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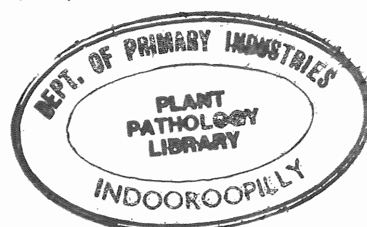

Chemical control of the tomato russet mite on tomatoes in the dry tropics of Queensland

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Abstract

Seven trials were conducted from 1982 to 1985 to test the efficacy of 14 acaricides in controlling the tomato russet mite, *Aculops lycopersici* (Massee), on tomatoes in north Queensland.

The most effective acaricides (dose rate in g a.i./ha) in controlling an established infestation of *A. lycopersici* were dicofol (500), SLJ 0312 (500), cyhexatin (200), azocyclotin (200), sulprofos (720), and monocrotophos (400, 600). Fenbutatin oxide (220) was moderately effective but demeton-S-methyl (275), dimethoate (300), DPX 3792 (300), endosulfan (735), methamidophos (1102), propargite (300), and sulphur (3000) were ineffective.

Weekly or fortnightly applications of an effective acaricide were necessary to prevent a damaging infestation of *A. lycopersici* from developing. Three-weekly or monthly applications were not sufficient. Dicofol (500) and cyhexatin (200) were the most effective preventative treatments, and sulprofos (720) and monocrotophos (400) were also effective. Sulphur (3000) was ineffective.

INTRODUCTION

The tomato russet mite, *Aculops lycopersici* (Massee), damages the foliage, stems, and fruits of tomato plants and can eventually kill the plants.

A. lycopersici was an important and common pest of tomatoes in Queensland before 1970 (Sloan 1938; Smith and Saunders 1956). During the decade to 1980 the incidence of *A. lycopersici* declined in commercial tomato plantings, and Smith (1977) attributed this decline to the use of dithiocarbamate fungicides such as maneb and propineb in disease control schedules on tomatoes. It is probable that the use of organophosphorous insecticides such as methamidophos for *Heliothis* spp. control also helped to suppress the mite.

Since 1980 the incidence of *A. lycopersici* infestations on tomatoes has increased despite the continued use of dithiocarbamate fungicides and methamidophos. In north Queensland infestations are most severe during the peak production months from July to November and specific control measures are essential to prevent severe damage to tomato crops.

Smith and Saunders (1956), in the most recently published trial work in Australia, found sulphur and parathion effective against *A. lycopersici* at Bowen. Demeton-S-methyl, dicofol, and sulphur were recommended to control the mite in Queensland (Anon. 1979).

The re-emergence of *A. lycopersici* as a serious pest and the apparent ineffectiveness of some of the recommended acaricides made it essential to reappraise control measures against the mite. This paper reports the results of a series of trials carried out from 1982

to 1985 in the Bowen and Ayr districts of north Queensland. The trials investigated the efficacy of a range of acaricides in controlling *A. lycopersici*, and the frequency of treatment necessary to prevent damaging mite infestations from developing.

MATERIALS AND METHODS

Seven trials were conducted. Trials 1 to 5 were carried out from August to November 1982, Trial 6 from August to October 1983, and Trial 7 from September to November 1985.

The tomato cultivar Flora-Dade was used in Trials 1 to 6, and a breeding line, 9-2-7-4, similar to Flora-Dade and to cultivar Delta Contender (D. McGrath, pers. comm. 1985) was used in Trial 7. In all the trials the tomatoes were grown as irrigated ground crops.

All trials were randomised block designs with four replicates. Chemical treatments were applied in 1000 L/ha of water using a Rega pneumatic sprayer fitted with a single hollow cone nozzle and operated at 200 to 250 kPa.

Acaricides used were:

azocyclotin	250 g/kg	wettable powder
cyhexatin	500 g/kg	wettable powder
demeton-S-methyl	250 g/L	emulsifiable concentrate
dicofol	240 g/L	emulsifiable concentrate
dimethoate	300 g/L	emulsifiable concentrate
*DPX 3792	250 g/kg	wettable powder
endosulfan	350 g/L	emulsifiable concentrate
fenbutatin oxide	550 g/L	suspension concentrate
methamidophos	580 g/L	emulsifiable concentrate
monocrotophos	400 g/L	emulsifiable concentrate
propargite	300 g/kg	wettable powder
sulphur	800 g/kg	wettable sulphur
sulprofos	720 g/L	emulsifiable concentrate
*SLJ 0312	500 g/kg	wettable powder

(* experimental chemicals)

Trials 1 to 4

These trials were done to quickly screen 14 potential acaricides in commercial tomato crops heavily infested with *A. lycopersici* and showing obvious damage. Dicofol was included in each trial as a standard treatment. Rates of chemical commonly used to control mites were used. Plot size was one row by 5 m. *A. lycopersici* numbers were counted at 1 day pre-treatment and at 3, 5 and 8 days post-treatment, except in Trial 1 in which one post-treatment count was done at 5 days.

Trial 5

In Trial 5 a heavily infested tomato crop was sprayed twice (on day 0 and day 6) with the seven most promising chemicals from Trials 1 to 4. Mites were counted on day −1, day 5, day 11, and day 14. Plot size was one row by 5 m.

Trial 6

Trial 6 investigated the frequency of acaricide application required to prevent an infestation of *A. lycopersici* from developing to damaging levels. Acaricide treatments, which began one week after seedlings were transplanted to the field, were weekly, fortnightly or monthly applications of dicofol, or three-weekly applications of sulprofos. Plot size was two rows by 10 m and plots were separated by single untreated guard rows. The whole trial area was sprayed at weekly intervals with permethrin to control *Heliothis* spp. and with mancozeb for disease control.

A. lycopersici numbers were counted at five weeks and nine weeks after field planting.

Tomatoes were harvested from 5 m of each row per plot; that is, 10m per plot, in two picks. Coloured and green-mature fruit were harvested in the first pick nine weeks after field planting, and all remaining fruit were harvested a week later. Fruit were counted and weighed, and results from the two picks were bulked for analysis. Fruit quality was not assessed.

Trial 7

In Trial 7 the efficacy of cyhexatin, dicofol, monocrotophos, sulphur and sulprofos as preventative treatments was investigated. Sulphur was included here as it is a commonly used miticide and it was deemed necessary to test its prophylactic properties despite its ineffectiveness in Trial 3. The acaricides were applied at fortnightly intervals starting one week after seedlings were transplanted into the field, and a total of five applications were made. Plot size was one row by 10 m. The whole trial was sprayed at weekly intervals with permethrin for *Heliothis* spp. control and mancozeb for disease control.

A. lycopersici numbers were counted at five, seven and nine weeks after field planting.

At nine weeks post-planting the lower stems of plants in each plot were examined for symptoms of *A. lycopersici* damage (lack of hairs and russetting of stem) and the plot was rated from 0 = no damage to 5 = severe damage.

Tomatoes were harvested from 6 m of row per plot in three picks, each separated by a week, starting ten weeks after field planting. Coloured and green-mature fruit were harvested in the first two picks, and all remaining fruit were harvested in the final pick. Fruit were counted and weighed, and the data from the three picks were bulked for analysis. Fruit quality was not assessed.

Numbers of *A. lycopersici*

The method of counting *A. lycopersici* was the same for each trial. Ten leaf discs (each 29 mm^2) per plot were punched from leaves near the base of plants if damage was not obvious, or from just above obvious plant damage. The leaf discs were placed with the underside of the leaf upwards in a holding card which was then wrapped in Glad Wrap® to prevent desiccation. The leaf punch and holding cards were similar to those described by Hoffman *et al.* (1970) for collecting and holding lepidopterous eggs. The cards were taken to the laboratory and the numbers of living *A. lycopersici* adults and nymphs on the leaf discs were counted with the aid of a Wild M8 stereomicroscope at 40× magnification. Counts for the 10 discs per plot were bulked. Mites on the underside only of leaves were counted as preliminary work had shown much higher numbers on the underside than on the topside of leaves.

Statistical analysis

Analyses of variance were used to test for treatment differences. If significant treatment differences were detected ($P<0.05$), pairwise comparisons were made using Student's

t-test. A logarithmic transformation was used on the mite count data before analysis. Pre-treatment mite count was used as a covariate in the analyses of post-treatment counts for Trials 1 to 5. The results of the covariance analysis were used only if the covariate was significant. Only back transformed means and the coefficient of variation from the analysis of the transformed data are presented in each table.

RESULTS AND DISCUSSION

Trials 1 to 4

The results of these trials (Table 1) allowed separation of the 14 miticides into those that were effective against *A. lycopersici* (cyhexatin, dicofol, monocrotophos at both rates, and sulprofos) and those for which the evidence was inconclusive (azocyclotin and SLJ 0312) or that were ineffective (fenbutatin oxide, dimethoate, demeton-S-methyl, DPX 3792, endosulfan, methamidophos, propargite, and sulphur).

The lack of effectiveness of methamidophos, propargite and sulphur in these trials confirmed the experience of commercial growers who reported poor results after using them. Abou-Awad and El Banhawy (1985) reported that *A. lycopersici* in Egypt had developed resistance to methamidophos which had been used for its control. The failure of methamidophos to control *A. lycopersici* in Trial 4, and in commercial situations, raises the possibility that the mite has developed resistance to the chemical in north Queensland.

Demeton-S-methyl, which had been recommended for *A. lycopersici* control (Anon. 1979) was ineffective in Trial 2 and in a subsequent small field test (I. R. Kay, unpub. data 1982).

The results of Trials 1 to 4 demonstrated that a single application of even the most effective acaricides was not sufficient to completely control an established infestation of *A. lycopersici*. Survivors and newly hatched nymphs meant unacceptable numbers of mites remained.

Trial 5

All chemicals caused some reduction in mite numbers compared to the untreated check after the first application, although the difference was not significant ($P>0.05$) in some cases and numbers in the fenbutatin oxide treatment actually increased (Table 2). After the second application all the chemical treatments, except fenbutatin oxide on day 11 had significantly fewer ($P<0.05$) mites than the untreated check. The second application of all chemicals provided improved control, except for monocrotophos where the numbers remained almost constant.

Dicofol, SLJ 0312, and cyhexatin were the most effective treatments against *A. lycopersici* after two applications. Azocyclotin performed better than in the previous trials. Sulprofos and monocrotophos gave reasonable control of *A. lycopersici* in this trial. Both these chemicals (monocrotophos at 1000 g a.i./ha) are effective in controlling *Heliothis* spp. on tomatoes (Kay 1983), and their use in a *Heliothis* control spray programme may obviate the need to apply specific acaricides to control *A. lycopersici.*

Trial 6

A. lycopersici numbers were low at five weeks post-planting but numbers were high and damage obvious at nine weeks post-planting. Numbers of *A. lycopersici* decreased significantly ($P<0.05$) with increasing frequency of acaricide application (Table 3). Control provided by weekly and fortnightly applications was good, but three-weekly and monthly applications allowed the mite population to increase.

Table 1. The effect of acaricide treatments on numbers of *A. lycopersici* in Trials 1 to 4

Treatment (g a.i./ha)	Mean number* of *A. lycopersici*			
	Pre-treatment	Day 3	Day 5	Day 8
Trial 1				
Untreated check	381*a*†	n.a.	514*a*	n.a.
Dicofol (500)	437*a*	n.a.	164*b*	n.a.
Sulprofos (720)	279*a*	n.a.	117*b*	n.a.
Cyhexatin (200)	287*a*	n.a.	150*b*	n.a.
Endosulfan (735)	340*a*	n.a.	205*b*	n.a.
CV‡	7.2	n.a.	10.9	n.a.
Trial 2				
Untreated check	98*a*	82*ab*§	151*ab*§	154*ab*
Dicofol (500)	93*a*	44*b*	96*b*	83*b*
Demeton-S-methyl (275)	101*a*	90*ab*	152*ab*	176*a*
Monocrotophos (600)	123*a*	6*c*	7*c*	5*c*
Azocyclotin (200)	100*a*	63*ab*	99*b*	89*b*
Propargite (300)	69*a*	116*a*	220*a*	192*a*
CV	9.9	12.2	8.3	10.5
Trial 3				
Untreated check	58*a*	72*a*§	146*a*	144*ab*
Dicofol (500)	56*a*	24*cd*	65*bc*	55*c*
Dimethoate (300)	54*a*	56*ab*	76*bc*	116*ab*
Monocrotophos (400)	75*a*	14*d*	20*d*	16*d*
Fenbutatin oxide (220)	52*a*	68*a*	57*c*	108*ab*
Cyhexatin (200)	52*a*	30*bc*	52*c*	51*c*
DPX 3792 (300)	73*a*	61*a*	108*ab*	206*a*
SLJ 0312 (500)	60*a*	58*ab*	71bc	85*bc*
Sulphur (3000)	71*a*	51*ab*	90*abc*	125*ab*
CV	7.4	12.2	9.2	10.3
Trial 4				
Untreated check	137*a*	170*a*	213*a*	337*a*
Dicofol (500)	135*a*	92*bcd*	81*cd*	72*c*
Sulprofos (720)	97*a*	57*d*	64*d*	72*c*
Endosulfan (735)	117*a*	172*a*	167*ab*	247*a*
Methamidophos (1102)	142*a*	129*ab*	176*a*	243*a*
SLJ 0312 (500)	129*a*	110*ac*	98*bcd*	106*bc*
Azocyclotin (200)	120*a*	78*cd*	126*ac*	110*b*
CV	5.0	7.2	6.5	5.4

* Back transformed means after $\log_e$ transformation.

† For each trial, in each column treatments not followed by the same letter are significantly different ($P<0.05$).

‡ Coefficient of variation of transformed data.

§ Covariate corrected means using pre-treatment count as covariate.

n.a. = not available.

The adverse effect of *A. lycopersici* on yield is demonstrated by the reduction in yield in the unsprayed check compared with any of the acaricide treatments. The monthly dicofol treatment yielded less than the weekly or fortnightly treatments. The three-weekly sulprofos treatment also had a significantly higher ($P<0.05$) yield than the monthly dicofol treatment, but it was not significantly different from the more frequent dicofol treatments. Improved control of *Heliothis* spp. provided by the sulprofos may have contributed to the high yield in this treatment despite the build up of mites.

Table 2. The effect of acaricide treatments on numbers of *A. lycopersici* in Trial 5. Treatments were applied on day 0 and day 6

Treatment (g a.i./ha)	Mean number* of *A. lycopersici*			
	Day−1	Day 5	Day 11	Day 14
Untreated check	165*a*†	310*a*	268*a*	281*a*
Fenbutatin oxide (220)	148*a*	211*ab*	111*ab*	77*b*
Monocrotophos (400)	214*a*	80*c*	51*bc*	64*b*
Sulprofos (720)	215*a*	204*ab*	47*bc*	38*bc*
Azocyclotin (200)	214*a*	163*ac*	38*bc*	15*cd*
Cyhexatin (200)	174*a*	92*c*	21*cd*	9*de*
SLJ 0312 (500)	225*a*	145*bc*	11*d*	5*de*
Dicofol (500)	189*a*	159*ac*	10*d*	4*e*
CV‡	6.1	9.8	20.6	25.8

* Back transformed means after $\log_e$ transformation.

† In each column treatments not followed by the same letter are significantly different ($P<0.05$).

‡ Coefficient of variation of transformed data.

Table 3. The effect of frequency of acaricide treatment on *A. lycopersici* numbers and tomato yield in Trial 6

Treatment (g a.i./ha)	Number of sprays applied	Mean number of *A. lycopersici*		Mean yield of tomatoes	
		5 weeks post-plant	9 weeks* post-plant	Weight (kg)	Number of fruit
Untreated check	0	0 (†)	284*a* (−)‡	27.3*a*	244*a*
Dicofol weekly (500)	9	0 (1)	1*e* (1)	40.1*c*	385*c*
Dicofol fortnightly (500)	5	0 (2)	5*d* (2)	38.0*c*	339*bc*
Dicofol monthly (500)	3	0.3 (4)	75*b* (4)	32.4*b*	313*b*
Sulprofos 3-weekly (720)	3	0 (1)	31*c* (2)	39.8*c*	378*c*
CV§		n.a.	14.6	10.1	12.4

* Back transformed means after $\log_e (x + 1)$ transformation.

† Weeks since last spray.

‡ In each column treatments not followed by the same letter are significantly different ($P<0.05$).

§ Coefficient of variation.

n.a. = Not analysed.

Trial 7

Numbers of *A. lycopersici* were low in all treatments at five and seven weeks post-planting and the counts were not analysed. Mite numbers had increased at nine weeks post-planting (Table 4) and plant damage was conspicuous in the field.

Sulphur was not effective as a preventative treatment, allowing mite numbers to increase and damage to occur. These results, coupled with those recorded in Trial 3, show that it is no longer effective against *A. lycopersici*. Sulprofos and monocrotophos adequately protected the plants from *A. lycopersici*, and dicofol and cyhexatin gave excellent control.

No significant differences in yield between the treatments were recorded. The harvest data show that over half of the fruit from the check and sulphur treated plots were harvested in the first two picks compared to between 27% and 39% for the other treatments. Since heavy rain fell between the second and third picks and caused loss of fruit due to rotting it is likely that the loss due to rotting was higher in the treatments with better

mite control. Hence it is likely that significant yield increases due to improved mite control were masked by the fruit loss due to rotting. Although fruit quality was not assessed it was obvious that many of the fruit in the untreated check and sulphur treatments were blotchy because of mite damage, and sunburnt because of defoliation resulting from mite damage to the leaves.

Table 4. The effect of acaricide treatments on *A. lycopersici* numbers, stem symptom rating, and tomato yield in Trial 7

Treatment (g a.i./ha)	Mean number of *A. lycopersici*			Stem symptom rating	Mean yield of tomatoes	
	5 weeks post-plant	7 weeks post-plant	9 weeks* post-plant		Weight (kg)	Number of fruit
Untreated check	0	1.5	63.2*a*†	4.6*a*	30.6*a*	254*a*
Sulphur (3000)	0	3.5	27.2*a*	3.9*a*	31.3*a*	267*a*
Sulprofos (720)	0.3	0	5.9*b*	2.6*b*	41.9*a*	345*a*
Monocrotophos (400)	0	0	5.5*b*	2.9*b*	37.9*a*	326*a*
Cyhexatin (200)	0	0	0.6*c*	0.5*c*	40.1*a*	345*a*
Dicofol (500)	0.3	0	0.4*c*	1.1*c*	36.6*a*	318*a*
CV‡	n.a.	n.a.	34.9	23.2	22.6	23.3

* Back transformed means after $\log_e (x + 1)$ transformation.

