Identification, vector relationships, epidemiology and control of virus and bacterial diseases of banana.

Final Technical Report for project A0507/X0285 (follow-on to A0217/X0285/A0365).

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Executive Summary

Virus and bacterial diseases are major constraints to banana production by both commercial and small-scale growers in the Philippines (and elsewhere in South-east Asia). The purpose of this project was to build on and link to the findings of a previous project (R5234) seeking more substantive information (in the Philippine context) on the vectors of these diseases and the factors influencing the vector population dynamics and movement, and hence the pattern and rate of spread of the diseases. The intention initially was to concentrate on banana bunchy top virus (BBTV) and Moko and Bugtok diseases caused by *Ralstonia (Pseudomonas) solanacearum* since these were considered some of the most important diseases of banana in the Philippines. A better understanding of the factors influencing the increase and spread of these diseases should allow the formulation of improved control strategies.

During the preceding project (R5234), four trial sites each consisting of at least 600 virus-indexed banana plants were established. During this project (R6579), at each of three of the sites each plant was assessed on a regular basis both visually and by enzyme-linked immunosorbent assay (ELISA) to monitor ingress and spread of BBTV at the sites. Climatic records were kept for each site, and the number of, and type of, aphids caught in traps or observed on a sample of banana pseudostems at each site were also recorded. Despite following the routine management practice employed in the area of digging out infected plants and replacing them with healthy ones, the monthly incidence of BBTV gradually increased in all three sites. However, only at one site (Panabo) was the incidence sufficiently high to readily observe aggregation of BBTV infections, indicating that some secondary spread of the disease was occurring. Alate *Pentalonia nigronervosa* (the aphid vector of BBTV) were caught most frequently on yellow sticky traps between ground level and 1m above ground level in the experimental plots of banana. Numbers caught on sticky traps or by a suction trap could not readily be correlated with population estimates based on counting apterae and/or alatae on the banana pseudostems. Further analysis is required to determine if aphid numbers can be related to climatic factors and BBTV incidence. The roguing practice followed in these trials, appeared not to be as efficient as previously believed since it was common to find re-growth of infected suckers at locations where infected mats had been rogued. Preliminary results obtained at the end of the project suggested that the "vampire" method whereby infected banana mats were treated by driving split bamboo skewers soaked in the herbicide "glyphosate" into the base of the pseudostems of the mother-plant and suckers might be an efficient means of eradicating infected mats.

Early in the preceding project (R5234) an assessment was made of the then available methods for detecting BBTV. This concluded that, on the basis of reliability, availability and cost, a serological assay (using the monoclonal and polyclonal antibodies produced by Dr. Thomas (QDPI)) was currently the most applicable for use in the field. Facilities to perform this assay were transferred to the Bureau of Plant Industry, Davao. Subsequently, a polymerase chain reaction(PCR)-based method for detecting BBTV was evaluated and used to confirm the presence of the virus in samples received from Malawi (Kenyon *et al.* 1997). The available antisera against banana bract mosaic potyvirus (BBrMV) were found not to be suitable for use in traditional micro-titre plate-based immuno-assays, and thus the equipment and reagents for protein electrophoresis and immuno-blotting were transferred to the BPI.

Information on the epidemiology of BBTV was also obtained by compiling and analysing disease incidence data from commercial banana farms in the Davao city region. From these data, models of a BBTV disease epidemic were developed to incorporate the two key features of

an epidemic in a plantation in the Philippines: an exponential increase in disease incidence over 10 years, and a declining gradient of incidence from the outside edge of the plantation to the centre. A non-spatial model consisted of three difference equations to describe the numbers of latently infected and of infectious plants in the plantation and the size of the inoculum source outside the plantation. In a spatial model the outside portion of the plantation was divided into 8 blocks running parallel to the outside edge. The dispersal gradient of the inoculum was assumed to be negative exponential. Analysis of the two models showed that for disease incidence to increase exponentially over time, the rate of disease progress could be dependent either on internal spread and roguing rate (proportion of diseased plants removed and replaced per unit time) or on the rate of increase of external inoculum pressure. The observed incidence gradient from the edge to the centre of the plot could be explained only by parameterising the spatial model so that external inoculum dominated. This model was also used to explore a variable roguing rate across blocks. Simulations indicated that this may produce small gains over the adoption of a constant roguing rate over all blocks, but was risky because a shift of roguing emphasis only slightly too far towards the outside blocks can result in a dramatic increase in disease (Smith *et al.* 1998).

