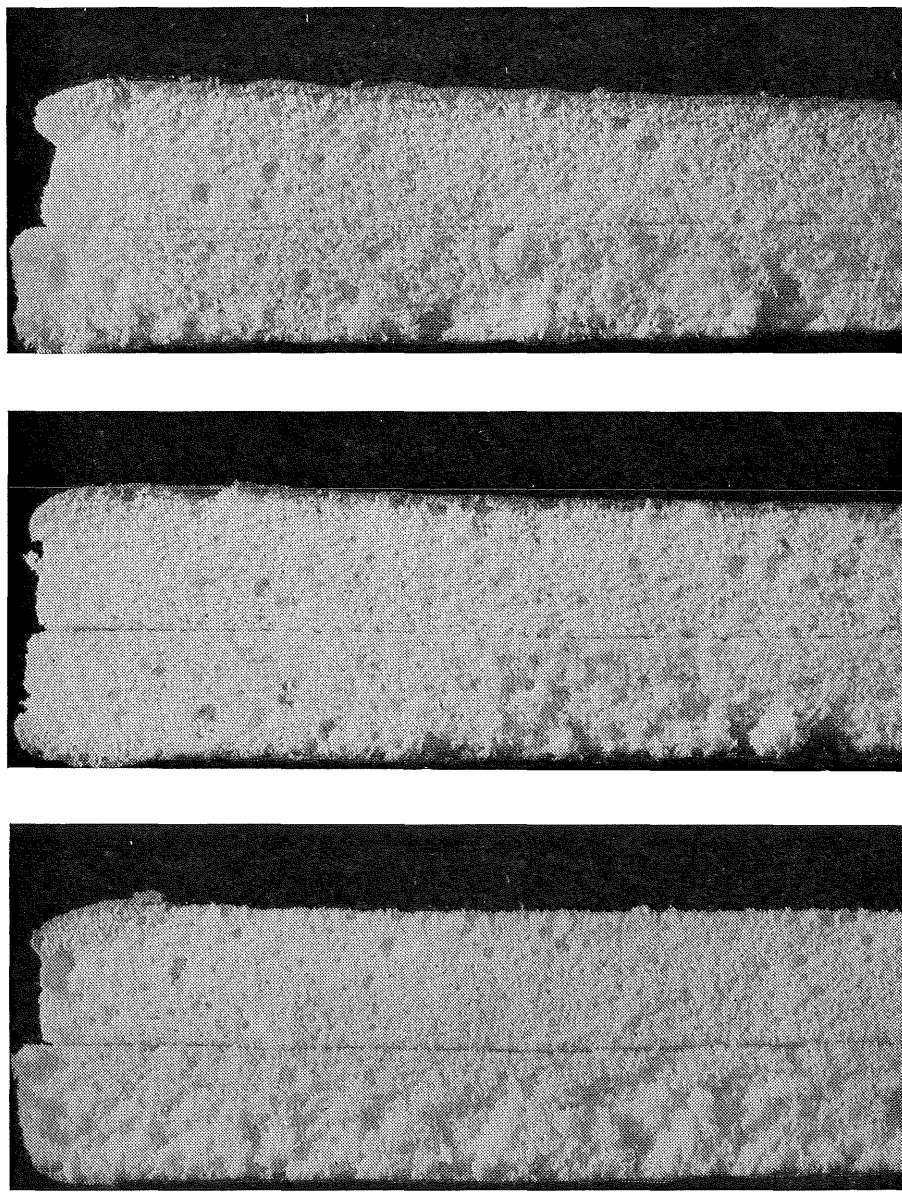

Fig. 1.—Sections of sponges baked with unpasteurized egg pulp (top), egg pulp pasteurized at 145°F (centre), and egg pulp pasteurized at 148°F (bottom).

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(Received for publication November 22, 1963)

COMPARISON OF DRYING OVENS FOR DETERMINING THE MOISTURE CONTENT OF CHEESE

As cheese moisture determinations comprise a considerable proportion of the work carried out in a dairy laboratory situated in a cheesemaking area, the efficiency of three types of drying ovens was investigated.

The methods used in determining the moisture content of cheese were as follows:—

(a) Vacuum oven.—The official A.O.A.C. method was used (A.O.A.C., 8th Ed. 278-15-129 and Method 1:31).

(b) Convection oven—as for (a).