† In each column treatments not followed by the same letter are significantly different ($P<0.05$).

‡ Coefficient of variation.

n.a. = not analysed.

Based on the results of these seven trials, recommendations have been made for the control of *A. lycopersici* on tomatoes. Dicofol is recommended to control (with two applications) or to prevent (with fortnightly applications) an infestation of the mite. Alternatively, the inclusion of sulprofos or monocrotophos, at least fortnightly, in a spray programme against *Heliothis* spp. is suggested to prevent the build up of damaging mite populations.

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Insecticidal control of white grubs (Coleoptera:Scarabaeidae) on the Atherton Tableland, with observations on crop losses

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Abstract

Eight chemical treatments were tested against large white grubs (*Lepidiota* spp.) in maize and peanuts on the Atherton Tableland, Queensland. Terbufos at 2 and 4 kg a.c./ha increased yield by up to 0.985 t/ha in maize and significantly decreased grub populations. In the same experiment EDB-treated areas had significantly fewer white grubs than the untreated plots. In peanuts, terbufos and phorate (both at 2 kg a.c./ha) reduced white grub populations 49 days after application but there were no differences in either crop yield or white grub populations at harvest. Chlorpyrifos was ineffective applied as either an emulsifiable concentrate or granules. The relationship between white grub numbers at harvest and peanut yield was linear, with one white grub per 3 m of row reducing nut in shell yield by 12 g. In a second trial in peanuts, where all chemicals were used at 3 kg a.c./kg, ethoprophos, fensulfothion, isofenphos, phorate and terbufos significantly reduced grub numbers compared to the untreated plots but aldicarb did not.

INTRODUCTION

The role of white grubs of the genus *Lepidiota* in pasture deterioration on the Atherton Tableland of north Queensland has been well documented (Atherton 1931, 1939; Smith 1936; Saunders 1958). Other species such as *Dasygnathus dejeani* Macleay (*D. australis* Boisduval of Atherton, 1931) also occur. Although primarily pasture pests, white grubs sporadically attack maize, peanuts and potatoes (Crosthwaite 1983 and present study). Despite the serious losses which may occur there is no information on either their control or the damage they cause.

White grubs are a perennial problem in sugar cane (Wilson 1969*a*; Hitchcock 1974) where traditionally they have been controlled by BHC (Buzzacott 1948; Mungomery 1948, 1949) and other organochlorines (Wilson 1969*b*). Recent studies on sugar cane have replaced these persistent chemicals with controlled release formulations of chlorpyrifos for long-term control (Hitchcock *et al.* 1984) and fensulfothion and ethoprophos for short-term control on *Lepidiota* spp. (B.E. Hitchcock, pers. comm. 1983). Insecticide trials against white grubs in sweet potato and maize were reported by Rolston and Barlow (1980) and McBride (1984) in the USA, by Ram and Yadava (1982) in peanuts in India and by Stewart (1984) in pasture in New Zealand.

Our aim was to evaluate insecticides for white grub control in field crops and, where possible, attempt an assessment of crop losses. Identification of the white grubs was difficult as they could not be bred to adults and circumstantial evidence is presented as to the identity of the larvae.

MATERIALS AND METHODS

Because of the sporadic nature of white grub attack trials could be set up only when opportunities arose. When signs of attack occurred early in a crop it was either ploughed

out and replanted (with chemical treatments) or insecticides were applied to the existing planting.

Some *Lepidiota* spp. have a two-year life cycle and thus large one-year-old white grubs (third instar) may be present when the crop is planted. Trials on such large larvae (probably *L. laevis* Arrow) were undertaken in peanuts (1979) and maize (1981) on a farm east of Atherton. In 1982 second instar *Lepidiota* larvae caused lodging in maize on a farm between Tolga and Rocky Creek; between Atherton and Mareeba. The soil type on both farms was a krasnozem formed on basalt. A trial was also conducted on large *Lepidiota* larvae attacking peanuts in a deep fine sand (Mulligan fine sand, McDonald 1976) near Dimbulah in 1982.

Peanut trial, Atherton, 1979

The trial was laid down in a heavily infested crop of Virginia Bunch peanuts on 9 February 1979 when the crop was six weeks old. The design was a randomised block with four chemical treatments and a control (Table 1) replicated five times. To overcome patchiness of the infestation, plots were assigned to the different blocks according to the number of white grubs in a pretreatment count. Each plot consisted of 3 rows each 3.5 m long. Plots were separated laterally by one guard row and along rows by 1.5 m between adjacent plots. At the pretreatment count a trench 0.5 m long × 0.25 m × 0.25 m was dug from the end of each of the three datum rows in each plot. The white grubs were unearthed and counted.

Chemicals (Table 1) were applied by digging trenches 100 mm wide and 100 mm deep on either side of rows and as close as possible to the plants without damaging them. The required quantities of granules or emulsifiable concentrate were sprinkled or sprayed into the trenches which were then refilled with soil. The amount of chemical per metre was calculated on 11 000 m of row per hectare.

The first assessment was carried out 49 days after treatment. Trenches 1 m long × 0.25 m × 0.25 m were dug at the end of each of the datum rows. White grubs were counted and the immature peanuts removed and their dry weight determined. At final harvest (14 May 1979), a further metre of trench 0.28 m × 0.3 m was dug in each datum row leaving 0.5 m of undisturbed row on either side. The numbers of white grubs and plants were counted and the yield of sun dried nut in shell determined for each plot. Despite precautions in setting up the trial, there was large plot to plot variation within blocks in the number of white grubs at final assessment and in yield of nut in shell. An examination of the relationship between white grub density per plot and yield was therefore undertaken using regression analysis. For each of the five treatments the slopes and intercepts of the lines relating the two variables were compared. As there were no significant differences data were pooled and a common regression equation calculated.

Maize trial, Atherton, 1981

In mid January 1981 severe white grub damage appeared in 20 ha of maize which had been planted in mid December 1980. The maize was ploughed out and the trial was established in the area at replanting. Three chemical treatments (Tables 2 and 3) and a control were laid out in a randomised block with five replicates. Plots were eight rows wide and 250 m long. Because of the required minimum two week waiting period between ethylene dibromide (EDB) application and planting and the lateness in the season, the controls and terbufos treatments were planted first on 2 and 3 February 1981.

A four row planter equipped with a Gandy granule applicator was used to plant the maize (QK230) in 0.81 m rows with a 100 mm granule band beneath the soil near the

seed. EDB 193 was applied at the rate of 15 L/ha mixed with 76 L/ha of water using a simple gravity fed applicator mounted behind a tractor. The applicator delivered the EDB into the soil behind six tynes and the soil was levelled after application. EDB was applied on 6 February 1981 and the maize was planted in the EDB treated plots on 20 February. Urea (46% N) was applied at 185 kg/ha to all plots in early March.

White grub density and plant size were determined 50 days after planting in the terbufos and untreated plots. Larval density was also determined in the EDB treated plots but no data on plant growth or on final yield was collected as the plants were 18 days younger than those in the rest of the trial. In the central four rows of each plot five 10 m lengths of row were chosen at random. The height of five plants selected randomly and the number of plants per 10 m of row were determined. A 2.5 m length of row was chosen at random from within the 10 m lengths and four plants complete with roots were removed. The plants were dried at 80°C for 3 days and weighed. A trench 2.5 m long, 0.3 m deep and 0.3 m wide was dug in each 10 m length and the number of white grubs counted. Cobs from three 10 m lengths of row were harvested from the centre of each plot in early July 1981 and the weight of grain at 14% moisture content recorded. The number of plants and the number of sterile plants in each 10 m length of row was also determined.

Peanut trial, Dimbulah, 1982

The crop of Virginia Bunch peanuts in which this trial was conducted was planted in December 1981 in land only recently prepared from Rhodes grass pasture. Within six weeks, damage by large white grubs was apparent. An experiment with six replicates and six chemical treatments (Table 4) was established in a randomised block design. The granules were sprinkled on the soil surface and covered with a light layer of sandy soil. Plots were three rows wide and 5 m long and the central metre of the central row was sampled 54 days after treatment. There was no assessment of final yield.

Observations in lodged maize, Tolga, 1982

In May 1982 widespread lodging occurred in six circular areas, each of 2 to 4 ha, in a 30 hectare field of mature QK657 maize. Within these areas all the plants were lodged. Examination of lodged plants showed numbers of second instar white grubs and damaged root systems. White grub numbers on lodged plants and adjacent unlodged plants were compared by sampling beneath 15 randomly selected plants in each area. A further 15 plants were examined for white grubs in an adjacent upright stand of well grown maize. To estimate yields, five lengths of row each five metres long were chosen at random in each of the three areas. All cobs were collected and shelled and the weight of grain at 14% moisture recorded. In addition 20 cobs were selected at random in one area of lodged plants and in one area of standing maize and the weights of the whole cobs recorded.

RESULTS

Peanut trial, Atherton, 1979

Granular formulations of phorate and terbufos significantly ($P<0.05$) reduced white grub populations compared with the untreated control 49 days after application. Both the granular formulation and the emulsifiable concentrate of chlorpyrifos were ineffective (Table 1). The mean dry weights of immature peanuts and the mean numbers of plants per metre at this time did not differ significantly (data not presented). Although the trend in white grub numbers at harvest followed that above, differences in populations were not significant at $P<0.05$ (Table 1). The ranges of larval density recorded indicates extreme variability in populations. There were also no significant differences in yields or in plant density. The ranges of yields per plot within treatments are also extreme, two fold variations being common (Table 1).

Table 1. Effect of insecticides on numbers of white grubs (*Lepidiota* sp. probably *laevis*), plant populations and peanut yield as nut in shell, Atherton, 1979

Insecticide formulation and treatment	Mean no. grubs/m 49 days after treatment	Mean no. grubs/m (range) at harvest	Mean field g/m (range) at harvest	Mean no. plant/m at harvest
Phorate (100 g/kg gran) 2 kg a.c./ha	2.7	3.2 (1.0–6.0)	199.6 (149.8–246.5)	10.7
Terbufos (100 g/kg gran) 2 kg a.c./ha	3.1	4.1 (2.7–5.3)	183.5 (150.7–252.5)	10.2
Chlorpyrifos (500 g/L e.c.) 2 kg a.c./ha	5.9	4.7 (1.7–8.0)	185.0 (134.6–271.2)	9.9
Chlorpyrifos (150 g/kg gran) 2 kg a.c./ha	6.3	7.0 (3.3–7.7)	152.5 (96.0–189.6)	9.6
Control (untreated)	6.3	5.6 (3.7–9.7)	173.5 (109.9–227.5)	8.9
LSD ($P = 0.05$)	2.9	n.s.	n.s.	n.s.

n.s. = not significant.

Regression lines relating yield to white grub numbers were calculated for each of the five treatments and the slopes and intercepts of the five lines compared. As there were no significant differences in either slopes or intercepts the data were pooled and a common regression calculated (Figure 1). Yield varied inversely with white grub numbers, the linear regression being: $y = 713.4 - 12.0\,x$ ($r = -0.75$, $n = 23$, $P<0.01$) where y is the weight of nut in shell per plot and x is the number of white grubs per plot (3 m of row) at harvest. An average density of one white grub per 3 m of row at harvest caused a yield loss of 44 kg/ha (assuming 11 000 m of row/ha).

As the larvae were present in the soil when the peanuts were planted it seems probable that the equation has some applicability as a predictive tool and future work could well aim at verifying this.

Maize trial, Atherton, 1981

Assessment 50 days after application showed that all three chemical treatments significantly ($P<0.05$) reduced white grub numbers (Table 2). Many recently killed larvae were recovered directly beneath maize plants in terbufos treated areas. In addition 36% of those white grubs recorded as alive from the terbufos treatments were moribund. The sampling trenches were wider than the chemically treated band and live white grubs were often found at the edge of the treated areas. Plants in the terbufos treated areas were significantly higher and heavier than those in the control. This advantage in plant growth continued to harvest when plots treated with terbufos at 2 kg a.c./ha outyielded the untreated controls by 0.985 t of grain/ha (Table 3). Although there were no differences in total plant populations between treatments there was a significant ($P<0.05$) decrease in the number of sterile plants in insecticide treated plots (2.5%) compared with untreated plots (9.1%) (Table 3).

There is no doubt that *Lepidiota* larvae can greatly reduce maize yields near Atherton but it seems unlikely that the number of larvae (1.34 per m) in the untreated area 50 days after planting could influence yield to the extent above. Although no pretreatment count was performed inspections before replanting revealed large numbers of white grubs. At sampling, diseased white grubs were recorded from the untreated areas and the survivors probably represented the tail of a larger population present when the crop was planted. Disease outbreaks were recorded among dense populations of white grubs on the Atherton Tableland by Smith (1937). Maize is planted sparsely at a density of 2.7 to 4.0 plants/m (I. C. Crosthwaite, pers. comm. 1987). In this trial it was grown at about three plants per

metre so that even a few white grubs as large as those sampled (1 to 3 g) could severely damage the plants early in the growing period. Many very small stunted plants (<0.20 m) were present in the untreated plots 50 days after planting, probably the result of severe white grub attack.

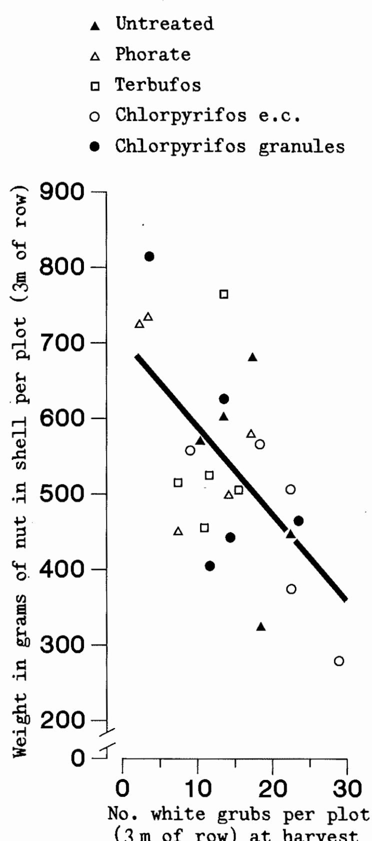

Figure 1. Relationship between white grub density at harvest and peanut yield at Atherton, 1979. Data are pooled from the following treatments: untreated, phorate, terbufos, chlorpyrifos e.c., chlorpyrifos granules at 2 kg a.c./ha.

Table 2. Effect of insecticides on number of white grubs (*Lepidiota* sp. probably *laevis*) and height and dry weight of maize plants, Atherton, 1981

Chemical formulation and treatment	Mean no. grubs/m 50 days after treatment	Mean plant height (mm) 50 days after planting	Mean plant dry weight (g) 50 days after planting
Terbufos (100 g/kg gran) 2 kg a.c./ha	0.32	751	41.6
Terbufos (100 g/kg gran) 4 kg a.c./ha	0.26	724	35.2
EBD (1930 g/L e.c.) 29 kg a.c./ha	0.33	n.a.	n.a.
Control (untreated)	1.34	520	19.7
LSD ($P = 0.01$)	0.56	49	14.9

n.a. = not available.

Conditions during this trial were particularly suitable for the action of the granular and fumigant chemicals. The soil was moist at application (18 mm of rain just before planting) and in the first month thereafter weekly rain falls of 80, 71, 101 and 73 mm were recorded. The tilth of the soil was fine and this, combined with high soil moisture, made an ideal seal to prevent the escape of EDB.

Table 3. Effect of insecticides on grain yield (at 14% moisture), number of sterile plants and total plant population in maize, Atherton, 1981

Insecticide formulation and treatment	Yield of grain (tonnes/ha)	No. sterile Plants/m	Plants/m
Terbufos (100 g/kg gran) 2 kg a.c./ha	3.822	0.060	2.98
Terbufos (100 g/kg gran) 4 kg a.c./ha	3.580	0.047	2.89
Control (untreated)	2.837	0.240	2.92
LSD (P=0.01)	0.634	0.15	n.s.

n.s.=not significant.

Peanut trial, Dimbulah, 1982

All chemicals except aldicarb significantly reduced numbers of white grubs (Table 4). Direct comparison with the Atherton trials is difficult because a different species of *Lepidiota* was involved and also the rate of chemical treatment was higher (3 kg a.c./ha *cf.* 2 kg a.c./ha). Nevertheless such good results were not obtained in the other trials reported here or on other trials on soil insects near Atherton (Gough and Brown, unpub. data 1979 to 1982). One possible explanation is that the chemicals were particularly effective in the Mulligan sands at Dimbulah. Nielsen and Boggs (1985) showed that soil insecticides were generally more toxic to first instar black vine weevil larvae in sand than in loam or muck, the LC_{50} increasing by about two to threefold in loam and up to 27 times in muck.

Table 4. Mean number of white grubs (*Lepidiota* sp.) in peanuts 54 days after application of granular insecticides at 3 kg a.c./ha, Dimbulah, 1982

Insecticide formulation	Mean no. white grubs/m
Ethoprophos (100 g/kg)	0
Fensulfothion (100 g/kg)	0
Isofenphos (50 g/kg)	0
Phorate (100 g/kg)	0
Terbufos (100 g/kg)	0.2
Aldicarb (150 g/kg)	1.7
Control (untreated)	1.2
LSD (P=0.05)	0.88

Observations in lodged maize, Tolga, 1982

The root systems of the lodged maize plants were attacked by significantly more white grubs than those of the upright plants (Table 5) and roots on the lodged plants were extensively pruned. This farm is exposed to the south east tradewinds and the reduction in the root system led to lodging in the windy period of mid May 1982. Lodging also occurs in sugar cane severely attacked by white grubs (Mungomery 1948).

Table 5. Comparison of numbers of second instar white grubs (*Lepidoptera* sp.) and grain yield (at 14% moisture) in one lodged and two upright stands of maize, Tolga, May 1982

Condition of crop	Mean no. white grubs/plant (±SE)	Mean wt (g) grain/5m of row (±SE)	Yield* tonnes/ha	Mean wt. (g) of whole cobs (±SE)
Upright—good stand	0.2±0.13	2400±175	5.28	n.s.
Upright—random	0.7±0.30	2092±144	4.61	233.1±11.9
Lodged—random	5.3±0.85	2057±80	4.53	246.0±10.9
LSD (P=0.05)	1.8	n.s.		n.s.

* Assuming 11 000 m of row/ha.

n.s.=not significant.

n.a.=not available.