The incidence of BBrMV also appeared to be increasing in the Philippines. Transmission studies (in association with a UNDP-funded project) showed that BBrMV is most efficiently transmitted by the aphid *Pentalonia nigronervosa,* but can also be transmitted by *Aphis gossipii and Rhopalosiphum maydis.* BBrMV is only distantly related to abaca mosaic potyvirus.

During experiments to try to identify the putative insect vectors of *R. solanacearum,* banana-infecting strains of the bacterium were isolated from individuals of the banana thrips and a number of hymenoptera caught on Bugtok-infected bunches of cooking banana. Study of the phylogenetic and pathogenicity relationships between Bugtok and Moko isolates of *R. solanacearum* is still underway, and will be the subject of a PhD thesis to be completed later this year (Thwaites 1998).

A study of small-holder banana growers perceptions of banana disease and knowledge and understanding of currently recommended control practices was initiated during the previous project and continued during this one. Most farmers had heard of BBTV but did not recognise it until the disease was at an advanced stage; young infected suckers were completely overlooked. BBrMV and CMV generally were not recognised, probably because they do not usually cause as much apparent damage. Farmers were confused over the different control measures used for different diseases and there was a lack of a clear extension messages. Recommended control measures of complete eradication of BBTV-infected plants were not adhered to, even when farmers were aware of them, as they tended to leave part of the infected root mats and young suckers in the ground after the infected mother plant had been removed. Farmers were unaware that this provided a source of disease inoculum which could then be spread to other plants. Even the farmers who knew that BBTV was a virus spread by aphids, and who had good contact with extension staff, did not appear to be aware of the risks of leaving infected suckers in the farm. New planting material usually came from the farmer's existing plants, or was bought from neighbouring farms. Tissue cultured material generally was not available to individual farmers. Where it was available it was often planted in amongst infected plants, and hence most of the benefits of clean planting material were soon lost.

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Acronyms and Abbreviations

APO(S)	Associate Professional Officer (Scheme)
ABTV	abaca bunchy top virus
AbaMV	abaca mosaic virus
BBrMV	banana bract mosaic potyvirus
BBTV	banana bunchy top virus
BPI	Bureau of Plant Industry, Philippines
CMV	cucumber mosaic cucumovirus
DAS	Double antibody sandwich (ELISA)
DFID	Department For International Development
DMV	dasheen mosaic potyvirus
DNCRDC	Davao National Crop Research & Development Centre
ELISA	enzyme-linked immunosorbent assay
HRI	Horticulture Research International
IMTP	International Musa Testing Program (INIBAP)
INIBAP	International Network for the Improvement of Banana and Plantain
IPGRI	International Plant Genetic Resources Institute
ISEM	immuno-sorbent electron microscopy
IRRI	International Rice Research Institute, Los Baños, Philippines
NRI	Natural Resources Institute
ODA	Overseas Development Administration
PCR	Polymerase chain reaction
PCARRD	Philippine Council for Agriculture, Forestry and Natural Resources Research and Development
PTA	Plate-trapped antigen (ELISA)
PVY	potyvirus (potato virus Y)
QDPI	Queensland Department of Primary Industries
SMAP	Southern Mindanao Agricultural Project (EU funded)
SDS-PAGE	sodium dodecyl sulphate - polyacrylamide gel electrophoresis
TAS	Triple antibody sandwich (ELISA)
TRRC	Twin Rivers Research Centre

Background

The Asia and Pacific region including the Philippines is considered to be the centre of origin of the family Musacea (bananas and plantains). It is hence likely to be the centre of origin and diversity of many of the pests and diseases attacking this family. Banana virus diseases, especially banana bunchy top (BBTV) have long been recognised as major constraints to banana production by both the commercial plantations and the small scale grower. In 1993 the International Network for the Improvement of Bananas and Plantains (INIBAP) ranked the diseases of banana in this region in the following order of decreasing importance: Panama disease (= Fusarium wilt, *Fusarium oxysporum* fsp. *cubense*), Sigatoka (*Mycosphaerella musicola* & *M. fijiensis*), BBTV, Bacterial wilt (Moko), cucumber mosaic virus (CMV) and nematodes. Perhaps because of changing cultural practices, the introduction of new/improved banana cultivars or the introduction of new diseases or races of already present diseases, the order of importance of these diseases may be changing; banana bract mosaic potyvirus (BBrMV) and banana streak badnavirus (BSV) appear to be increasing in importance both in the Philippines, Sri Lanka on the Indian sub-continent.