(c) Meihuizen quick-drying oven.—Samples of approximately 2 g of the grated cheese are weighed out in the special dishes provided. These are placed in a vertical rack, which is lowered into the oven, where it is surrounded by a paraffin-oil jacket electrically heated through a thermostatic control. The cheese samples are heated in the oven for 27 min at 165°C, cooled in a desiccator and reweighed.

The results obtained are shown in Tables 1–3.

TABLE 1

COMPARISON OF MEIHUIZEN OVEN AND CONVECTION OVEN, USING THE SAME 19 SAMPLES

—	Meihuizen Oven	Convection Oven
Average difference between duplicates within method (per cent. moisture)	0·10	0·13
Range of differences between duplicates within method (per cent. moisture)	0·00 to 0·39	0·00 to 0·42
Average difference between methods (per cent. moisture)	+ 0·03	− 0·03
Range of differences between methods	− 0·42 to + 0·32	− 0·32 to + 0·42

TABLE 2

COMPARISON OF CONVECTION OVEN AND VACUUM OVEN, USING THE SAME 13 SAMPLES

—	Convection Oven	Vacuum Oven
Average difference between duplicates within method (per cent. moisture)	0·16	0·19
Range of differences between duplicates within method (per cent. moisture)	0·01 to 0·60	0·01 to 0·65
Average difference between methods (per cent. moisture)	+ 0·006	− 0·006
Range of differences between methods (per cent. moisture)	− 0·32 to + 0·6	− 0·6 to + 0·32

TABLE 3

COMPARISON OF CONSISTENCY FOR CONVECTION OVEN AND VACUUM OVEN

—	Convection Oven	Vacuum Oven
Number of samples	161	97
Range of difference between duplicates (per cent. moisture)	0·00 to 0·83	0·01 to 0·95
Average difference between duplicates (per cent. moisture)	0·194	0·218

Table 1 shows that moisture values obtained in the quick-drying oven are only very slightly higher than those obtained in the vacuum oven and the convection oven. As the quick-drying oven provides a rapid method of obtaining a moisture percentage, it could be extremely useful in compositional control at cheese factories, where it is sometimes desirable to conclude a moisture determination on the previous day's batch of cheese before the manufacture of the current day's batch has commenced. With matured cheese, however, the results could be raised by the higher temperature employed and the fact that the moisture dishes are in direct metallic content with the oven, causing some loss of volatiles other than moisture.

Table 2 indicates that for the same cheese the convection oven is a little more consistent than the vacuum oven. The average difference between duplicates was 0·19 per cent. in the vacuum oven and 0·16 per cent. in the convection oven, and the maximum variation between duplicates was 0·65 per cent. and 0·60 per cent. respectively.

Table 3 confirms that the convection oven is a little more consistent than the vacuum oven. However, the average differences between duplicates of 0·194 and 0·218 per cent. are small and serve to show that both ovens give fairly consistent results. It is possible that in the vacuum oven different levels of heat are obtained as a result of uneven movement of air.

Though no thorough investigation has been carried out, some evidence has been obtained to suggest that the official method for determining cheese moistures can be shortened without affecting the accuracy of the results. In another laboratory outside the Department, the cheese moisture dishes are not placed on the steam bath at all and they are given only 4 hr in the convection oven. The results obtained in a very small number of checks made were within 0·3 per cent. moisture lower than those obtained in this laboratory using the official methods.

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(Received for publication January 6, 1964)

A SIMPLE LABORATORY GLASS STILL WITH AUTOMATIC WATER CONTROL

In some laboratories, the demand for glass-distilled water is often erratic and short-term, and the cost of purchasing an automatic all-glass still is not always warranted. Distillation of water in a simple still requires frequent refilling and attention. The still described, which is readily assembled from standard "Quickfit" glassware, overcomes the difficulty of maintaining a constant level in the evaporating vessel and thus reduces the amount of attention needed.

A photograph of the unit is given in Figure 1, which shows a board A, 26 in. x 11 in. x 1 in., placed on a fulcrum F mounted on a base board B, 16 in. x 11 in. x 1 in., so that A may move freely with a rocking motion. A piece of 2-in. angle-iron 6 in. long serves as a suitable fulcrum. A heating mantle with a round-bottom, 2-necked, 2-l flask is placed on one side of board A and connected via a splash head with inlet tube to a coil condenser P, which acts solely as a preheater for the feed water, and then to a Liebig condenser C. The weight of the mantle, flask and water is counter-balanced by weight W, approximately 6 lb.

An increase in volume of water in the evaporating flask will cause that side of the board A to move downward and the opposite side upward. As water evaporates, the flask will become lighter and the movement is reversed. These movements are utilized through lever L and connected rods to close and open stopcock S. A small tension spring T dampens the movement and helps establish equilibrium.