Yield and cob size of the lodged plants were not reduced by the presence of larvae and the pruned roots (Table 5). This may be explained by the feeding habits of white grubs of which *L. frenchi* Blackburn is probably typical. The first instar lasts for about 60 days (Jarvis 1917) and during this time larvae subsist on organic matter rather than on living roots, as do many young white grubs (Wilson 1969*a*). That there was no reduction in yield before the maize reached physiological maturity at about 125 days after planting (Crosthwaite 1983) is therefore to be expected, as the larvae were generally too small to cause significant damage until the maize plants were nearly mature. Second instar larvae then caused damage to the root system as the plants senesced and harvest maturity was approached, resulting in lodging. No assessment of crop loss was made but the farmer experienced extreme difficulty in harvesting the lodged maize plants.

DISCUSSION

These data demonstrate that white grubs can cause substantial crop losses in areas of the Atherton Tableland. Experimental chemical control applied in line with commercial practice was successful using banded applications of terbufos at 2 kg a.c./ha. Rolston and Barlow (1980) and McBride (1984) also found terbufos to be effective against *Phyllophaga* spp. in the USA. Phorate, ethoprophos, fensulfothion and isofenphos showed promise, the latter three and terbufos also being effective against *Heteronyx* spp. (small white grubs with a one-year life-cycle) on peanuts in the South Burnett area of Queensland (D. J. Rogers and H. B. Brier, pers. comm. 1984). The failure of both formulations of chlorpyrifos was surprising as it has proved effective in slow release formulations against other *Lepidiota* larvae in sugar cane (Hitchcock *et al.* 1984). McBride (1984) found chlorpyrifos among the least effective chemicals in preventing stand losses in corn. Aldicarb was the only ineffective chemical in the Dimbulah trial and was also ineffective against larvae of white fringed weevil (*Graphognathus leucoloma* (Boheman) (Gough and Brown unpub. data). EDB has been used successfully against cicada nymphs attacking the roots of sugar cane (Chandler 1981).

As control is expensive, it is imperative that chemicals only be applied in areas where significant economic losses will occur. If the relationship between white grub density and yield in peanuts is correct, treatment would be warranted at, or above, average densities of about one larva per metre of row. In maize only very dense populations of white grubs may be worth treating.

Most damage is caused by one-year-old white grubs coming up from deeper in the soil to feed for a second year. The soil is often hard and dry during the fallow and sampling before planting may be difficult. However, it is possible to predict conditions

under which the likelihood of attack is high. Thus crops planted in hastily prepared areas immediately after pasture when one-year-old white grubs may already be present are at risk. Crops in certain local areas are commonly attacked so that individual farms, including two of those above, are at risk year after year. Such sites were immediately adjacent to large areas of pasture from which adult beetles flew in to oviposit in newly planted crops. This behaviour is not restricted to *Lepidiota* spp. and was recorded for white grubs in the USA by McBride (1984).

The attractiveness of peanut plants as oviposition sites should be examined. In the Atherton trials, both the infested maize and peanut crops followed peanut crops in which the eggs were laid a year earlier. The maize crop attacked by younger second instar white grubs initially contained an extremely high density of peanut volunteer plants although these were subsequently destroyed by disease. The attractiveness of peanut plants for some melolonthids was well demonstrated by *Heteronyx piceus* Blanchard near Rocky Creek. Adults hid in the soil at the base of the plants where the eggs were laid, emerging at night to feed on the foliage (Gough and Brown unpub. data 1979). The preference for peanut foliage was again shown at this site in the summer of 1980 when half a uniform field was sown to peanuts and half to maize. A large beetle population emerged in the maize which was an unsuitable diet. They moved out to feed and where the two crops met the peanut plants were completely defoliated. *H. piceus* also occurs in the South Burnett where adults have a similar behaviour in peanuts (D. J. Rogers and H. B. Brier, pers. comm. 1987).

Attempts to predict the sporadic attacks by white grubs must take into consideration the two-year life-cycle. Adult emergence from the soil depends on rainfall in the period from September to December. Smith (1936) examined rainfall records for a series of odd and even years. He showed that a dry spring could prevent adult emergence and severely reduce subsequent populations of white grubs in that series. The population of white grubs would then take some years to build up. At the same time, the population one year out of phase was often high because two very dry springs in succession are rare on the wet tablelands. This two year pattern was evident on the farm near Atherton where heavy attacks occurred in 1979 and 1981. When these crops were sampled, almost all the white grubs were third instar, suggesting that beetle flights and egg laying occurred in late 1977 and 1979 but not in late 1978 and 1980.

Positive identification of the species of *Lepidiota* in this study proved impossible as white grubs collected in crops and removed to the laboratory for rearing died of fungal or mite infections before adult emergence. However, circumstantial evidence exists as to their specific identity. Third instar larvae collected from the extensive infestations in maize and peanuts near Atherton could not be separated from those of *L. frenchi*, yet it seems unlikely they belong to this species but rather *L. laevis*, the larvae of which have not been described. Adults of both species (and therefore their larvae) are of similar size. Light traps on this farm yielded only *L. laevis*, which is recorded as a major pest of pastures in a very restricted area, including the farm in question (Atherton 1939). Extensive collections of *Lepidiota* spp. from Atherton made by J. H. Barrett and others commonly include *L. laevis* but *L. frenchi* is absent (QDPI Collection). *L. frenchi* was recorded from Mareeba and near Ravenshoe but not from Atherton (Britton 1978). The species of *Lepidiota* near Tolga and at Dimbulah are not known.

Because of the sporadic nature of attack, data on control and yield losses are difficult to acquire. This paper may contribute to a more complete study in the future. Chemical control of white grubs on the Atherton Tableland clearly is possible. Treatment of maize is warranted when white grub numbers are very high, but a more accurate assessment of the influence of grub density on yield is needed. This study has dealt mainly with crops

in continuously cultivated areas, however, attention should be given to white grub damage in areas near Atherton where maize is planted immediately following pasture. In peanuts, future work should aim at verification of the economic thresholds proposed above.

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A revised host list of fruit flies (Diptera : Tephritidae) from the Northern Territory of Australia

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Abstract

Host records for 19 indigenous fruit flies in the Northern Territory are reported. One species, *Dacus aquilonis* (May), has recently extended its host range and developed into a major pest of cultivated fruit in the Darwin area. *D. jarvisi* (Tryon) also appears to have the potential to increase in economic importance. Although 11 host fruits have been infested concomitantly by these two sympatric species, there is no evidence that interspecific competition occurs between them.

Two other species (*D. bryoniae* (Tryon) and *D. cucumis* French) recorded as being economic in other localities of the South Pacific region have not been reared from commercial hosts in the Northern Territory.

INTRODUCTION

Occurrences of fruit fly species have quarantine implications for horticultural industries within Australia and overseas. Although several of the species recorded in this paper are apparently confined to some coastal areas of the Northern Territory (NT) most are more widely distributed within Australia (May 1953). During the past decade, research work on fruit flies in the NT has resulted in: the detection and description of seven new species (Drew 1979; Drew *et al.* 1981; Drew and Hardy 1981; Drew 1988); the preparation of host records for many of the indigenous fruit flies (Allwood and Angeles 1979); and ecological studies on several species (Fitt 1981*a*, 1981*b*, 1983).

Allwood and Angeles (1979) reviewed fruit fly work in the NT up to 1978 and listed the known hosts and recorded localities for 13 fruit flies. Those records were compiled from older collections and intensive fruit sampling between 1975 and 1978. In April 1985, the native species *Dacus aquilonis* (May) suddenly expanded its host range in the Darwin area to include many cultivated fruits. This species is extremely difficult to separate taxonomically from Queensland fruit fly, *Dacus tryoni* (Froggatt) and will produce viable offspring when crossed under laboratory conditions (Drew and Lambert 1986). The emergence of *D. aquilonis* as a pest prompted the collection of more introduced fruit samples than previously when the emphasis had been on the collection of native plant hosts.

To date, 26 species of tephritids have been recorded in the NT, seven of which are of the subfamily Trypetinae and 19 of the subfamily Dacinae. This paper includes those records published earlier (Allwood and Angeles 1979; Drew 1979; Fitt 1981*a*). Records of exotic fruit fly species reared from Quarantine interceptions of infested fruit grown outside the NT are not included. Botanical nomenclature of hosts is as listed in Dunlop (1987).

MATERIALS AND METHODS

Fruits of native hosts were collected at localities representative of a large area of the NT between 1976 and June 1987. Where possible, mature fruits were collected both from

trees and the ground. Subsamples were allocated for identification by the Botany Section of the Conservation Commmission of the NT.

Cultivated hosts were collected in a similar manner. However, from March 1986 to June 1987, emphasis was placed on regular weekly sampling of fruiting species in an experimental orchard of introduced tropical fruit species located at Berrimah Research Farm near Darwin, and regular or more frequent samplings of other introduced fruits in urban and rural situations near Darwin than had previously occurred.

After collection, fruits were counted and held for pupation and adult emergence in clear plastic boxes with gauze covered aeration holes in the lid. Environmental conditions in the rearing room were maintained at 25°±4°C and 70%±15% relative humidity (r.h.) with natural daylight supplemented during the day by a bank of fluorescent tubes. Sieved sawdust (moistened as necessary) was provided as a pupation medium. Fleshy fruit was removed after 12 days (by which time larvae would have emerged from the fruit) to avoid a build up of infestations of mites and *Drosophila* sp. and to avoid excessive moisture in the sawdust.

When flies emerged, they were offered water and a sugar–protein mix for 3 to 4 days to allow development of colour, then killed and identified. Several specimens of each species from each host were mounted and retained in a reference collection. After 30 days, numbers of each fruit fly species and of emerging parasites were recorded and the fruit remnants and sawdust discarded.

RESULTS

More than 2500 samples of introduced and native fruits representing 285 plant species from 77 plant families were collected for fruit fly rearing. Native hosts have been recorded for 19 species of NT fruit flies and are listed in Tables 1 to 3. Many samples of fruit yielded two species of fruit fly and the frequency of multiple species infestation increased after March 1985 when *D. aquilonis* emerged as a significant pest species. Host fruits which were infested simultaneously with more than one species of fly are listed in Table 4.

Table 1. Recorded plant hosts of some Tephritidae in the Northern Territory

Fruit fly species	Plant host species	Host Family	Number of occurrences		
			Host sampled	Flies sampled	Multiples
Trypetinae					
Adrama biseta Malloch	*Barringtonia acutangula* (L.) Gaertner	Lecythidaceae	10	3	0
Adrama sp.	*Ipomoea abrupta* R. Br.	Convolvulaceae	1	1	0
Callistomyia horni Hendel	*Micromelum minutum* (Forster f.)Wight & Arn.	Rutaceae	2	2	0
	Glycosmis pentaphylla (Retz.) DC.	Rutaceae	7	2	1
	Glycosmis trifoliata (Blume) Sprengel	Rutaceae	15	5	3
	Glycosmis sp.	Rutaceae	2	2	0
Ceratitella sp.	*Amyema maidenii* (Blakely) Barlow	Loranthaceae	2	2	0
Gen. et sp. nov.	*Capparis* sp.	Capparaceae	1	1	0

Table 1. Recorded plant hosts of some Tephritidae in the Northern Territory

Fruit fly species	Plant host species	Host Family	Number of occurrences		
			Host sampled	Flies sampled	Multiples
Dacinae					
Callantra axana (Hering)	*Luffa cylindrica* (L.) M. Roemer	Cucurbitaceae	3	2	1
Dacus aquilonis (May)	See Table 2—64 spp.	Table 2			
Dacus bryoniae (Tryon)	*Diplocyclos palmatus* (L.) C. Jeffrey (=*Bryonopsis laciniosa*)	Cucurbitaceae	Fitt 1981*a*	Fitt 1981*a*	
	Passiflora suberosa L.	Passifloraceae	2	1	0
	Strychnos lucida R. Br.	Loganiaceae	49	3	0
Dacus cucumis French	*Luffa cylindrica* (L.) M. Roemer	Cucurbitaceae	3	1	1
	Passiflora edulis Sims	Passifloraceae	13	1	0
Dacus decurtans (May)	*Carallia brachiata* (Lour.) Merr.	Rhizophoraceae	14	10	0
Dacus sp. nov. (sp. C)	*Diospyros maritima* Blume	Ebenaceae	26	8	1
Dacus hardyi Drew	*Cynanchum* sp.	Asclepiadaceae	Drew 1979	Drew 1979	
Dacus jarvisi (Tryon)	See Table 3—21 spp.	Table 3			
Dacus mendosus (May)	*Pouteria sericea* (Aiton) Baehni	Sapotaceae	4	4	0
Dacus opiliae Drew and Hardy	*Mangifera indica* L.	Anacardiaceae	131	2	0
	Terminalia ferdinandiana Exell	Combretaceae	Fitt 1981*a*	Fitt 1981*a*	
	Mukia maderaspatana (L.) M. Roemer	Cucurbitaceae	4	2	2
	Opilia amentacea Roxb.	Opiliaceae	42	22	0
Dacus pallidus (Perkins and May)	*Hibiscus tiliaceus* L.	Malvaceae	5	1	0
	Nauclea orientalis (L.) L.	Rubiaceae	25	11	0
Dacus tenuifascia (May)	*Planchonella arnhemica* (F. Muell.) P. Royen	Sapotaceae	Fitt 1981*b*	Fitt 1981*b*	
	Planchonella pohlmaniana (F. Muell.)	Sapotaceae	15	10	0
Dacus signatifer (Tryon)	*Capparis sepiaria* L.	Capparaceae	1	1	0
	Capparis sp.	Capparaceae	3	2	0
Dacus sp. nov. (sp.B.)	*Secamone elliptica* R. Br.	Asclepiadaceae	6	1	0

Table 2. Recorded host plants of *Dacus aquilonis* (May) in the Northern Territory

Plant host species	Host Family	Number of occurrences to March 1985			Number of occurrences after March 1985		
		Host sampled	Flies emerged	Multiple fly spp.	Host sampled	Flies emerged	Multiple fly spp.
Anacardium occidentale L.	Anacardiaceae	4	0	n.a.	8	2	0
Mangifera indica L.	Anacardiaceae	68	0	n.a.	64	21	2
Spondias cytherea Sonn.	Anacardiaceae	2	0	0	23	4	1
Annona muricata L.	Annonaceae	2	0	n.a.	19	9	1
Annona reticulata L.	Annonaceae	0	0	n.a.	6	1	0
Annona squamosa L.	Annonaceae	0	n.a.	n.a.	5	2	0
Polyalthia australis (Benth.) Jessup	Annonaceae	4	1	0	0	n.a.	n.a.
Rollinia deliciosa Saff.	Annonaceae	0	n.a.	n.a.	3	3	0
Rollinia mucosa Baill.	Annonaceae	0	n.a.	n.a.	4	4	0
Livistona humilis R. Br.	Arecaceae	8	3	0	14	0	n.a.
Maranthes corymbosa Blume	Chrysobalanaceae	9	7	1	0	n.a.	n.a.
Terminalia catappa L.	Combretaceae	2	0	0	14	14	0
Terminalia erythrocarpa F. Muell.	Combretaceae	6	2	0	0	n.a.	n.a.
Terminalia ferdinandiana Exell	Combretaceae	81	40	0	45	17	0
Terminalia grandiflora Benth.	Combretaceae	5	1	0	1	0	n.a.
Terminalia platyphylla F. Muell.	Combretaceae	7	1	0	0	n.a.	n.a.
Diospyros ebenaster L.	Ebenaceae	0	n.a.	n.a.	30	6	0
Diospyros maritima Blume	Ebenaceae	26	1	1	0	n.a.	n.a.
Elaeocarpus grandis F. Muell.	Elaeocarpaceae	1	1	0	0	n.a.	n.a.
Petalostigma pubescens Domin	Euphorbiaceae	20	1	0	13	0	n.a.
Phyllanthus acidus (L.) Skeels.	Euphorbiaceae	1	0	n.a.	17	2	0
Flacourtia jangomas (Lour.) Rauschel	Flacourtiaceae	0	n.a.	n.a.	7	1	0
Flacourtia rukam Zoll. & Mor.	Flacourtiaceae	0	n.a.	n.a.	16	1	n.a.
Cryptocarya cunninghamii Meissner	Lauraceae	2	1	0	0	n.a.	n.a.
Persea americana Mill.	Lauraceae	1	0	n.a.	6	2	0
Malpighia glabra L.	Malpighiaceae	0	n.a.	n.a.	22	20	0
Malpighia puniciflora L.	Malpighiaceae	0	n.a.	n.a.	13	5	0
Aglaia rufa Miq.	Meliaceae	4	1	0	0	n.a.	n.a.
Musa acuminata Colla	Musaceae	2	0	n.a.	4	3	1
Musa acuminata x *M. balbisiana* cv. Lady's finger	Musaceae	0	0	n.a.	1	1	0
Acmena hemilampra (F. Muell. ex Bailey) Merr. & Perry	Myrtaceae	6	1	0	0	n.a.	n.a.
Acmenosperma claviflorum (Roxb.) Kausel	Myrtaceae	2	2	0	0	n.a.	n.a.
Psidium guajava	L.Myrtaceae	55	6	0	133	72	18
Psidium littorale Raddi var. littorale Bail.	Myrtaceae	0	n.a.	n.a.	11	11	2

Table 2. Recorded host plants of *Dacus aquilonis* (May) in the Northern Territory—*continued*

Plant host species	Host Family	Number of occurrences to March 1985			Number of occurrences after March 1985		
		Host sampled	Flies emerged	Multiple fly spp.	Host sampled	Flies emerged	Multiple fly spp.
Syzygium aqueum (Burm) Alston	Myrtaceae	0	n.a.	n.a.	4	4	0
Syzygium armstrongii (Benth.) B. Hyland	Myrtaceae	13	1	1	0	n.a.	n.a.
Syzygium angophoroides (F. Muell.) B. Hyland	Myrtaceae	2	1	0	0	n.a.	n.a.
Syzygium fibrosum (Bailey) Hartly & Perry	Myrtaceae	8	1	0	4	2	0
Syzygium forte (F Muell.) B. Hyland	Myrtaceae	2	2	1	0	n.a.	n.a.
Syzygium jambos (L.) Alston	Myrtaceae	0	n.a.	n.a.	4	4	0
Syzygium malaccense (L.) Merr. & Perr	Myrtaceae	2	0	n.a.	1	1	0
Syzygium operculata Roxbg.	Myrtaceae	4	1	0	0	n.a.	n.a.
Syzygium suborbiculare (Benth.) Hartly & Perry	Myrtaceae	39	8	5	4	3	1
Eugenia uniflora L.	Myrtaceae	1	0	n.a.	1	1	0
Averrhoa carambola L.	Oxalidaceae	6	0	n.a.	201	143	0
Ziziphus mauritiana Lam.	Rhamnaceae	3	0	n.a.	6	4	0
Eriobotrya japonica (Thunb.) Lindl.	Rosaceae	0	n.a.	n.a.	1	1	0
Malus sylvestris Mill.	Rosaceae	0	n.a.	n.a.	2	2	0
Prunus persica (L.) Batsch	Rosaceae	2	1	0	8	2	0
Ixora klanderana F. Muell.	Rubiaceae	4	1	0	4	0	n.a.
Citrus limon (L.) Burm. f.	Rutaceae	3	0	n.a.	54	14	0
Citrus grandis (L.) Osbeck	Rutaceae	0	0	n.a.	12	5	0
Citrus paradisi Macf.	Rutaceae	6	1	0	32	11	1
Citrus reticulata Blanco	Rutaceae	2	0	n.a.	23	2	0
Citrus sp.	Rutaceae	3	3	0	0	n.a.	n.a.
Fortunella crassifolia Swingle (Meiwa var.)	Rutaceae	0	n.a.	n.a.	6	1	0
Glycosmis pentaphylla (Retz.) DC.	Rutaceae	6	2	1	0	n.a.	n.a.
Glycosmis trifoliata (Blume) Sprengel	Rutaceae	10	3	1	2	2	2
Micromelum minutum (Forster f.) Wight & Arn.	Rutaceae	23	3	0	7	0	n.a.
Blighia sapida Koenig	Sapindaceae	0	n.a.	n.a.	11	1	0
Chrysophyllum cainito L.	Sapindaceae	0	n.a.	n.a.	2	1	0
Manilkara zapota (L.) Van Royen	Sapindaceae	0	n.a.	n.a.	1	1	0
Capsicum annuum L.	Solanaceae	0	n.a.	n.a.	4	4	0
Lycopersicon esculentum Miller	Solanaceae	4	0	n.a.	16	9	0

n.a.=not applicable.