Crop protection measures can account for up to 70% of the production costs for bananas in commercial plantations in the Philippines. Depending upon the location, local conditions and age of crop this figure can be divided up more or less equally between control of weeds, nematodes, insects, fungal and bacterial diseases, and virus diseases (personal communication with growers in Mindanao region). Similar costs are probably incurred by commercial growers in other countries of the region.

Control of virus diseases in intensive banana plantations used to rely heavily on the blanket spraying of insecticides to control the insect vectors. However, the increasing awareness of the hazard this approach can pose to the environment, plantation workers and end consumers, means that growers are under mounting pressure to reduce pesticide applications. Excessive use of pesticides is also likely to result in the selection of pesticide resistance in the pests, while reducing the populations of natural enemies. Insecticide sprays are unlikely to be of any practical use in smallholder plantings unless very well timed and targeted, and anyway would be generally too expensive.

Many viruses, as systemic pathogens, are also readily disseminated and perpetuated in vegetatively propagated crops adding further complication to control strategies. Because the different viruses that can infect banana in the region have different vectors and different rates of spread and multiplication in the host, each may require a slightly different control strategy. In order to develop economically and environmentally sound control strategies for use in small holdings and commercial plantings, simple and reliable methods for detecting and distinguishing between the viruses, and a better understanding of the epidemiology of the viruses are required.

Of the four major virus diseases of banana, BBTV is regarded as the most damaging, and has received the most research attention. However, this effort has mainly been directed at the etiology and vector relationships, and little has been done on the factors affecting its spread and increase. In Australia, where these factors were studied, BBTV was practically eliminated by enforcing a strict policy of identifying and quickly destroying all occurrences of the disease in each plantation. Attempts to use this strategy appear at best only to be keeping

the disease at bay in the commercial plantations of the Philippines and neighbouring countries. This is probably for two reasons; (1) there are numerous small-hold plantings of banana where recommendations to destroy infected stands go unheard or unheeded, and which act as sources of the disease, and (2) the local climatic and environmental conditions and the varieties grown and cultural practices differ from those encountered in Australia and may influence the numbers and activity of the aphid vectors.

Bugtok and Moko disease of cooking and desert banana respectively are both increasing in the Philippines and are both caused by the bacteria *R. solanacearum*. As yet, there is no clear explanation as to why the organism causes such different diseases in such closely related host species. The suggestion is that there may be Bugtok- and Moko-strains of the pathogen, that it may be related to different modes of transmission, that it may be related to the morphological and genetic differences between cooking and desert bananas, or a combination of these.

In the Philippines, bananas are grown under conditions ranging from large multinational (monocropping) plantations to small, mixed stands in small-holdings and back-yards. What disease management is practised (if any at all) is likely to be dependant on the size of the enterprise and the wealth (and education) of the grower. However, because of the ways the diseases can move and be transmitted, what is practised by one grower is likely to also influence what happens to his neighbours crop.

Project Purpose

The overall purpose of this project was to **contribute to improving yields and enhancing sustainability of banana cultivation** by cost-effective reduction in losses caused by virus diseases through the formulation of improved control practices. The aim was to build on and link to the previous project on banana virus identification and epidemiology (A0217/X0285) based in the Philippines, and the UK-based project on banana bacterial diseases (A0365). The specific objectives at the start of this follow-on project were:

1. To maintain the trials already established in the Davao region of the Philippines, and to continue to monitor the ingress and spread of banana bunchy top virus (and if feasible, other virus and bacterial diseases). The data collected from the trials to be used to assess the factors influencing the distance, rate and pattern of spread of banana bunchy top virus (and possibly other virus diseases) in this region.