Lever L is made from 18-gauge sheet metal, one arm 2 in., the other arm 4 in. long. An axle, made by cutting off the head of a 2-in. nail and soldering the nail through a hole in the lever, is sleeved with short pieces of copper tubing. Two saddle clamps secure the axle under board B so that the longer arm projects through a slot, approximately $\frac{1}{4}$ in. wide and $1\frac{3}{4}$ in. long, which has been cut into the board $1\frac{1}{4}$ in. from its outer edge. The shorter arm of the lever is attached to board A by a rod approximately 3 in. long. The longer arm is connected to stopcock S by two linked rods, so that the oblique movement of the longer rod (attached to lever L) is changed into a horizontal movement in the shorter rod, which is guided by a screw-eye K to prevent sideways movement.

An extension arm $1\frac{1}{2}$ in. long, made of wire, is fitted to the stopcock by means of a thin sheet-metal clamp bent around the stopcock and secured with a suitable adhesive. An eyelet at the end of this extension arm links the stopcock via the two rods to the lever L. All connecting rods are made from 12 S.W.G. galvanized wire, formed into eyelets where required.

In order to overcome the vapour pressure in the preheater and feed line, a head of at least 4 ft is necessary, and the carboy R used as a feed reservoir in this laboratory is placed on a shelf 4 ft above the still. This carboy is

conveniently filled from floor level by connecting it to a vacuum pump, and utilizing the difference in pressure to fill it with water, previously distilled in a metal still. By this double distillation, water of low conductivity is obtained.