Table 3. Recorded host plants of *Dacus jarvisi* (Tryon) in the Northern Territory

Plant host species	Host Family	Number of occurrences to March 1985			Number of occurrences after March 1985		
		Host sampled	Flies emerged	Multiple fly spp.	Host sampled	Flies emerged	Multiple fly spp.
Mangifera indica L.	Anacardiaceae	68	26	0	64	7	2
Spondias cytherea Sonn.	Anacardiaceae	2	1	0	23	1	1
Annona muricata L.	Annonaceae	2	0	n.a.	19	1	1
Carica papaya L.	Caricaceae	5	2	0	14	1	0
Maranthes corymbosa Blume	Chrysobalanaceae	9	1	1	0	n.a.	n.a.
Terminalia arostrata Ewart & O.B. Davies	Combretaceae	2	0	n.a.	1	1	0
Terminalia catappa L.	Combretaceae	2	1	0	14	0	0
Mukia maderaspatana (L.) M. Roemer	Cucurbitaceae	4	2	2	0	n.a.	n.a.
Planchonia careya (F. Muell.) Knuth	Lecythidaceae	61	32	0	7	7	0
Musa acuminata Colla	Musaceae	2	0	n.a.	4	1	1
Psidium guajava L.	Myrtaceae	55	13	0	133	30	18
Psidium littorale Raddi var. littorale Bail.	Myrtaceae	0	n.a.	n.a.	11	2	2
Syzygium armstrongii (Benth.) B. Hyland	Myrtaceae	13	4	1	0	n.a.	n.a.
Syzygium eucalyptoides ssp. *bleeseri* (O. Schwarz) B. Hyland	Myrtaceae	2	2	0	0	n.a.	n.a.
Syzygium malaccense (L.) Merr. & Perry	Myrtaceae	2	1	0	1	0	n.a.
Syzygium forte (=*S. rubiginosum*) (F. Muell.) B. Hyland	Myrtaceae	2	1	1	0	n.a.	n.a.
Syzygium suborbiculare (Blume) Hartley & Perry	Myrtaceae	39	29	5	4	1	1
Syzygium sp.	Myrtaceae	6	2	0	0	n.a.	n.a.
Averrhoa bilimbi L.	Oxalidaceae	0	n.a.	n.a.	9	1	0
Punica granatum L.	Punicaceae	2	1	0	1	0	n.a.
Citrus paradisi Macf.	Rutaceae	6	0	n.a.	26	1	1
Citrus sinensis (L.) Osbeck	Rutaceae	9	2	0	8	0	n.a.

n.a.=not applicable.

DISCUSSION

Hosts of five Dacine species (namely *Callantra aequalis* (Coquillet), *D. allwoodi* Drew, *D. bellulus* Drew and Hancock, *D. newmani* (Perkins) and *Dacus* sp. D*) which have been collected at male lure traps in the NT are as yet unknown while several species of Trypetines, occasionally collected at lure traps, are unlikely to damage fruit. In addition, fruit sampling produced evidence of a non-indigenous species of fruit fly *Ceratitis capitata* (Wiedemann) (Mediterranean fruit fly) which had infested cultivated hosts in Alice Springs. The latter had been detected and field outbreaks successfully eradicated in two separate control programmes mounted from December 1976 to April 1977 and December 1981 to March 1982 at Alice Springs.

* Three new species identified here as *Dacus* sp. B, *Dacus* sp. C and *Dacus* sp. D. are described in Drew (1988).

Table 4. Fruit samples from which more than one species of fruit fly emerged

Fruit fly species	Plant host species	Host Family	Number occurrences
Callantra axana *Dacus cucumis*	*Luffa cylindrica*	Cucurbitaceae	1
Dacus opiliae *Dacus jarvisi*	*Mukia maderaspatana*	Cucurbitaceae	2
Dacus aquilonis *Dacus* sp. nov. (sp. C)	*Diospyros maritima*	Ebenaceae	1
Dacus aquilonis *Callistomyia horni*	{ *Glycosmis pentaphylla* { *Glycosmis trifoliata*	Rutaceae Rutaceae	1 3
Dacus aquilonis *Dacus jarvisi*	*Annona muricata* *Citrus paradisi*	Annonaceae Rutaceae	1 1
	Mangifera indica	Myrtaceae	2
	Maranthes corymbosa	Chrysobalanaceae	1
	Musa acuminata (cv. Cavendish)	Musaceae	1
	Psidium guajava	Myrtaceae	18
	Psidium littorale	Myrtaceae	2
	Spondias cythera	Anacardiaceae	1
	Syzygium armstrongii	Myrtaceae	1
	Syzygium forte (=*S. rubiginosum*)	Myrtaceae	1
	Syzygium suborbiculare	Myrtaceae	6

D. aquilonis

This species occurs only in north-western Australia and is geographically separated from closely related species in eastern Australia (Drew and Lambert 1986). Allwood and Angeles (1979) found that *D. aquilonis* showed more diversity in host range than other indigenous fruit flies, having 12 native (*Pouteria sericea* was listed in error) and 4 cultivated hosts. Since their list was compiled, the known host range of this pest has increased to 63 species (Table 2) of which 40 are cultivated or introduced plants and 23 are native plants. These hosts range over 21 plant families and include 14 species in Myrtaceae. However, of the 40 cultivated hosts listed in Table 2, 34 have been recorded since April 1985 and all within a limited area extending up to 80 km from Darwin. *D. aquilonis* is now recognised as a pest species which has eclipsed *D. jarvisi* (Tryon) in importance in the NT.

The reason for the sudden and dramatic change in host preference remains unknown but a damaging strain of *D. aquilonis* would appear to exist which is still expanding both its territorial and host range. Specimens reared from this strain are morphologically indistinguishable from the original strain but *D. aquilonis* has not been reared from cultivated hosts outside this limited territorial range since April 1985 whereas it is consistently reared from at least some of these hosts within the range. The presence of the noxious strain is readily detected by infestations in the widely planted host *Averrhoa carambola* and in *Mangifera indica*, the most commonly grown domestic and commercial fruit trees in the tropical region of the NT. Prior to April 1985, these fruits were not attacked by *D. aquilonis* (Table 2) but since that time, 71% (143/201) of *A. carambola* samples and 33% (21/64) of mango samples were infested. These infestation rates could have been even higher since many samples were taken from commercial plantings where chemical spraying had been carried out and were also diluted by samplings from areas where the harmful strain had not yet reached.

D. jarvisi

D. jarvisi also increased its host range from nine (four cultivated) species in 1979 to 21 (12 cultivated) species (Table 3). However, as Fitt (1986) showed, this species strongly prefers its native host *Planchonia careya* for oviposition and most of the records of *D. jarvisi* from cultivated fruit occurred outside the fruiting season of this native host. It is probable that *D. jarvisi* will increase in numbers and extend its range when more cultivated fruit becomes available so that it will be supported from season to season by cultivated hosts as occurs in coastal Queensland (May 1963).

D. cucumis French

Until recently very few specimens and no hosts of *D. cucumis* had been recorded in the NT although the species was regarded as a potential pest of commercial cucurbits (Fitt 1980). The species is of major importance in Queensland where it infests cucurbits, tomatoes and pawpaws (Drew 1982). *D. cucumis* was reared from cultivated *Luffa cylindrica* and a single specimen from passionfruit (*Passiflora edulis*). Following the collection of numerous specimens on leaves of *Ficus racemosa* in August 1986, a laboratory culture was established and has been readily maintained by females ovipositing into and larvae rearing in cut cucumber. In the NT, *D. cucumis* has also been collected from Katherine, over 200 km inland.

D. bryoniae (Tryon)

Records of *D. bryoniae* from capsicum, mango and passionfruit in Queensland (Drew 1982) are incorrect (Drew 1988). This species is known only from banana in Papua New Guinea (Drew 1982) and from three native hosts in the NT. It is possible that it could develop into a commercial pest there.

D. opiliae Drew and Hardy

D. opiliae (=*Dacus* sp. A—Allwood and Angeles 1979) has now been recorded from three native hosts but has not been reared from mangoes since 1969 (Allwood and Angeles 1979). Fitt (1981*a*) indicates that this fly is unlikely to develop into an economically important species.

Callistomyia horni Hendel

There are now three known native hosts of *Callistomyia horni* (*Barringtonia acutangula* was recorded incorrectly by Allwood and Angeles (1979)) but this species is unlikely to develop into an economic pest.

All other species listed in Table 1 are probably monophagous and possibly univoltine and are therefore very unlikely to develop into pest species of commercial fruit.

The rapid change and expansion in host range of *D. aquilonis* is also evident in the dual infestations recorded in Table 4. Allwood and Angeles (1979) reported two species of fruit fly from the same fruit in three host fruits. In each instance *D. aquilonis* was one of the species. The list (Table 4) has now been increased to 16 host fruits, 14 of which include *D. aquilonis*. Of these 14 species, seven were recorded to March 1985 and seven from March 1985 to June 1987. The former included only native fruits while the latter were all cultivated. This was despite the more frequent sampling of these cultivated fruits (135 samples) in the period to April 1985 than the corresponding native fruits (105 samples).

The most favoured hosts infested by *D. jarvisi* were *Planchonia careya*, *Psidium guajava* and *Syzygium suborbiculare* while *D. aquilonis* regularly infested many hosts, including *Annona muricata*, *Averrhoa carambola*, *Citrus paradisi*, *Malpighia glabra*, *Man-*

gifera indica, *Psidium* spp., some *Syzygium* spp. and *Terminalia* spp. As indicated by Fitt (1987), there is little evidence that interspecific competition occurs between these two sympatric species. For example, *D. jarvisi* was reared from 23.6% (13/55) of guava samples collected before March 1985 and from 22.6% (30/133) of samples collected since that time and was unaffected by competition from *D. aquilonis* which infrequently (6/55) infested guava before March 1985 but emerged from 54.1% (72/133) of samples collected post March 1985 and 18 of these 133 samples (=13.5%) had dual infestation of both fruit fly species.

Similar results were reported in a study involving other sympatric fruit flies in Queensland where Gibbs (1967) showed that, although using the same host fruits for oviposition and larval development, *D. tryoni* and *D. neohumeralis* Hardy did not exert any deleterious effect on one another.

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Boron requirements of flue-cured tobacco and soil residual effects from repeated applications to a granitic sand in north Queensland

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Abstract

The effect of boron on flue-cured tobacco yield and leaf quality was investigated by spraying 'Solubor' (20.5% boron) onto the soil at 0, 2, 4, 8 and 16 kg/ha (0, 0.41, 0.82, 1.64 and 3.28 kg B/ha). No significant differences in yield, leaf quality or the principal chemical attributes of the leaf were observed for the varieties Hicks Q46 and ZZ100, though, at the highest rate of application, root weights were significantly reduced. No accumulation of boron in the soil profiles to a depth of 1.2 m occurred following three years of application. Boron uptake ranged from 12 to 14 mg per plant (or 0.23 to 0.25 kg/ha for a plant density of 19 000/ha) without applied boron to 18 to 21 mg per plant (0.34 to 0.41 g/ha) at the highest rate of application. Boron accumulation occurred primarily in the leaf.

INTRODUCTION

Bartholomew and Nicholson (1976) identified cases of boron deficiency in the Dimbulah area which were corrected with a spray of 0.2% Solubor (20.5% B). A recent survey (T. B. Jacobsen pers. comm. 1983) of fifty tobacco farms in the Mareeba–Dimbulah area indicated that 96% of growers were applying boron (as Solubor) to crops to correct a physiological disorder known as leaf drop. The average rate of boron application was 0.86 kg/ha, with a small percentage (12%) of the growers applying above 1.43 kg/ha.

According to McCants and Woltz (1967), boron has been the most extensively studied of the micronutrients with respect to the nutrition and fertilisation of tobacco. Some of the reports in the literature, however, are not consistent.

For example, Bacon *et al.* (1950) noted that boron toxicity was caused by an application of 2.5 kg B/ha when tobacco grew in soil that already had a hot water soluble (HWS) concentration of 14 mg B/kg. On the other hand, Le Lacheur (1972) reported that tobacco did not exhibit toxicity symptoms when grown in soil with a concentration of 25 mg HWS B/kg. Reisenauer *et al.* (1973) claim that the range between adequate and toxic levels of boron is smaller than for any other nutrient element. Hutcheson and Woltz (1956) concluded that concentrations of 15 to 16 mg B/kg in bud leaves at flowering were near the deficient level for flue-cured tobacco, after observing toxicity on young plants when boron was applied at 1.0 kg/ha. Matthews and McVickar (1946) obtained a five per cent increase in yield and value of flue-cured tobacco with an application of 0.28 kg B/ha, but the claim of increased yield and quality has since been refuted by Hutcheson and Woltz (1956), Terry and Terrill (1969) and Jones and Leslie (1986). These three groups of researchers applied boron at rates up to 1.34, 0.56 and 1.68 kg/ha respectively, which are comparable to the rates applied by north Queensland tobacco growers.

Some tobacco growers in the Mareeba–Dimbulah area are currently diversifying into deeper rooted orchard crops. Concern was expressed by Departmental officers that prolonged use of boron on tobacco soils may lead to a toxic accumulation of the element in the lower soil profiles.

The objectives of this study were to investigate the effects of annual applications of boron (at rates up to 3.3 kg/ha) on tobacco yield, cured leaf quality and the incidence of leaf drop over a three year period. Soil boron to a depth of 1.2 m was also monitored during the experiment to study whether accumulation of applied boron in soil was occuring.

MATERIALS AND METHODS

Location

The field experiment was conducted at Southedge Tobacco Research Station (16°58′S; 145°21′E) on a red earth soil of granitic origin, locally known as Morganbury Loamy Sand (Gn 2.14, Northcote 1974). Some soil chemical attributes (0 to 150 mm) were: pH (1:5 H_2O) 5.8; organic carbon (Walkley and Black 1934) 0.6%; exchangeable cations (M NH_4Cl, pH 7.0) K 0.30, Ca 0.87 and Mg 0.23 cmol/kg; and HWS boron <0.1 mg/kg (Berger and Truog 1939).

A chacteristic of the soil was the high percentage of gravel ($>$2mm), with depth intervals of 0 to 150, 150 to 300, 300 to 600, 600 to 900 and 900 to 1200 mm containing 21%, 17%, 25%, 59% and 56% respectively; bulk densities in these profile increments were 1.61, 1.85, 1.88, 1.75 and 1.80 g/cc respectively.

Design and cultural data

Five rates of Solubor, 0, 2, 4, 8 and 16 kg/ha (0, 0.41, 0.82, 1.64 and 3.28 kg B/ha) were replicated four times in a randomised block design. The Solubor was applied as an aqueous solution just prior to planting. The varieties grown were Hicks Q46 in 1983 and ZZ100 in 1984 and 1985. Each plot comprised 72 plants (0.0038 ha), at a plant density of 19 000 /ha. The basal fertiliser was a commercial NPK mixture (9.7:5:28.8) which provided 70 kg N/ha. A sidedressing of sodium nitrate was applied four weeks after transplanting to increase the nitrogen application to 100 kg N/ha. Maleic hydrazide was used for sucker control at the rate of 16 L/ha. The Departmental reccommendations for other cultural practises such as irrigation and pesticide application were followed. Following transplanting, irrigation is withheld for 30 days then the crop is irrigated every 6 to 8 days with 20 mm water (K. H. Ferguson pers. comm. 1983). Leaf drop was regularly assessed during the experiments. A severe wind and hail storm in 1984 completely destroyed the mature crop in the second year with the result that this paper reports the findings of two years cultural data and three years soil data.

Plant analysis

Three plants were selected from each plot at budding on the basis of conformity with overall plot development. Over successive harvests, leaves were removed from these plants in a manner which simulated the harvest pattern for the remainder of the plot. The inflorescence (top), suckers and leaves of each plant were kept separate during the harvest period. At the completion of each experiment, the plants were carefully removed from the field, thoroughly washed to remove all soil and then divided into stem and root. All plant material (leaf, root, stem and top plus suckers) was dried at 65°C, weighed and ground to pass through a 0.8 mm sieve.

Total and saleable cured leaf weights were recorded in each experiment. Leaf quality was assessed by an officer of the Tobacco Leaf Marketing Board and a grade assigned.

The reserve price for each treatment was determined as the weighted average price of assigned grades in the 1984 and 1986 Grade and Price Schedule for Hicks Q46 (1983) and ZZ100 (1985) respectively.

Soil analyses

Soil was sampled at 0 to 150, 150 to 300, 300 to 600, 600 to 900 and 900 to 1200 mm on three occasions by compositing soils from three cores taken from within the rows of each treatment. Sampling times were January 1984, May 1985 and May 1986. Soil samples were air dried, sieved through a 2 mm screen and hot-water extractable boron (Berger and Truog 1939) determined using an auto-analyser (Basson *et al.* 1974).

Analytical methods

The four plant parts and cured leaf samples were analysed for boron by the method of Basson *et al.* (1974). The cured leaf samples were analysed for total alkaloids (Griffith 1957), reducing sugars (Harvey *et al.* 1969) and nitrogen (Varley 1966).

RESULTS

Cured leaf yield, quality and the dollar return per hectare were not significantly affected by the application of boron (data not shown). Mean total cured leaf yields of 4648 and 3361 kg/ha and mean dollar return per hectare of 18 487 and 14 993 were produced by Hicks Q46 (1983) and ZZ100 (1985) respectively. These yield differences and the resultant differences in mean monetary return between the two years are attributable to variety and season.

Toxicity symptoms were observed on young plants in the 3.28 kg/ha treatment for Hicks Q46 but plants recovered after irrigation commenced. No toxicity symptoms were observed on plants for the variety ZZ100.

Application of boron at up to 3.28 kg/ha had no significant effect on reducing sugar, total alkaloid and nitrogen content of the cured leaf. The total alkaloid level, however, was generally lower in the 3.28 kg/ha treatment for both varities (data not shown). Boron concentrations in cured leaf were increased by application of boron (Table 1). The boron level in the control plots was similar for each variety (Table 1).

For both varieties, between 10% and 15% of the applied boron was taken up when 0.41 kg/ha boron was applied (Table 1). For an application of 3.28 kg/ha boron only three to five per cent of the application was taken up by the plant. The plant component that was most effected by treatment was the root system. With Hicks Q46 the root weight was reduced at boron rates of 3.28 kg/ha (P=0.05), whereas, for ZZ100 the root weight increased then fell as more Solubor was applied; no significant changes were observed for leaf, stem and the tops (plus suckers) component. Boron concentration in the leaf increased as the application of boron increased (Table 1), whereas the changes in the other plant components were either not significant or small when compared with the control. This resulted in a higher proportion of the boron in the plant being present in the leaf component (Table 2).

Concentrations of boron found in the soil after successive applications of boron (Table 3) show, that even at the highest rate of application (3.28 kg B/ha/yr), accumulation in the top 900 mm of soil was small, and well below the concentrations considered to be toxic.

Leaf drop for all three experiments was very slight, amounting to only a few leaves for the whole of each experiment.

Table 1. Dry matter yield and boron content in components of tobacco plants grown with various rates of applied boron

Treatment kg B/ha	Cured leaf mg/kg boron				Plant component, dry weight (g)				B in plant component (mg/kg)				B in Whole Plant (mg)	Uptake of B g/ha	% of applied B†
	X*	C	L	T	Leaf	Root	Stem	Top	Leaf	Root	Stem	Top			
Hicks Q46															
0.00	28	21	23	44	258.4	146.8	162.3	20.3	32	10	15	49	13.9	253	
0.41	36	30	35	53	260.4	131.0	162.7	22.5	40	10	16	46	15.3	293	9.8
0.82	37	34	42	64	264.0	143.2	160.3	20.2	42	10	16	45	15.7	301	5.9
1.64	50	40	44	62	258.9	125.1	159.8	19.7	53	10	17	48	18.6	356	6.2
3.28	81	56	58	73	233.1	91.0	167.7	20.9	70	10	17	44	21.2	408	4.7
LSD $P = 0.05$	14	6	7	8	n.s.	35.3	n.s.	n.s.	9	n.s.	n.s.	n.s.	3.1	59	
ZZ100															
0.00	30	26	45	54	169.6	108.2	109.4	116.2	34	9	13	33	12.0	231	
0.41	37	29	50	61	200.9	138.8	121.5	114.3	41	9	13	35	15.2	291	14.7
0.82	43	39	63	66	190.2	125.2	111.4	112.2	44	9	14	33	15.0	289	7.1
1.64	49	46	64	72	189.4	135.4	115.0	89.3	47	9	15	37	15.3	294	3.8
3.28	73	57	82	80	173.2	113.1	106.4	95.5	62	10	16	41	17.6	338	3.3
LSD $P = 0.05$	12	10	10	15	n.s.	21.1	n.s.	n.s.	11	n.s.	1	4	n.s.	n.s.	

* X = Lugs, C = Cutters, L = Leaf and T = Tips.

† Calculation of % of applied boron recovered = $\frac{\text{Uptake of } B_T(g) - \text{Uptake of } B_O(g)0}{B \text{ applied } (g)} \times 100\%$

where B_T = Boron uptake at a particular treatment.
B_O = Boron uptake by control treatment.

n.s. = not significant.

Table 2. Boron in plant component as a percentage of total plant boron

Treatment kg B/ha	Hicks Q46 (1983)				ZZ100 (1985)			
	Leaf	Root	Stem	Top	Leaf	Root	Stem	Top
0.00	62.7	11.2	18.4	7.7	48.1	8.2	12.1	31.6
0.41	67.5	8.8	17.0	6.7	55.6	8.7	10.8	24.9
0.82	68.9	9.2	16.2	5.7	57.0	7.4	10.7	24.9
1.64	74.1	6.7	14.4	4.8	58.7	7.7	11.0	22.6
3.28	77.2	4.6	13.6	4.6	62.3	6.0	9.3	22.4

Table 3. Effect of applied boron on concentration of hot water extractable B (mg/kg) in the soil profiles

Treatment kgB/ha	January 1984 B concentration (mg/kg) at various depths (mm)					May 1985 B concentration (mg/kg) at various depths (mm)					May 1986 B concentration (mg/kg) at various depths (mm)				
	00/150	150/300	300/600	600/900	900/1200	00/150	150/300	300/600	600/900	900/1200	00/150	150/300	300/600	600/900	900/1200
0.00	0.17	0.13	0.14	0.14	0.17	0.14	0.13	0.11	0.07	0.06	0.13	0.13	0.10	0.12	0.10
0.41	0.26	0.18	0.12	0.14	0.10	0.14	0.13	0.08	0.07	0.08	0.12	0.14	0.16	0.14	0.07
0.82	0.34	0.28	0.17	0.12	0.11	0.23	0.20	0.17	0.08	0.06	0.13	0.14	0.15	0.12	0.11
1.64	0.45	0.29	0.17	0.14	0.10	0.27	0.23	0.16	0.10	0.06	0.18	0.19	0.17	0.11	0.07
3.28	0.66	0.35	0.19	0.13	0.09	0.25	0.27	0.20	0.17	0.08	0.24	0.26	0.28	0.23	0.09
LSD $P = 0.05$	0.22	0.10	n.s.	n.s.	n.s.	n.s.	0.06	0.08	n.s.	n.s.	n.s.	0.08	n.s.	n.s.	n.s.

n.s. = not significant.

DISCUSSION

The current results demonstrate that flue-cured tobacco will tolerate high levels of applied boron when grown in coarse sandy soils of low boron status and that a significant accumulation of boron does not occur in the soil (Table 3). The lack of a marked increase in soil boron concentration after three successive applications was possibly due to the heavy summer rainfall which leached the boron beyond the 1.2 m depth.

In May 1986, for the highest rate of application of boron, 4.8 kg B/ha was present in the 0 to 1200 mm soil profile and 0.5 kg B/ha had been removed from the field in the cured leaf. At the same sampling of the nil control treatment 2.4 kg B/ha remained in the soil profile and 0.3 kg B/ha had been removed in the cured leaf. By subtracting these plant and soil amounts, an estimate of the amount of boron remaining in the soil from the applied boron was found to be 2.6 kg B/ha or 27% of the total application. In a similar way, it was found that 56% of the boron appied in the 3.28 kg/ha treatment was present in the soil after the first tobacco crop. The reason for the disparity between these percentages was possibly that greater quantities of rainfall were recorded between transplanting and the time of soil sampling for the two ZZ100 crops. These amounts were 913 and 1000 mm respectively compared with 519 mm of rainfall recorded in the six months to January 1984. The fact that only 56% of applied boron could be accounted for in plant and soil after 519 mm of rainfall, indicates either that this soil is highly susceptibile to leaching because of its high gravel content, particularly below 600 mm, or our sampling intensity was not sufficient to detect applied boron.

The study found no agronomic benefit in terms of cured leaf yield, quality or monetary return from applying boron. The site for this experiment was chosen because of its low hot water soluble boron status. Because leaf drop in all plots, including the control, was very low some other unknown factor must be responsible for the disorder.

Boron removed from the field in cured leaf was 0.11 to 0.16 kg/ha, and 0.25 to 0.3 kg/ha was accounted for in the whole plant. The latter quantity would not be supplied by tobacco fertilisers which contain 10 to 20 mg B/kg (G. Price, Consolidated Fertilisers Limited, pers. comm. 1982) since only 12 to 15 g B/ha would be applied at current rates. The amount of Solubor required to provide the 0.25 to 0.3 kg B/ha requirement is 1.1 to 1.2 kg/ha. In view of previous recorded instances of deficiency and the highly leachable nature of boron in this soil, tobacco growers are advised to maintain the current recommendation of applying Solubor at a rate of 2 kg/ha.

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Bioassay of phosphorus deficiency in Queensland wheat soils by the *Azotobacter* plaque method

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Abstract

The *Azotobacter* plaque method was tested as a bioassay of phosphorus deficiency in Queensland soils used for wheat growing. The method was modified by using seven concentrations of supplied phosphate, inoculating replicate plaques with a pure culture of *Azotobacter chroococcum*, supplying other nutrients where required, and rating *Azotobacter* growth after three incubation periods. Tests were conducted on nine soils of varying phosphate status for which data on wheat response to rate of phosphate fertiliser application were available from a glasshouse experiment. From semi-quantitative curves of *Azotobacter* growth in response to increasing levels of supplied P, I assessed each soil for concentration of supplied P giving maximum growth of *Azotobacter*, concentration of P giving half-maximum growth, and growth rating at the lowest concentration of supplied P as a percentage of maximum growth.

Azotobacter in the plaques required greater concentrations of P for maximum growth than did wheat in pot culture. Concentration of supplied P resulting in half-maximum growth of natural *Azotobacter* in plaques after 5 days' incubation correlated best with parameters of wheat response to applied phosphate (r=0.86, P<0.01 with maximum yield increase and r=0.82, P<0.01 with linear response trend of wheat to applied P). *Azotobacter* response parameters to applied P were generally better correlated with water soluble phosphate in soil and P sorption measures than with acid- or bicarbonate-extractable P.

INTRODUCTION

In a previous study (Thompson 1987*a*), a soil plaque method based on growth of naturally occurring *Azotobacter* (Winogradsky 1928) was used in an attempt to bioassay 'long fallow disorder' in a black earth soil. The method proved sensitive to phosphorus and sulphur deficiencies but not to zinc deficiency. Since black earths (Stace *et al.* 1968) and other cracking clay soils or vertisols (Soil Survey Staff 1975) are extensively used in Queensland and northern New South Wales for wheat growing, the *Azotobacter* plaque method was tested as an aid to prediction of phosphorus fertiliser requirements on a range of vertisols for which response data of wheat to P fertiliser was available (Whitehouse and Hibberd 1969). Some modifications that were made to published *Azotobacter* plaque methods included the use of several rates of applied phosphorus to establish semi-quantitative response curves, the addition where necessary of 'complete nutrients' other than P, and tests conducted both with natural populations of *Azotobacter* and an inoculated culture of *Azotobacter chroococcum.*

MATERIALS AND METHODS

Soils

Nine soils from the wheat belt of Queensland selected to cover a range of phosphate levels and assessed for wheat response to phosphorus fertiliser in a glasshouse experiment (Whitehouse and Hibberd 1969) were assayed for phosphorus deficiency by the *Azotobacter* plaque method (Winogradsky 1928). A tenth soil tested in plaque experiments described by Thompson (1987*a*) was used in the development of the method. All samples were of

topsoil (0 to 0.1 m) that had been air-dried, crushed to <2 mm and stored in sealed glass jars for 3 months (soils 1 to 9 were subsamples of the soils used in the glasshouse experiment) or 14 months (soil 10). Some characteristics of these ten soils are given in Table 1.

Table 1. Some characteristics of soils used in the experiments

No.	Great soil group*	Soil association†	Locality	pH	Pa‡ (mg/kg)	Pb§ (mg/kg)	Colour	Texture
1	Black earth	Condamine	Daandine	7.3	27	21	dark grey	clay-loam
2	Black earth	Mywybilla	Norwin	6.9	73	20	dark grey	clay
3	Black earth	Mywybilla	Norwin	6.9	15	19	dark grey	clay
4	Black earth		Warwick	7.8	55	24	dark grey	clay
5	Black earth	Condamine	Haystack	7.3	15	22	dark grey	clay
6	Black earth	Condamine	Dalby	7.5	146	44	dark grey	clay
7	Brown clay		Biloela	6.5	74	51	brown	clay-loam
8	Black earth		Willowvale	8.3	11	9	dark grey	clay
9	Brown clay		Inglewood	8.2	54	40	dark brown	silty clay-loam
0	Black earth	Waco	Mt. Maria	8.5	380	70	dark grey brown	clay

* Great soil group (Stace *et al.* 1968).

†Soil association (Beckmann and Thompson 1960).

‡Pa=phosphate extracted with 0.02M H_2SO_4 (Kerr and von Stieglitz 1938).

§Pb=phosphate extracted with 0.5M $NaHCO_3$ (Colwell 1963).

Preparation of plaques

To prepare a plaque, the soil (20 g oven-dry equivalent) was mixed with 1 % w/w of carbon source (sucrose or glucose) by shaking in a polythene bag. The soil was spread in a clean Petri dish and solutions of the required rate of phosphorus as NaH_2PO_4 and other inorganic nutrients if needed, were added as evenly as possible by pipette to the soil. An additional, pre-determined volume of deionised water required to bring the soil to the 'sticky point' was added. The soil was moulded by hand to a putty-like consistency, working from the lowest to the highest concentration of added P; hands were washed and rinsed in alcohol between soils. The moulded soil was pressed into a 50 mm diameter Petri dish and the surface smoothed with a moistened stainless steel spatula to an 'iced', convex finish. All treatments were prepared in duplicate and plaques were smoothed in order from the lowest to the highest rate of phosphorus with thorough cleaning of the spatula and sterilisation by flaming in alcohol between soils. The plaques were placed in glass desiccators containing 0.5 M H_2SO_4 to absorb atmospheric ammonia and incubated at 28°C. *Azotobacter* growth on the plaques was rated after three incubation periods on colony size (Thompson, 1987*a*) with a rating system from 0=no visible growth to 5=mean colony diameter approximately 1.2 mm.

Experiment 1: Comparison of glucose and sucrose as carbon source for assessing *Azotobacter* response to a range of concentrations of applied P

The effect of either glucose or sucrose as carbon source on *Azotobacter* growth in response to six rates of phosphorus; that is, 0, 50, 100, 200, 400 and 800 µg P/g soil supplied as NaH_2PO_4 was tested in Waco soil (soil 10, Table 1). All treatments received a basal dressing of 22 µg S/g soil as Na_2SO_4 because this soil required sulphur for maximum

growth of *Azotobacter* (Thompson 1987*a*). A further treatment comprised a 'complete nutrient treatment' additional to 800 µg P/g soil and the basal sulphur. The 'complete nutrient' solution consisted of (g/L): $CaCl_2.2H_2O$ 0.162; KCl 0.084; $MgCl_2.6H_2O$ 0.368; $Fe_2(SO_4)_3$ 0.157; $CuSO_4.5H_2O$ 0.017; $ZnSO_4.7H_2O$ 0.039; $MnSO_4.4H_2O$ 0.036; $Na_2MoO_4.2H_2O$ 0.056; which when added at the rate of 10 mL per plaque supplied the following elements in µg/g soil: Ca 10, K 10, Mg 10, Fe 10, Cu 1, Zn 2, Mn 2 and Mo 5. Plaques were rated after 3, 4 and 7 days' incubation.

Experiment 2: Response of natural *Azotobacter* in a range of soils to a single rate of phosphorus and complete nutrients

This experiment was designed to determine for a number of potentially phosphorus deficient soils whether the natural populations of *Azotobacter* were sufficient to conduct the plaque tests, and whether additional inorganic nutrients were required to obtain maximum response in *Azotobacter* growth to applied phosphorus. Soils 1 to 9 (Table 1) were tested. All plaques were prepared with 1 % w/w sucrose and 1600 µg P/g soil as NaH_2PO_4. Each soil was treated in two ways; that is, no further nutrient addition or complete nutrients similar in composition to that applied in Experiment 1 but also containing Na_2SO_4 sufficient to supply 22 µg S/g soil. Growth of *Azotobacter* on the plaques was rated after 2, 3 and 5 days' incubation.

Experiment 3: Response of natural and introduced *Azotobacter* to multiple rates of phosphorus in a range of soils

This experiment was designed to bioassay the phosphorus status of soils 1 to 9 (Table 1) by assessing the response of *Azotobacter* to seven rates of supplied phosphorus; that is, 0, 50, 100, 200, 400, 800 and 1600 µg P/g soil as NaH_2PO_4. Based on the results of Experiments 1 and 2, all soils were supplied with 1 % w/w sucrose and soils 4, 6 and 8 were supplemented with the complete nutrients used in Experiment 2. Because two of the soils had too small a natural population of *Azotobacter* a second set of plaques of all soils was inoculated with a pure culture of *Azotobacter chroococcum*, strain WR-68 (Thompson 1977), which had been isolated from the rhizosphere of wheat growing in a Mywybilla black earth (Beckmann and Thompson 1960). To prepare suitable inoculum, growth of WR-68 from a 2-day-old slope culture of maintenance medium 22 (Thompson and Skerman 1979) was suspended in 20 mL sterile deionised water, shaken with glass beads to disaggregate the cells, then washed twice in 20 mL deionised water by centrifugation, decantation and resuspension of the cells. To inoculate plaques, the stainless steel spatula was dipped in the washed *Azotobacter* cell suspension instead of deionised water before smoothing the plaque surface. A separately prepared cell suspension was used for each soil to avoid transfer of nutrients between soils. Growth of *Azotobacter* on the plaques was rated after 2, 3 and 5 days' incubation.