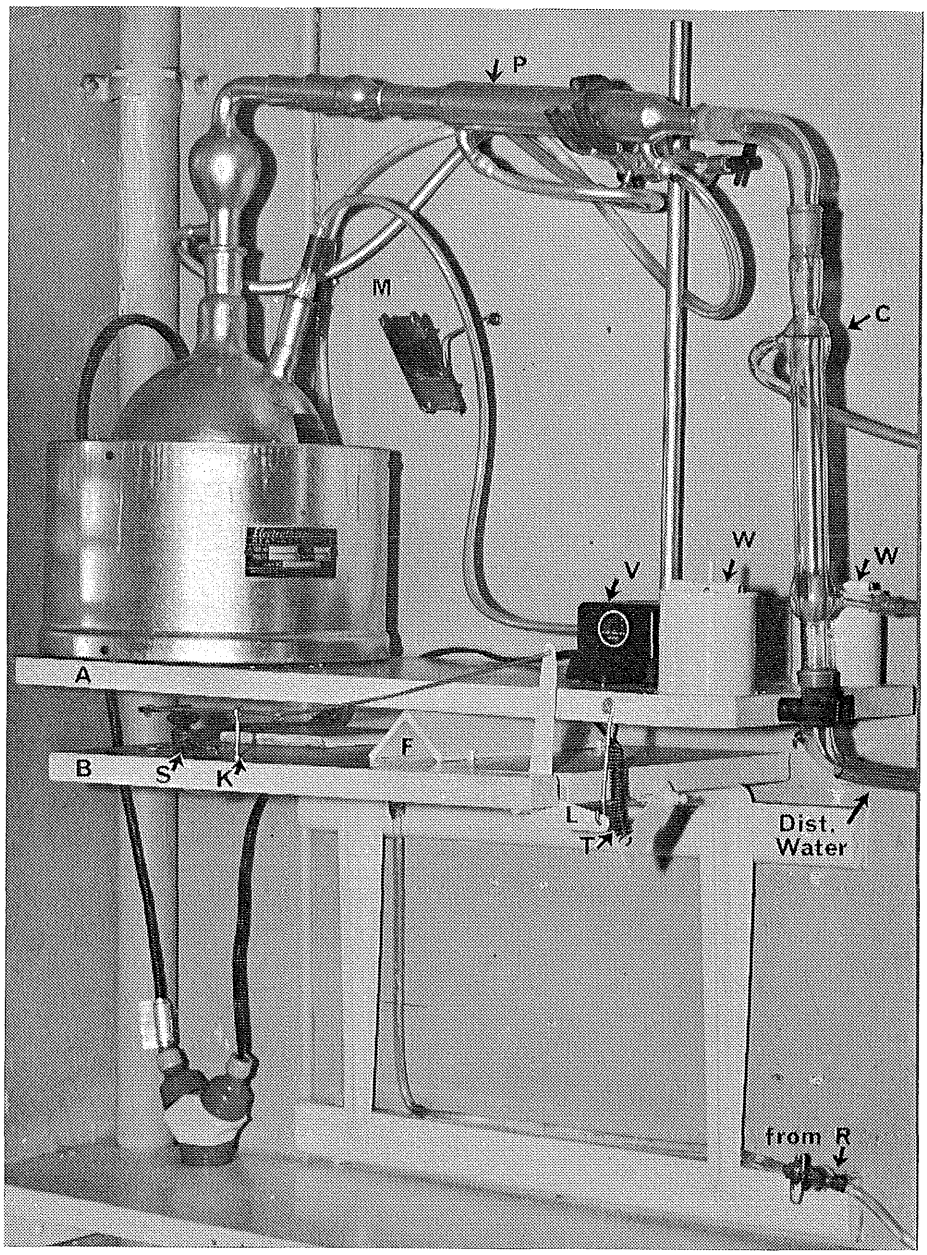

Fig. 1.—Details of still unit.

To prevent superheating and bumping in the evaporating vessel, a small vibrator pump V, such as is commonly used to aerate aquaria, passes a stream of fine air bubbles through the water from a sintered-glass gas diffuser fitted into the second neck of the boiling vessel. This method appears to be the most durable and satisfactory. Other boiling aids were tried, but were found to require frequent replacement.

By using a heating mantle of 500 w capacity and applying an insulating coat of aluminium paint to the evaporating flask and preheater, an output of approximately 650 ml per hr can be expected. The water level may be observed through a window in the paint, and a small mirror M at an appropriate angle facilitates this observation if the apparatus has to be installed above eye level.

A bench-model may be constructed by fixing suitable supports under base board B so that sufficient clearance is maintained to operate lever L. Such a model can easily be assembled and dismantled for irregular use.

To convert this still to automatic operation, a device as described by Joliffe (1963) may be incorporated in the feed reservoir or receiving vessel as required.

Valuable help and advice from Messrs. G. G. Crittall and K. Scott is gratefully acknowledged.

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(Received for publication January 3, 1964)

MOLAR PROGRESSION AND MACROPOD AGE

In the Macropodidae the changing position with age of the molars in both jaws relative to the remainder of the skull has been described qualitatively by several authors, including Thomas (1888), Tate (1948) and Troughton (1954).

Twenty-six grey kangaroos (*Macropus major* Shaw) of both sexes and known ages up to 33 months were sacrificed, and this "molar progression" was