Semi-quantitative response curves of *Azotobacter* growth to increasing level of supplied phosphorus were constructed from results for both uninoculated and inoculated plaques of each soil. From these curves I determined for each soil at the three rating times: concentration of supplied phosphorus giving maximum growth of *Azotobacter*; concentration of phosphorus giving half-maximum growth; and growth rating at the lowest concentration of supplied phosphorus as a percentage of the maximum growth rating. Correlation coefficients were calculated between these parameters of *Azotobacter* response and soil phosphate status as determined by chemical analysis and parameters of wheat response to four levels of applied phosphate; that is, 0, 15, 30 and 60 µg P/g soil in a glasshouse experiment (Whitehouse and Hibberd 1969).

RESULTS

Experiment 1: Comparison of glucose and sucrose as carbon source for assessing *Azotobacter* response to a range of concentrations of applied P

Azotobacter colonies on plaques grew somewhat faster and larger with sucrose than with glucose as a carbon source. Complete nutrients also stimulated early growth above that attained with P and basal S. However, maximum growth attained at 7 days with all P rates above 200 µg P/ g soil was similar irrespective of the nature of the carbon source or the addition of complete nutrients. As sucrose appeared a somewhat superior carbon source for *Azotobacter* growth in plaques to glucose as used previously (Thompson 1987*a*), sucrose was used in all subsequent experiments.

Experiment 2: Response of natural *Azotobacter* in a range of soils to a single rate of phosphorus and complete nutrients

The response to complete nutrients in growth of *Azotobacter* in the various soils after 2, 3 and 5 days' incubation is given in Figure 1. Although, soils 3, 5 and 9 contained few *Azotobacter*, their colony size could be rated. The other soils all contained large numbers of *Azotobacter*. Of these, soils 4, 6 and 8 required complete nutrients for maximum growth of *Azotobacter*.

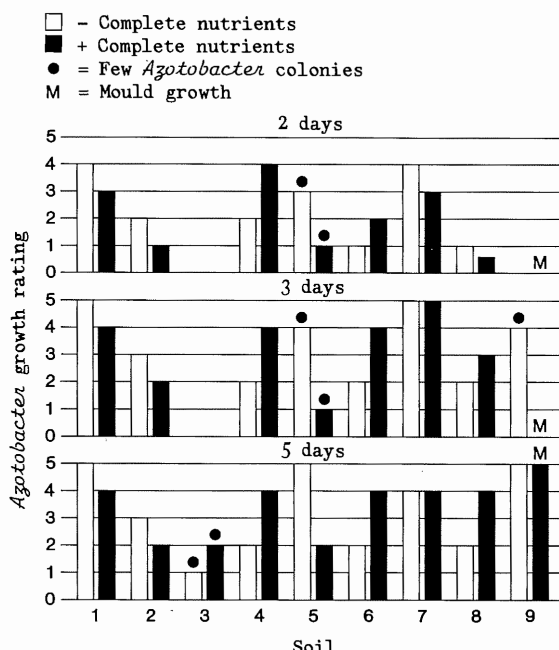

Figure 1. Responses of naturally occurring *Azotobacter* to addition of 'complete nutrients' including sulphur, in plaques of nine soils (descriptions given in Table 1) after 2, 3 and 5 days' incubation. All plaques were supplied with sucrose (1% w/w) and phosphorus (1600 µg P/g soil).

Experiment 3: Response of natural and introduced *Azotobacter* to multiple rates of phosphorus in a range of soils

The growth of *Azotobacter* at 2, 3 and 5 days in both uninoculated and inoculated plaques of the nine soils is given in Figures 2, 3 and 4. Naturally occurring *Azotobacter* were present in all soils but colonies were not evident in soils 3 and 9 until after 5 days'

incubation. Ratings of *Azotobacter* growth on uninoculated and inoculated plaques were similar in some soils but somewhat different in others. All soils responded to increasing rate of phosphorus addition with some showing reduction in growth at the highest rates of phosphorus.

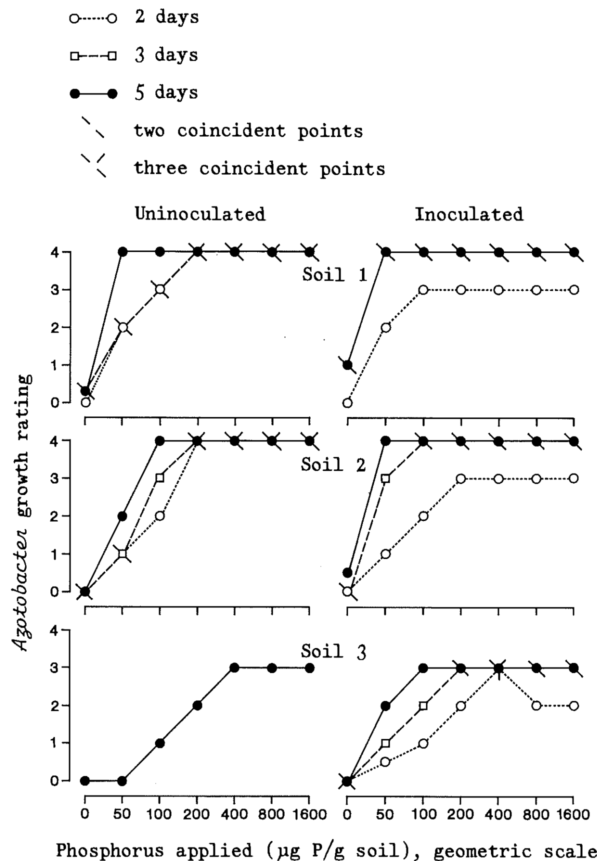

Figure 2. Response of *Azotobacter* to rate of phosphorus addition in uninoculated and inoculated plaques of soils 1 to 3 (descriptions given in Table 1) after 2, 3 and 5 days' incubation. All plaques were supplied with sucrose (1% w/w).

Maximum growth of *Azotobacter* in the various soils was attained with rates of P from 50 to 800 μg P/g soil in the uninoculated plaques and from 50 to 400 μg P/g soil in the inoculated plaques. Correlation coefficients between concentration of applied P for maximum and half maximum response of *Azotobacter* and some chemical measures of soil phosphate (M. J. Whitehouse and D. Hibberd, unpub. data 1970) are given in Table 2. The concentration of applied P giving maximum response of either natural or inoculated *Azotobacter* at 2 or 3 days (Table 2, code numbers A1–2, A7–8) were generally well correlated positively with chemical measures of P sorption (Table 2, code numbers C5–7). The concentration of applied P giving half maximum response of natural *Azotobacter* (A4–6) also was well correlated positively with chemical measures of P sorption (C5–7) and additionally was well correlated negatively with concentrations of phosphate in a water leachate (C3) and a water extraction (C4) of soil. Similar trends but with generally lower correlation coefficients were evident between concentration of applied P giving half maximum response of inoculated *Azotobacter* (A10–12) and water leachates and extracts

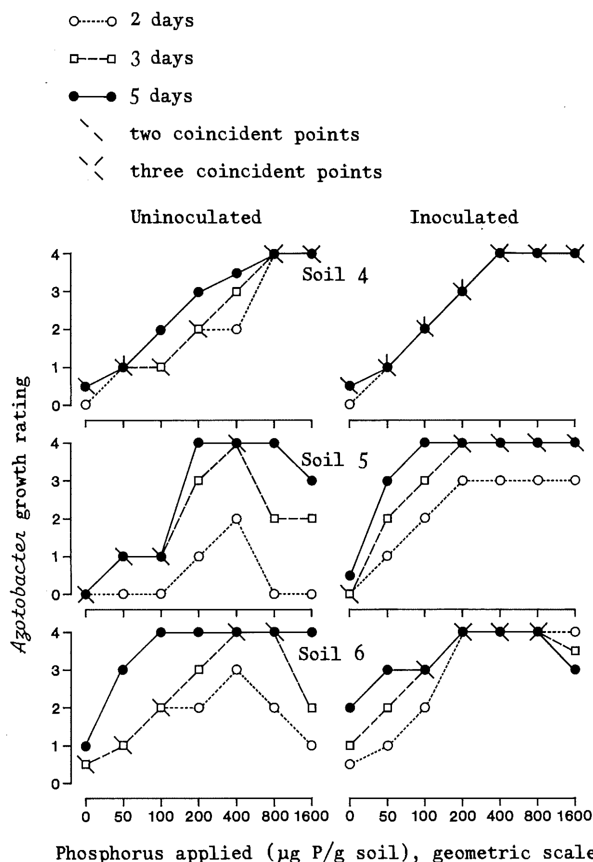

Figure 3. Response of *Azotobacter* to rate of phosphorus addition in uninoculated and inoculated plaques of soils 4 to 6 (descriptions given in Table 1) after 2, 3 and 5 days' incubation. All plaques were supplied with sucrose (1% w/w) and soils 4 and 6 received 'complete nutrients'.

Table 2. Correlation matrix between some measures of *Azotobacter* response to applied phosphorus and some chemical measures of soil phosphate status

Chemical measure of soil phosphate		Measure of *Azotobacter* response to phosphorus											
	Inoculation:	Uninoculated						Inoculated					
	P concentration for:	Maximum response			Half maximum response			Maximum response			Half maximum response		
	Rating time (days):	2	3	5	2	3	5	2	3	5	2	3	5
Code No.	Variable / Code No.:	A 1	A 2	A 3	A 4	A 5	A 6	A 7	A 8	A 9	A 10	A 11	A 12
C 1	0.005 M H_2SO_4 extractant	0.00	−0.02	−0.26	−0.41	−0.26	−0.67	−0.17	−0.14	−0.04	−0.29	−0.20	−0.46
C 2	0.5 M $NaHCO_3$ extractant	−0.18	−0.18	−0.20	−0.37	−0.34	−0.44	−0.43	−0.29	−0.30	−0.50	−0.07	−0.44
C 3	Water leachate (at field capacity)	−0.61	−0.56	−0.70*	−0.91**	−0.79*	−0.81**	−0.64	−0.65	−0.53	−0.62	−0.63	−0.83**
C 4	Water extract (1:10)	−0.46	−0.44	−0.59	−0.68	−0.61	−0.77*	−0.47	−0.38	−0.32	−0.44	−0.44	−0.67*
C 5	Sorbed P at 0.1 µg P/mL	0.74	0.71	0.47	0.86*	0.88**	0.75*	0.67*	0.66*	0.53	0.64	0.46	0.44
C 6	P buffer capacity at 0.1 µg P/mL	0.81*	0.81*	0.47	0.80	0.88**	0.57	0.66*	0.67*	0.59	0.47	0.42	0.35
C 7	Linear P sorption trend	0.81*	0.82*	0.47	0.81*	0.88**	0.58	0.66*	0.67*	0.58	0.45	0.41	0.35

* Statistically significant at $P<0.05$.

** Statistically significant at $P<0.01$.

and P sorption measures (C3–4). Generally, measures of *Azotobacter* response to applied phosphate were poorly correlated with concentrations of phosphorus in soil extracts with 0.005 M H_2SO_4 (C1) or 0.5 M $NaHCO_3$ (C2). One exception was a significant negative correlation between the concentration of applied P giving half-maximum response of naturally occurring *Azotobacter* (A6) and the P concentration in 0.005 M H_2SO_4 extracts (C1). The third measure of *Azotobacter* response; that is, growth rating at the lowest concentration of supplied phosphorus as a percentage of the maximum growth rating, was not significantly correlated with any chemical measure of soil phosphate.

The greatest correlations between measures of *Azotobacter* response and wheat yield response to applied P were between the rate of applied P giving half maximum response of natural *Azotobacter* at 5 days and on the maximum yield increase of wheat to applied P (r=0.855, P<0.01), relative yield of wheat; that is, yield without P as a percentage of maximum yield with P fertiliser (r=−0.70, P<.05) and the linear response trend, b, (r=0.817, P<.01) from a fitted equation of form $y=a+bx+cx^2$ where y=wheat yield and x=rate of applied phosphorus. Most other correlations between measures of *Azotobacter* response and wheat yield response to applied P were not statistically significant.

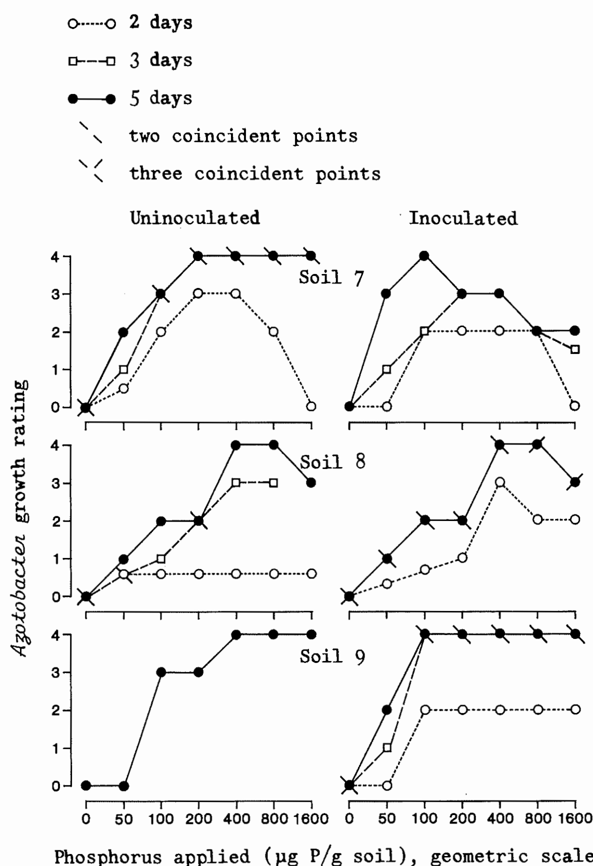

Figure 4. Response of *Azotobacter* to rate of phosphorus addition in uninoculated and inoculated plaques of soils 7 to 9 (descriptions given in Table 1) after 2, 3 and 5 days' incubation. All plaques were supplied with sucrose (1% w/w) and soil 8 received 'complete nutrients'.

DISCUSSION

The vertisols studied here were suitable for application of the *Azotobacter* plaque method for bioassay of phosphorus deficiency, because their chemical and physical properties are naturally favourable for growth of *Azotobacter*. Thus, there is no need for major amendments that could modify phosphorus availability, such as raising the pH of acid soils (Young 1933) or enriching sandy soils with kaolinite (Sackett and Stewart 1931). Although some of the soils contained few *Azotobacter*, any possible problem was overcome by inoculating a second set of plaques with a pure culture of *Azotobacter chroococcum*. In previous studies with soils from temperate areas the method has tested growth of *Azotobacter* at a single high rate of added phosphate in comparison with a control without added phosphate (Winogradsky and Ziemecka 1928; Sackett and Stewart 1931; Ziemecka 1932; Young 1933; Halversen and Hoge 1942). The degree of phosphorus deficiency was then assessed from the relative growth of *Azotobacter* colonies on the two treatments. However, Greene (1933) indicated that *Azotobacter* growth and nitrogen-fixation in culture media follow the law of decreasing increment or Mitscherlich function to increasing rate of phosphorus. He claimed that the *Azotobacter* plaque method related best to plant response in the field for extremes of deficiency and sufficiency but less well for intermediate levels. In the present study, a range of phosphorus rates was used in the plaques to obtain better discrimination between soils of varying degrees of phosphorus deficiency.

Azotobacter in the plaques required greater concentrations of added phosphorus for maximum growth than did six-week-old wheat plants in pot culture (60 μg P/g soil) (Whitehouse and Hibberd 1969). The apparently greater phosphorus requirements of *Azotobacter* in plaques than of crops has been noted before; for example, Young (1933) and Wieringa (1939). This possibly reflects the high demand by nitrogen-fixing *Azotobacter* for phosphorus; for example, Becking (1961) found 65 and 130 μg P/mL culture medium were required for maximum nitrogen fixation by a temperate and a tropical strain of *A. chroococcum* respectively. Probably, the even higher concentrations of added phosphorus needed for maximum growth of *Azotobacter* on plaques is also due to the poor mobility of phosphate in soil and the inability of an *Azotobacter* colony to move to undepleted soil once it has depleted the available phosphorus in the soil it contacts. In contrast, plant roots can grow to undepleted zones of soil and this process is greatly aided if the roots are colonised with mycorrhizal fungi as demonstrated for Queensland vertisols by Thompson (1987*b*).

Although the absolute requirements for phosphorus of *Azotobacter* in soil plaques and wheat in pots of soil may differ, correlations between parameters of the respective response curves to applied phosphorus could make the plaque method useful for predicting the fertiliser requirements of wheat. The greatest correlation coefficients between parameters of wheat response to phosphorus and *Azotobacter* response were obtained with the concentration of applied P giving half maximum response of natural *Azotobacter* after 5 days' incubation. The correlation coefficients were not as great as those obtained by Whitehouse and Hibberd (1969) between parameters of plant response and H_2SO_4 extractable phosphorus (r=0.89 to 0.97).

Because measures of *Azotobacter* response were better correlated with either a water leachate or a water extract than with acid or bicarbonate extracts of soil phosphate, *Azotobacter* seems more sensitive to intensity of phosphate in the soil solution than to the capacity of the soil to supply phosphate from its labile reserves. Possibly this reflects the relatively short time (2–5 days) of the *Azotobacter* test, whereas with the longer times involved in plant growth, replacement of phosphate removed from solution becomes a more important factor. Measures of *Azotobacter* response to applied phosphorus were also correlated with chemical measures of phosphorus sorption by the soils. These correlations

probably reflect the extent to which the various soils' sorption properties reduce the concentration of phosphate remaining in soil solution from the phosphorus applications and hence still available for *Azotobacter* growth.

Although the results indicate some interesting relations between measures of soil phosphate, *Azotobacter* response and wheat response to applied phosphate, they indicate that the *Azotobacter* plaque method offers no advantage over chemical methods for predicting phosphate availabity to wheat. However, the results may partly depend on the paricular set of test soils. Although Whitehouse and Hibberd (1969) found for this set of soils that acid-extractable phosphate was the best predictor of wheat response to phosphorus fertiliser, Whitehouse (1970) later found bicarbonate-extractable phosphate was better for a larger set of vertisols. Likewise, density of naturally occurring *Azotobacter* populations in another set of vertisols (J. P. Thompson, unpub. data 1971) was better correlated with bicarbonate-extractable phosphorus than other chemical measures of soil phosphate.

The system of rating *Azotobacter* growth and derivation of parameters to semi-quantitative response curves was adequate for the present purposes. However, more definitive results might be obtained if the method was quantitative to allow a better mathematical treatment of the *Azotobacter* response curves. Nitrogen fixed by *Azotobacter* is directly related to cell growth, and measuring nitrogen fixed in the soil plaques in response to rates of applied P would make the method quantitative. This might be achieved with Kjeldahl analysis for total nitrogen content. However, despite the active nitrogen fixation resulting from the addition of sucrose, total nitrogen content might be too insensitive a measure at low rates of applied P because of the large background of combined nitrogen in the soil organic matter. A preferable method might be to assay the nitrogenase in the *Azotobacter* cells on the soil plaques or within a mass of incubated soil by the acetylene reduction method as already applied to a Queensland black earth by Okafor and Macrae (1973). If the assay were to measure the activity of *Azotobacter* cells throughout the mass of soil instead of only those colonies on the plaque surface, the problem of poor phosphorus movement to the *Azotobacter* colonies might be reduced. The test might then better relate to plant response to phosphorus. Plants have mycorrhizal networks of roots and fungal hyphae that permeate the soil mass to overcome the limitation of poor mobility of phosphorus in soil.

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Inheritance of leaf and fruit characteristics in *Cucurbita maxima* Duch. cv. Queensland Blue × *C. ecuadorensis* Cutler and Whitaker

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Abstract

Inheritance of cotyledonary pattern of venation, bitterness of petioles and immature fruit, silver mottle on the leaf, hardness of rind and leaf shape (lobing) were investigated in parental, F_1, F_2 and backcross progeny of *Cucurbita maxima* cv. Queensland Blue×*C. ecuadorensis*. Bitter fruit and silver mottle were inherited as dominant traits as in other *Cucurbita* spp. A dominant gene (Hi) occurred in *C. maxima* and inhibited the development of hard rind. Lobed leaf was controlled by a single dominant gene and linked to bitterness of fruit. Bitterness of the petiole and fruit were also linked. Two genes appeared to control pattern of venation. Petiole bitterness was controlled by more than one gene.

INTRODUCTION

Cucurbita ecuadorensis, a wild South American species, is highly resistant to at least four viruses which infect cultivated pumpkin, *C. maxima* (Provvidenti *et al.* 1978, 1984; Pitrat and De Vaulx 1979; Greber and Herrington 1980). The two species readily hybridise. *C. ecuadorensis* is thus a valuable source of resistance, but the species also exhibits many undesirable characteristics including hard rind (Cutler and Whitaker 1969) and bitter flesh (Metcalf *et al.* 1982). Selection for a desirable trait or to eliminate undesirable traits would be easier if their inheritance was known. Wall and Whitaker (1971) investigated the inheritance of polymorphic enzymes but not other plant characteristics. Our work reports information on the inheritance of some fruit and leaf characteristics in the cross *C. maxima*×*C. ecuadorensis.*

MATERIALS AND METHODS

Parental, F_1 hybrid and segregating populations were derived by controlled pollination of plants from commercial seed of *C. maxima* cv. Queensland Blue 'Selected Strain' and seed of *C. ecuadorensis*. Parents were inbred for three generations before use and were uniform for the characters investigated. Seed was sown on 22 August 1985 in pots in the glasshouse and transplanted to the field 28 days later. Originally the number of plants of P_1 (*C. maxima*); P_2 (*C. ecuadorensis*); F_1 (*C. maxima*×*C. ecuadorensis*); F_2; BC_1P_1 (F_1×*C. maxima*) and BC_1P_2 (F_1×*C. ecuadorensis*) populations were 12, 12, 20, 144, 48 and 48, respectively. Because we wished also to determine the inheritance of resistance to papaya ringspot virus type W (PRV-W) from our population, all plants were inoculated with PRV-W at the cotyledonary stage.

The infection of plants with PRV-W was not expected to affect the results for the characters we studied, except that few fruit would set on P_1. Prior to field planting the pattern of venation on cotyledons was recorded and in the field, silver mottle on the leaf, fruit and leaf bitterness, leaf shape, and rind hardness were classified as described later.

Segregation patterns of plants in parental, F_1 and segregating populations were fitted to major gene models and goodness of fit tested using a Chi-square test (Srb *et al.* 1965). Associations among characters were investigated using contingency tables. Only the significant ($P<0.05$) associations are reported. Data on mature fruit characteristics were obtained only from plants with mature fruit, the number of plants being about 4, 20, 86, 35 and 45 for P_2, F_1, F_2, BC_1P_1 and BC_1P_2 populations, respectively. Data on fruit characteristics of P_1, *C. maxima,* were collected from a separate plant since there was no fruit set on P_1 in the trial area due to viral infection.

Venation

The pattern of venation at the base of expanded cotyledons was classified as similar to *C. maxima* (separated veins) or *C. ecuadorensis* (fused veins) 11 days after sowing.

Silver mottle on leaf

Silver mottle in the axils of veins was considered present if leaves of any age showed this character 75 days after sowing. A general uniform light silvering present over the entire leaf of some plants was considered as a separate character.

Bitterness

Bitterness of petiole of a plant was classified by tasting one (if bitter) or two (if non-bitter or uncertain) petioles at the junction of lamina and petiole of the sixth leaf from the terminal apex 89 to 92 days after sowing. Placental tissue (Jaworski *et al.* 1985) of one or two (if non-bitter or uncertain) immature fruit was similarly classified for bitterness, within five days and in most cases within one day of the flower's opening, 95 or 103 days after sowing. Bitterness of mature fruit was assessed on a sample of 55 fruit about 60 days later. To avoid fatigue (Andeweg and DeBruyn 1959) when assessing bitterness of petiole or fruit, the mouth was rinsed four times between samples, each rinse was with 45 mL, of water; only 25 to 50 samples were assessed at each session of 40 to 90 minutes; and there was a break of at least one and usually two hours between tasting sessions.

Leaf lobing

Leaf shape (lobing) was described as a 'lobe index', (L), from measurements on the tenth leaf from the terminal apex of each of two branches on each plant; $L=2D/(A+B)$ where A is the distance from leaf base to the tip of the midrib lobe, B is the distance from leaf base to the tip of next lobe and D is distance from leaf base to the closest point of the depression between the lobes. Where only one branch was available measurements were made on leaf 10 and 11, 84 to 88 days after sowing. A leaf was lobed when $L=<0.8$.

Hardness of rind

Soft rind (skin) of fruit was readily cut by a knife when the fruit was mature. Hard rind was very difficult to cut and was usually 3 mm to 5 mm thick.

RESULTS AND DISCUSSION

Thayer (1934) found the pattern of venation on the blade of cotyledons of *C. pepo* was controlled by more than one gene. In the present study the pattern of venation at the base of cotyledons was similarly not controlled monogenically but was consistent with duplicate dominant epistasis with dominance of the phenotype of *C. maxima* (P of F_2 Chi-square=0.49). However this needs confirmation with larger populations as there was an apparent deviation from expectation (P of Chi-square=0.047) in the backcross to the recessive parent.

The segregation pattern, with respect to the presence or absence of silver mottle in the vein axils, among plants in populations derived from *C. maxima*×*C. ecuadorensis* is consistent (*P* of F_2 and BC_1P_1 Chi-squares=0.11 and 0.47 respectively) with previous reports in other species of *Cucurbita* spp. Silvering is conferred by a single dominant gene (Robinson *et al.* 1976) although modifers occur (Shifriss 1982). Leaves of *C. ecuadorensis* have the silver mottle. This character could provide a useful marker to test the extent of cross pollination in attempted hybridisations.

Bitterness of immature fruit was conferred by a single gene with bitterness dominant. This is consistent (*P* of F_2 and BC_1P_1 Chi-squares=0.43 and 0.65 respectively) with inheritance of bitterness in *C. pepo* (Robinson *et al.* 1976). By contrast segregation pattern of bitterness of petiole was consistent (*P* of Chi-squares=0.81) with bitterness being conferred by recessive complementary epistasis in the F_2 but segregation in the backcross was not consistent. The degree of bitterness differed between plants (data not shown). This aspect requires further investigation.

Bitterness of immature fruit was completely associated with bitterness of mature fruit in the 55 plants assessed. Thus selection for fruit bitterness could be achieved at early flowering. Further, the strong association (*P* of Chi-square=<0.01) of bitter petiole and bitter fruit in F_2 and BC_1P_1 populations may allow selective elimination, even before the female flower opens, of more than two thirds of the plants destined to produce bitter fruit.

Hard rind in *C. pepo* and *C. andreana*×*C. maxima* is conferred by a single dominant gene (Robinson *et al.* 1976). By contrast, in the cross *C. maxima*×*C. ecuadorensis* hard rind was recessive to soft rind in the 20 plants of the F_1. Plants of *C. moschata*×*C. ecuadorensis* have hard rind (M. E. Herrington unpub. data 1983). Therefore we conclude that *C. maxima* cv. Queensland Blue has a dominant gene (Hi is proposed) which inhibits the expression of hard rind. None of the 35 plants of BC_1P_1 had hard rind but 27 of 45 plants of BC_1P_2 produced hard rind. The lack of fit in the F_2 population where 44 plants produced hard rind, may be due to incomplete expression of Hi, or linkage of Hi with sterility (Wall and Whitaker 1971) or some unknown factor which prevented maturation of fruit; only 86 of 144 F_2 plants matured fruit. These unknown factors may also have contributed to the association (for F_2, *P* of Chi-square=0.03) between an absence of silver mottle on the leaf and hard rind. The phenotype of *C. maxima* was lacking.

Table 1. Segregation pattern for lobing* of leaf in plants of *C. maxima* × *C. ecuadorensis*

	Numbers of plants			Expected ratio of Lobed: not lobed	χ^2	*P*
	Total	Lobed	Not lobed			
P_1 (*C. maxima*)	11	0	11	0:1	n.a.	n.a.
P_2 (*C. ecuadorensis*)	12	12	0	1:0	n.a.	n.a.
F_1	20	20	0	1:0	n.a.	n.a.
F_2	141	105	36	3:1	0.02	0.88
BC_1P_1	47	17	30	1:1	3.6	0.06
BC_1P_2	48	47	1†	1:0	n.a.	n.a.

* Ratio (*L*) of length of depression between lobes: average length of midrib and next lobe. A plant is classed as 'lobed' when *L*=<0.8.

† This entry is believed due to a misclassification as under the proposed hypothesis it should not exist.

n.a. = not available.

Lobed leaf (Table 1) was dominant to entire leaf, controlled by a single gene and linked to bitterness of immature fruit (Table 2). By contrast, lobed leaf in *C. maxima* is

controlled by a single recessive gene (Dyutin 1980). This difference indicates more than one genetic system controls leafshape in *Cucurbita* spp.

Table 2. Association of bitterness of immature fruit and lobed leaf

Bitterness of immature fruit	F_2 Leaf lobe*		Total
	Lobed	Not Lobed	
Not-bitter	10	16	26
Bitter	64	31	95
Total	74	47	127
$\chi^2_1 = 7.18$; $P = 0.01$			

* 1 (Lobed) when $2D/(A+B)$ <0.8, and not lobed when $2D/(A+B)$ is >0.8. Where A is the distance from leaf base to tip of midrib lobe, *B* is distance from base to tip of next lobe and *D* is the distance from leaf base to closest point of depression between lobes. P_1 (*C. maxima*) =not-bitter, not lobed and P_2 (*C. ecuadorensis*)=bitter, lobed.

These results indicate that inheritance of some characters in *C. maxima*×*C. ecuadorensis* differs from those reported elsewhere for *Cucurbita* spp. Such information on inheritance should be useful in choosing breeding strategies when it is desired to transfer characteristics from *C. ecuadorensis* into *C. maxima.*

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Partial resistance to bacterial leafspot in pepper cultivar Hungarian Yellow

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Abstract

Field experiments were used to determine the resistance of *Capsicum annuum* L. cv. Hungarian Yellow to bacterial leaf spot (*Xanthomonas campestris* pv. *vesicatoria*). In separate trials leaf fall was correlated (r^2=0.62, 0.8 and 0.9) with yield (t/ha) and provided a measure of resistance. The cultivar Hungarian Yellow was partially resistant, having less leaf fall than red bell peppers. The resistance was evident in the hybrid; cv. Hungarian Yellow×cv. Northern Belle. This suggests selection of hybrid genotypes similar to the F_1 should be possible in backcross populations but further genetical studies are required to clarify this.

INTRODUCTION

Bacterial leaf spot (BLS) caused by *Xanthomonas campestris* pv. *vesicatoria* (Doidge 1920) Dye 1978 is a destructive disease of bell pepper (*Capsicum annuum* L.) in Queensland (Simmonds 1966; Hibberd and Gillespie 1982). At least two races of BLS occur naturally in Florida (Cook and Stall 1969, 1982). Only race 1 has been found here (A. M. Hibberd, pers. comm. 1987). Resistance to BLS has been identified in a number of accessions (Sowell 1960; Sowell and Dempsey 1977; Hibberd *et al.* 1979; Hibberd and Gillespie 1982).

The cultivar, Hungarian Yellow, is reported to be field tolerant to bacterial leaf spot (Hibberd *et al.* 1979). The origin of this tolerance is not known but the cultivar is not considered to carry genes *Bs1*, *Bs2* or *Bs3* (Hibberd *et al.* 1987; A. M. Hibberd pers. comm. 1987) which confer resistance through hypersensitivity. Tolerance implies an ability of a plant to sustain a substantial amount of disease with little or no effect on yield while resistance limits the development of the disease (Russell 1978). The degree of a cultivar's resistance or tolerance would influence its usefulness in a breeding programme. However, quantitative comparisons of the disease levels and yield of cv. Hungarian Yellow with those of other cultivars have not been reported. This paper reports these comparisons.

MATERIALS AND METHODS

In a preliminary field experiment, in Spring 1975 to compare cultivars and determine a method to measure resistance, 46 cultivars from the Redlands Research Station (RRS) collection were evaluated in ten plant plots as two replications. The standard commercial cultivar, Northern Belle (syn. Yolo Y) was included. The cultivar Hungarian Yellow also was included, because it has been reported to have field tolerance to BLS and also has the ability to set large numbers of fruit under widely varying weather conditions (Hibberd *et al.* 1979). The response of each cultivar to natural infection with bacterial leaf spot was determined 17 weeks after sowing, about one week before harvesting commenced.

In the second experiment in early spring 1977, the response of cvv. Hungarian Yellow, Canape, Sheba, Northern Belle, Florida VR-2 and the F_1 (Hungarian Yellow×Northern Belle) were compared within separate blocks of plants naturally infected, or plants spray-inoculated when 6 to 8 expanded leaves were present, with a bacterial leaf spot suspension supplied by Dr M. Moffett, (Department of Primary Industries, Indooroopilly). The BLS was most likely race 1, as race 2 has not been reported in Queensland (A. M. Hibberd, pers. comm. 1987). Each regime of infection consisted of three replicates of 15 plants of each cultivar. The cultivars had been chosen following the first experiment as likely to give a range of reactions when infected with BLS. The number of leaves, expressed as a percentage, having BLS lesions and/or having fallen was visually estimated on each of five plants in each plot 18 weeks after sowing. This was one week before harvest. All 15 plants were harvested. Where fewer plants occurred in a plot marketable yields were adjusted by covariate analyses. Leaf fall was assumed to be due to BLS and thus the leaf spot estimate included both leaf fall, and leaves with lesions. Size of lesion was not estimated. Plants were grown using standard cultural practices.

Data were subjected to analysis of variance and where the *F*-test was significant means were compared using a *t*-test. Correlations were calculated using mean values.

RESULTS AND DISCUSSION

Cultivars differed in the severity of leaf symptoms, leaf fall and yield in both experiments (Tables 1 and 2). In the first experiment yield (t/ha), was moderately well predicted by leaf fall (r^2=0.62, P<0.01). In the second experiment similar correlations were also high (r^2=0.9 and 0.8 (P<0.05) for inoculated and naturally infected plants, respectively. This suggests leaf fall may be a suitable indication of resistance or tolerance. In the first experiment cv. Hungarian Yellow had less BLS (P<0.05) than only six cultivars and higher yields (P<0.05) than 15 cultivars, however, on the basis of leaf fall it was one of the most resistant cultivars. In the second experiment, when assessed on leaf spot, leaf fall or yield, cv. Hungarian Yellow was more resistant than the bell peppers, cvv. Northern Belle and Florida VR-2 irrespective of the method of infection (Table 2). Although cv. Hungarian Yellow had the lowest values for leaf spot and leaf fall these were, with one exception, not significantly different (P<0.05) from those of cvv. Sheba and Canape. The latter is a F_1 hybrid. Following inoculation, leaf fall on cv. Sheba was higher than cv. Hungarian Yellow (Table 2). The lower incidence of leaf spot compared with that on bell peppers indicates that in fact cv. Hungarian Yellow has higher resistance to BLS. Cultivar Hungarian Yellow is widely adapted (Hibberd *et al.* 1979), the performance of cvv. Sheba and Canape in Queensland is not well known but all appear to be potential sources of resistance to BLS. Because of the greater leaf fall (Table 2), cv. Sheba is the least desirable source of resistance to BLS.

On all criteria evaluated the F_1 hybrid (Northern Belle×Hungarian Yellow) was more resistant than cv. Northern Belle and not significantly different from cv. Hungarian Yellow except in leaf spot incidence under natural infection. While this suggests that resistance to BLS in cv. Hungarian Yellow is controlled as a dominant character the actual (non-significant) values for yield, leaf fall and leaf spot (Table 2) suggest additive gene action may occur. Further studies to clarify the genetic control of this partial resistance are desirable. Methods of studying inheritance in combination with a breeding programme have been outlined by Bassett and Woods (1978). The large difference between the reactions of the F_1 and the susceptible parent suggests that if resistance is simply inherited

identification of heterozygous plants in segregating backcross populations should be possible and allow the transfer resistance from cv. Hungarian Yellow to bell peppers.