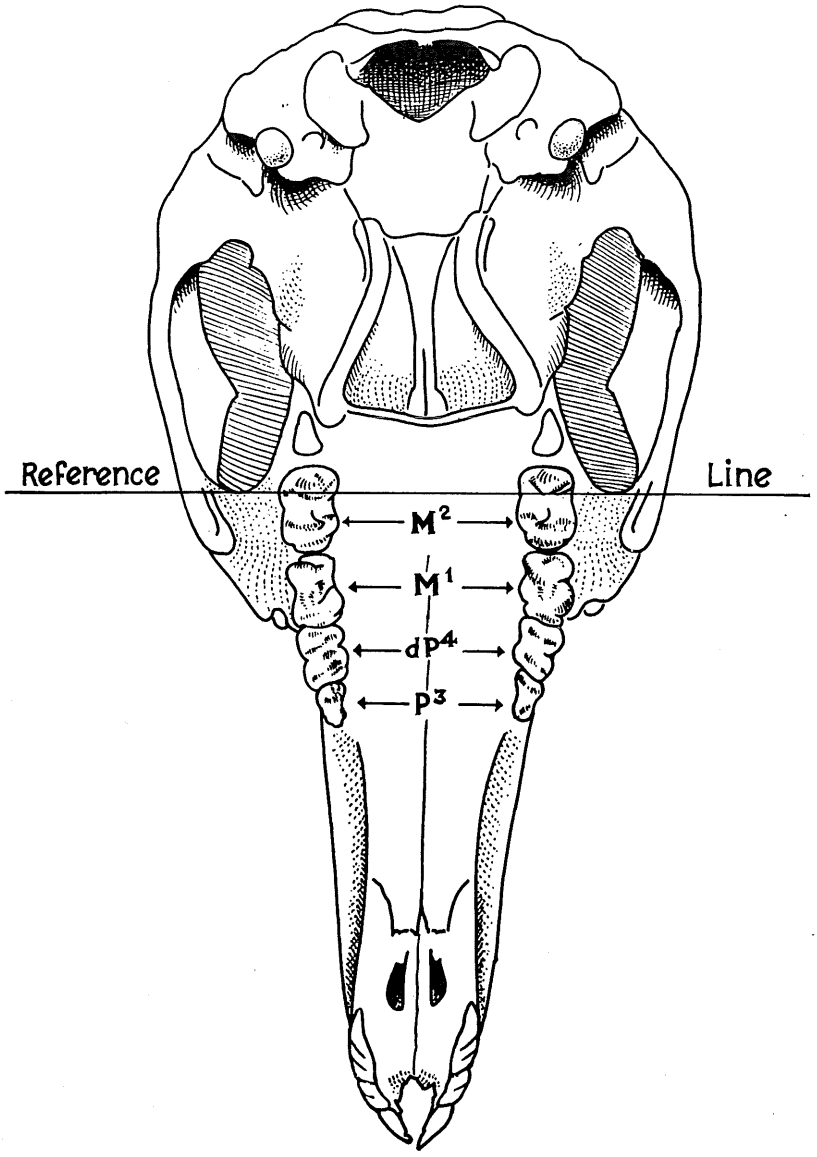

Fig. 1.—A grey kangaroo skull with a molar index of 1·7.

measured from the cleaned skulls as follows: A perspex plate etched with finely ruled lines scaled to 1/20 the length of a molar was placed over a skull held in palatal view, the palate at 90° to the line of vision. A reference line, tangential to the anterior rims of the orbits, was used. Both molar rows were considered: measurements were usually identical but where differences occurred an average was taken. A molar index (M.I.) of 2·05 would mean that the 1st and 2nd molars and 1/20 the length of the 3rd molar were anterior to the reference line. The M.I. of the skull in Figure 1 is 1·7.

The regression equation relating age to molar index in these animals was:

$$\text{Age (days)} = 219{\cdot}1 + 378{\cdot}0 \text{ (M.I.)}.$$
$$r = {\cdot}9982$$
$$\text{s.e. est.} = \pm 11{\cdot}8 \text{ days}.$$

Using additional skulls, this age criterion has been found sound for subadults (i.e. during the 2nd and 3rd years of life) of both sexes, and has also been confirmed by molar indices (Figure 2) from radiographs of live animals.

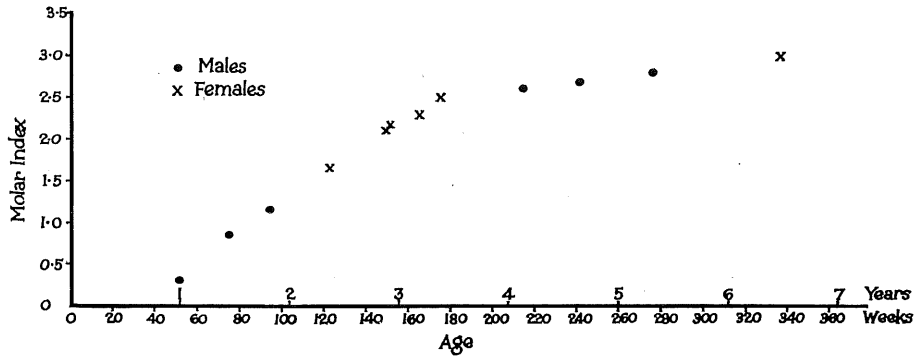

Fig. 2.—Molar indices from radiographs of live grey kangaroos plotted against known age.

The extension of this work to adults has been limited by the availability of suitable animals of known age. Figure 2, however, does illustrate what may be expected. The change of direction of the curve at the threshhold of adulthood is noteworthy. The molar index is a qualitative measure and there is no difference between sexes. Other age criteria, based on absolute measurements to be published later, diverge for sexes at an early age; these curves also tend to level out during the fourth year.

Molar indices beyond 4·0 are possible by presuming a fifth molar of equal length to the fourth; in fact, such a molar was present in four out of every 1000 skulls from a random field sample. The highest M.I. recorded from the field was 4·8; on present knowledge this animal was in its 16th–18th year.

Data on molar progression from prepared skulls and radiographs of the brush wallaby (*Wallabia rufogrisea* Desmarest), a wallaroo (*Osphranter robustus* Gould) and the red kangaroo (*Megaleia rufa* Desmarest) indicate that M.I. will be a useful indication of age in these species also.

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(Received for publication January 6, 1964)

S. G. REID, Government Printer, Brisbane