Table 1. Response of cultivars following natural infection with bacterial leaf spot

Cultivar	Leaf Spot* (%)	Leaf fall* (%)	Yield†
Burpee Fordhook	72	38	7.69 (8.1)
Golden Belle	74	39	8.05 (11.6)
Canape	66	39	8.63 (20.7)
Burpee Sunnybrook	76	42	7.93 (10.3)
Hungarian Yellow	70	42	8.67 (21.6)
Glory	73	42	7.54 (6.9)
Sweet Banana	65	45	8.75 (23.5)
Long Sweet Yellow	62	47	8.62 (20.5)
Sheba	84	48	7.92 (10.2)
Early Bountiful	76	53	8.22 (13.8)
Aconcagua	76	54	7.52 (6.8)
Long Green Sweet	79	55	7.90 (10.0)
Saitama Early	71	56	6.10 (1.6)
Pick-a-Peck	77	57	7.50 (6.7)
Jade	71	58	3.10 (0.1)
World Beater Thickwalled	70	59	5.93 (1.4)
Burpee Bellringer	72	59	3.01 (0.1)
Red Cherry Small	63	60	6.39 (2.2)
Burpee Tasty	73	61	7.52 (6.8)
Staddons Select	67	61	6.20 (1.8)
New Ace	72	62	6.79 (3.3)
Ace	76	62	7.30 (5.5)
Cubanelle	82	63	7.71 (8.3)
Calwonder 300	72	63	0 (0)
Grand Bell	72	64	0 (0)
Golden Calwonder	78	64	6.66 (3.0)
All Big	71	64	6.69 (3.0)
Miss Belle	78	66	6.48 (2.4)
Pimiento Select	74	66	7.26 (5.3)
Super Set 19	76	66	7.57 (7.2)
California Wonder	74	67	0 (0)
Earliest	74	67	3.52 (0.1)
Florida VR-2	79	67	2.80 (0.1)
Florida Giant	74	68	5.47 (0.9)
Cadice	75	68	3.37 (0.1)
Bell Boy	74	68	5.45 (0.9)
Titan	74	68	3.18 (0.1)
Midway	80	69	6.28 (2.0)
Northern Belle	73	69	5.56 (1.0)
Keystone Resistant Giant	76	70	2.90 (0.1)
Green Giant	80	70	0 (0)
Mercury	76	70	0 (0)
Market Giant	78	70	2.44 (0)
Emerald Giant	75	70	4.74 (0.4)
Yolo Wonder	82	70	2.44 (0)
Harris Early Giant	83	70	2.44 (0)
LSD P=0.05	9	14	3.87

* Estimated number of leaves affected 17 weeks after sowing as a percentage.

†Natural log transformation of kg/plot. Parenthesis encloses yield in t/ha.

This resistance is not complete but its incorporation into a bell pepper will likely result in useful reductions in the rate of disease progress through a crop. This would reduce losses from BLS epidemics.

Table 2. Response of selected cultivars following inoculation and natural infection with bacterial leaf spot

Cultivar	Inoculated			Natural infection		
	Leaf* spot	Leaf* fall	Yield (t/ha)	Leaf* spot	Leaf* fall	Yield (t/ha)
Hungarian Yellow	29	7	17.3	6	2	17.8
Canape	40	18	14.5	16	5	18.4
Northern Belle× Hungarian Yellow (F_1)	42	10	19.4	34	18	25.3
Sheba	56	40	7.6	31	10	16.8
Northern Belle	84	70	6.1	84	80	7.7
Florida VR-2	90	88	1.4	91	61	8.0
LSD P=0.05	39	21	5.3	26	27	7.2

* Estimated percentage (number) of leaves affected 18 weeks after sowing.

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Dentition in beef cattle in northern Australia

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Abstract

Estimation of age of cattle is important to north Australian cattle producers who sell cattle on the basis of carcass classification, by description or to premium markets. Age at eruption of permanent incisor teeth of 52 Shorthorn and 42 Brahman×British steers grazing spear grass pastures in north Queensland was determined. Shorthorn steers cut each pair of incisor teeth when younger than Brahman×British steers ($P<0.01$). The 95% ranges for age at eruption in days for the four pairs were: 631 to 823, 772 to 1066, 951 to 1321 and 1181 to 1611 for Shorthorn and 679 to 871, 848 to 1142, 1055 to 1425 and 1306 to 1736 for Brahman×British, respectively. This large variation and overlap between successive pairs shows the limitations of dentition as an indicator of age of cattle for use in marketing.

INTRODUCTION

The need to assess age of cattle accurately, especially in north Australia, has not been of economic significance because the beef produced is sold to markets which do not currently link age of carcass with price paid. However, age will become important if north Australian cattlemen wish to use marketing options such as classification or sale by description. There are suggestions that premium markets for beef might impose age restrictions. Dentition, as an indication of age, is one criterion on which cattle and carcasses are categorised when birth dates are not known. In view of the wide variation in age at tooth eruption as a result of factors such as individuality, plane of nutrition and breed, the practice is considered to have limitations as a guide to the age of cattle (Burns 1959; Steenkamp 1970; Andrews 1973).

There is a paucity of published data on how accurately the number of erupted permanent incisor teeth indicates age of cattle under Australian conditions. No data are available for animals run in tropical regions or for the commonly used *Bos indicus* infused cattle.

This paper reports age ranges for the eruption of permanent incisor teeth in Shorthorn and Brahman×British steers grazing native pastures in north Queensland.

MATERIALS AND METHODS

Location

The study was conducted at Swan's Lagoon Beef Cattle Research Station which is 110 km south of Townsville. The pastures and climate have been described previously by Winks *et al.* (1974).

Animals

Age at eruption of permanent incisor teeth of 52 Shorthorn and 42 Brahman×British steers was determined. The British component (40 to 60%) of the crossbred steers was either Hereford or Shorthorn.

The steers were born between June and September 1972 and their birth dates and birth weights recorded. When observations commenced during April 1974 the mean ages of the Shorthorn and Brahman×British steers were 596±6.5 (SE) and 633±7.2 days, respectively.

Before weaning the steers were segregated by breed and grazed either native pasture or Townsville stylo pasture fertilised with superphosphate. The steers were weaned in May 1973 and then managed as a common group until all had eight permanent incisor teeth. Grazing was predominantly black spear grass (*Heteropogon contortus*), giant spear grass (*H. triticeus*) and golden beard grass (*Chrysopogon fallax*) with some access to tassal blue grass (*Dicanthium tenuiculum*) and forest blue grass (*Bothriochloa bladhii*). A molasses–urea (230 and 60 g/hd/d respectively) supplement was fed during winter and spring each year.

Measurements

The steers were mustered every three weeks, weighed, and then restrained in a crush or head bail to record the number of permanent incisor teeth. A tooth was considered to have erupted when it had broken through the gum and a pair to have erupted when the first tooth had broken through the gum.

Statistical analysis

Data were analysed by analysis of variance. Range in age at eruption of each pair of teeth was indicated for each genotype by 95% confidence intervals. The mean age for each steer with a given number of pairs of incisors was the age mid way between that for eruption of two successive pairs.

The effects of genotype, birth weight and liveweight gain on ages at tooth eruption were examined. Correlations between age at eruption of the first pair and age at eruption of subsequent pairs were determined.

RESULTS

Shorthorn steers cut each pair of incisor teeth when younger than Brahman×British steers ($P<0.01$) but within each genotype there was a wide range in the ages at which a given pair of incisor teeth erupted (Table 1).

Table 1. Mean age and range in age at eruption of each pair of incisor teeth and mean interval between eruption of teeth within each pair

Dentition	Breed	Mean age at eruption (days)	95% Range for age at eruption (days)	Interval between teeth erupted within pairs
1st pair	Shorthorn	727	631–823	n.r.
	Brah×Brit	775	679–871	n.r.
	LSD $P=0.05$	19.8		
2nd pair	Shorthorn	919	772–1066	3
	Brah×Brit	995	848–1142	17
	LSD $P=0.05$	30.4		9.2
3rd pair	Shorthorn	1136	951–1321	25
	Brah×Brit	1240	1055–1425	36
	LSD $P=0.05$	38.1		12.5
4th pair	Shorthorn	1396	1181–1611	24
	Brah×Brit	1521	1306–1736	49
	LSD $P=0.05$	44.3		20.8

n.r. = not recorded.

There was a positive relationship ($P<0.01$) within both genotypes between age at eruption of the first pair of incisor teeth and age at eruption of subsequent pairs with correlation coefficients ranging from 0.42 to 0.65. Steers which cut their first pair of incisor teeth when younger than other steers also cut subsequent pairs when younger than their contemporaries.

Mean intervals between eruptions of successive pairs of incisor teeth were less in Shorthorn than in Brahman×British steers for all pairs but the difference was significant ($P<0.05$) for intervals between the first and second and second and third pairs only.

The interval between the eruption of the two teeth of the first pair was not recorded. Mean intervals between the eruptions of individual teeth in subsequent pairs were shorter for Shorthorn than for Brahman×British but differences were significant ($P<0.05$) for the second and fourth pairs only.

The mean ages of steers with one pair, two pairs, or three pairs of permanent incisor teeth were 822, 1027 and 1267 days,respectively for Shorthorn steers; and 885, 1118 and 1381 days, respectively for Brahman×British steers. Corresponding pooled standard deviations were 54.3, 77.0 and 92.6 days, respectively.

Birth weight was negatively correlated with age at eruption of incisor teeth in Shorthorn steers, the coefficients varying from −0.23 to −0.43. These relationships were significant for the first and second pairs ($P<0.01$) and the fourth pair ($P<0.05$). The corresponding relationships in Brahman×British steers were low and not significant, varying from −0.03 to 0.17.

Liveweight gain from birth to the commencement of the study was not correlated with age at eruption of incisor teeth. Average daily gains were 0.305 kg/hd for Shorthorn and 0.465 kg/hd for Brahman×British steers.

DISCUSSION

This study highlights the limitations of using the number of permanent incisor teeth as an indication of age of cattle and is in agreement with other observations (Burns 1959; Tulloch 1962; Steenkamp 1970; Andrews 1973).

In this study Brahman×British steers with one pair of incisor teeth had mean ages in the range 777 to 993 days but could have been as young as 679 days or as old as 1141 days. With the mean daily growth rate of 0.465 kg/hd recorded for Brahman×British steers in this observation, an extreme liveweight difference of 215 kg is possible.

Burns (1959) found that first and second pairs of incisor teeth erupted approximately one and three months later, respectively, amongst females than amongst contemporary Polled Hereford males. The range in ages at which these pairs of incisors erupted tended to be greater amongst females than males. Therefore, data from steers may not be appropriate for contemporary females.

The age at which incisor teeth erupt in Shorthorn steers has also been recorded by other authors. Tulloch (1962) reported that teeth in Shorthorns erupted earlier than in Herefords but at the same age as in Angus steers. Mostert (1972) found that teeth in Shorthorns erupted earlier than in a range of genotypes including Angus, Hereford, Africander and Bonsmara. The Bonsmara is a Zebu (Africander) British cross breed and it may be comparable to Brahman×British crossbreds.

The strong positive correlation between age at eruption of the first pair of incisors and age at eruption of subsequent incisors recorded in this observation suggests that beef

cattle have a pre-determined pattern of tooth emergence. We are unaware of similar findings with cattle but Adler (1963) has documented such a pattern with humans and suggested that it has a genetic background.

The intervals between eruptions of teeth within a pair were shorter than those recorded by Andrews (1974). This difference may be a function of the recording techniques used, as Andrews estimated when incisor teeth were fully erupted. In our observation the intervals between eruption within pairs of incisors increased from the second to the fourth pair and were greater in Brahman×British steers. The greater variation in the crossbreds may be a function of genetic variation but whatever the reason it highlights the importance of a consistent recording technique.When comparing our data with those of other studies, a variation of 49 days is possible if assessing the age of Brahman×British steers on the basis of 7 or 8 incisor teeth erupted.

The negative correlation between birth weight and age of eruption of permanent incisor teeth in Shorthorn steers indicates that heavier calves tended to cut their teeth at younger ages than calves of lower birth weight.

Commercial implications

The Australian meat industry is currently striving to objectively classify bovine carcasses as a means of improving market transactions and identifying carcasses of superior eating quality. Dentition is currently used as an indication of age. This study supports other studies which have shown dentition to be an imprecise indicator of age of cattle and provides specific information on Shorthorn and Brahman×British cattle in the north Australian environment. The large variation in ages at eruption of incisor teeth recorded here results in considerable overlapping of the distributions for successive pairs. This indicates that low priority be given to pricing carcasses on the basis of dentition and that biologists be encouraged to search for a more accurate method of assessing age of cattle or alternatively meat tenderness.

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High concentrate feeding and growth promotants for Brahman crossbred steers

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Abstract

Two experiments using *Box indicus*×*Bos taurus* steers were carried out to investigate the effectiveness of three growth promotants; zeranol, oestradiol-17β and oestradiol benzoate-progesterone in association with high concentrate feeding. In contrast to most reported work none of the three treatments significantly ($P>0.05$) affected growth rate, final liveweight or fat depth.

INTRODUCTION

As lot feeding costs are high there is a need to maximise growth rate. Growth promotants are commonly used for this purpose. Although their effectiveness has been widely demonstrated, there have been some reports, particularly from feedlots in Queensland, of failure to increase gain, (Venamore *et al.* 1982 and Hodge *et al.* 1986).

Information on the product oestradiol benzoate-progesterone under Queensland conditions is scarce and more so its effectiveness in Brahman crossbred steers under feedlot conditions. Therefore we investigated the effectiveness of this compound, zeranol and oestradiol-17β in feedlots.

MATERIALS AND METHODS

The two experiments were carried out in a feedlot 45 km south-east of Springsure (24°23′S, 148°21′E) Queensland. The steers used were bred and reared on native pastures on the property. At the time of introduction into the feedlot the cattle were vaccinated against the major clostridial diseases. All growth promotants were implanted in the ear, subcutaneously, at the manufacturers recommended sites.

The time of slaughter was determined by unfasted liveweight and the manager's estimate of fat cover. Therefore, a number of drafts of animals were slaughtered on different occasions in each experiment.

Approximately one hour after slaughter, carcass weight and subcutaneous fat depth at the sacral crest site (Johnson and Vidyadaran 1981) were measured.

Experiment 1

One hundred and thirty-two Brahman crossbred (1/2 to 3/4 *Bos indicus*×*Bos taurus*) steers aged 3 to 3.5 years were introduced into the feedlot and treatments were imposed nine days later. Steers were fed an initial ration and over a period of 8 days progressively

brought onto a 90% sorghum ration. At commencement of the experiment, the steers (457.7±44.2 kg) were allocated by stratified randomisation to the following treatment groups on unfasted liveweight:

1. Control, C, 34 steers;
2. 20 mg oestradiol benzoate and 200 mg progesterone 100 day implant (Synovex-S), OB-P, 34 steers.

The average feeding period for the steers in Experiment 1 was 75 days.

Experiment 2

One hundred and thirteen Brahman crossbred steers *Bos indicus*×*Bos taurus* steers aged 2 to 2.5 years were used in this experiment.

All steers in this experiment were treated with injectable levamisole (Nilverm®) at commencement of the experiment.

The introductory feeding regime took 26 days to reach a 90% sorghum diet. After 58 days 2% molasses was added to the ration and the grain changed from 90% sorghum to 40% sorghum and 48% wheat for the rest of the experiment.

At the start of the experiment the steers (411.2±34.6 kg) were allocated by stratified randomisation to the following treatment groups on unfasted liveweight:

1. Control, C, 25 steers;
2. 36 mg zeranol implant (Ralgro®), ZERA, 28 steers;
3. 20 mg oestradiol benzoate and 200mg progesterone implant, OB-P, 30 steers; and
4. 24 mg oestradiol-17β implant (Compudose 200), O-17β, 30 steers.

The average feeding period for the steers in Experiment 2 was 75 days.

Statistical analysis

The data from both experiments were analysed by a least squares analytical model for unequal cell numbers using initial unfasted liveweight as a covariate (Harvey 1960), to correct for bias in initial treatment means. Pairwise differences between treatments were tested using a protected least significant differences technique.

RESULTS

The results are shown in Tables 1 and 2. In no group was there a significant ($P<0.05$) response to treatment with a growth promotant.

Table 1. Experiment 1: Effect of growth promotant on feedlot gain, sale liveweight, carcass weight and sacral crest fat depth

Parameter	Control	OB-P
Number of steers	34	34
Feedlot gain (kg/day)	1.51	1.87
Sale liveweight (kg)	588	614
Carcass weight (kg)	333	339
Fat depth (mm)	11.2	13.8

Table 2. Experiment 2: Effect of growth promotants on feedlot gain, sale liveweight, carcass weight and sacral crest fat depth

Parameter*	Control	ZERA	OB-P	0-17β
Number of steers	25	28	30	30
Feedlot gain (kg/day)	2.15	2.16	2.25	2.22
Sale liveweight (kg)	566	567	573	569
Carcass weight (kg)	299	298	300	294
Fat depth (mm)	10.1	11.6	11.9	12.8

* F values for each parameter were not significant ($P<0.05$).

DISCUSSION

Overseas workers have reported significant ($P<0.05$) positive responses to growth promotants under feedlot conditions Perry *et al.* (1970), Kahl *et al.* (1978), Wagner *et al.* (1979), Mathison and Stobbs (1983), Van Der Wal and Berende (1983) and Brown (1983). Similarly, significant ($P<0.05$) positive responses to ZERA and 140 mg trenbolone acetate combined with 20 mg oestradiol-17β (TBAO) have been recorded under feedlot conditions in Australia (Hodge *et al.* 1986; A.W. Plasto pers. comm. 1986) in *Bos indicus*×*Bos taurus* and *Bos taurus* steers. However, Venamore *et al.* (1982), have documented results from two feedlot sites where *Bos taurus* steers, *Bos indicus*×*Bos taurus* and *Bos indicus* steers, respectively, were fed high grain feedlot rations and ZERA implants failed to produce significant ($P>0.10$) responses over periods of 82 and 74 days, respectively. Hodge *et al.* (1986) and A. W. Plasto (pers. comm. 1986) have both recorded cases where there have been no significant ($P>0.05$) responses to ZERA implants in *Bos indicus*×*Bos taurus* steers fed high energy rations under feedlot conditions for periods of 72 days and 102 days, respectively. While these failures to respond to treatment have a common denominator in genotype and feed, the same genotypes given similar feed have in other reported research have had increased liveweights as would be expected (Hodge *et al.* 1986).

The comparitively short period of feeding may have been a contributory cause.

Treatment is not without some undesirable treatment effects. We observed side effects of bulling, preputial 'tipping', prolapses and elevated tail heads with the growth promotants used. Similar side effects were reported by Dickie and Forsyth (1982) and Knights and Venamore (1985). The side effects did not appear to affect growth rate or carcass composition in these experiments but if preputial prolapses were traumatised and strictures and urinary retention occured, this could affect liveweight gain (T. J. Tierney pers. comm. 1986).

The uncertainty of response, and possibility of undesirable side effects must be considered when deciding whether or not to treat Brahman steers, in feedlots, with the compounds we used.

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