2. To evaluate alternative methods for detecting/identifying viruses of banana, and where applicable transfer these to the Philippines.

3. To establish cultures of *Pentalonia nigronervosa* and other aphids at BPI and use these to study the transmission of bract mosaic virus using banana, abaca and other hosts.

4. To investigate procedures for following the population dynamics and movement of aphids in the field.

5. To collect disease incidence data from banana growers in the Davao region and use these to assess the effect of different factors on the rate and pattern of spread of the disease.

6. To investigate the identity and phylogenetic relationship between the bacteria causing Bugtok and Moko, and attempt to identify the principal vectors of these two diseases in the Davao region of the Philippines.

7. To conduct further socio-economic studies on farmers' perceptions and attitude towards banana diseases and on the factors that influence the type and source of the planting material used and the role of micro-propagation.

Research Activities

(1) Field trials for studying epidemiology of banana viruses

During the earlier projects (A0217/X0285) towards the end of 1994, four plots each of about 600 plants of the local desert banana "Lakatan" (AA/AAA) were planted at different locations in the Davao City region of Mindanao (Lot-18 BPI station Bago-Oshiro, Kadalian, Catalunan Grande and Panabo). The plants were grown from tissue culture and were indexed for BBTV prior to planting out in the field. Plant spacing was 3m x 3m, and each plant in each plot was given a unique identity number. Cultural management at each site varied slightly depending on the management practices of the particular grower.

The plots were monitored on a regular basis during the original project for occurrence and spread of virus diseases. For this follow-on project, the monitoring was continued for all the sites except Kadalian, which had been found to be performing poorly owing to corm weevils (*Cosmopolites sordidus*) and Panama disease. Similarly, daily records of rainfall, maximum and minimum temperature and humidity for the previous 24 hour period continued to be taken for each of the three remaining sites. The three sites were also used for assessing methods for monitoring aphid populations and flight activity (see 4 below and annex 2).

For the last 6 months of the project, the alternative roguing practice of inserting split bamboo skewers, previously soaked in the herbicide glyphosate (Roundup) into the base of the pseudostem and suckers of BBTV-infected plants (vampire technique) was used in the Panabo trial.

(2) Tests for detecting and identifying viruses of banana

In the original project, a selection of antisera against virus types known to infect banana and thought to be present in the Philippines were obtained from a variety of sources including some commercial enterprises (Final report A217/X0285, 1996). At that time the focus of the work was BBTV, and equipment and reagents for enzyme linked immunosorbent assay (ELISA) of banana viruses (primarily BBTV) were transferred to the laboratories of the Bureau of Plant Industry (BPI), Bago-Oshiro, Davao, Philippines. ELISA using the monoclonal antisera produced by J Thomas (QDPI) against BBTV continued to be used on an occasional basis to monitor/confirm the incidences of BBTV in the three trial plots maintained during this follow-on project.

During the original project, antisera against BBrMV, BSV and CMV had been tested for use in ELISA for detecting these viruses. However, none was sufficiently specific to be reliable in this format of assay for detecting BBrMV or BSV. During this follow on project it became apparent that, in order to support the transmission studies (see below) a more reliable assay was required for BBrMV. To this end, more antisera against BBrMV was obtained (through a collaboration with J Thomas (QDPI) and M-L Iscra-Caruana (CIRAD), and equipment for protein electrophoresis and immuno-blotting (= western- blot) was set up at BPI Davao.

During the period of the original project, several groups had reported the development of molecular biological methods for the detection and characterisation of BBTV. From

assessment of the reports it was concluded that a polymerase chain reaction (PCR) assay incorporating a pair of oligonucleotide primers specific to DNA component 1 of BBTV was likely to be the most straight-forward and reliable of these methods. Consequently, the specific primers were synthesised and the test was established at NRI. The test was then used on banana leaf samples sent to NRI from Malawi by P Khonje.

(3) Transmission studies on BBrMV and AbMV

Observations from India, Sri Lanka and the Philippines indicated that BBrMV was becoming more common in all three countries and could be causing significant loss in production and fruit quality in some areas (see A0217 FTR). Since knowledge of the biology of BBrMV and its relationship to abaca mosaic virus (AbMV - another potyvirus infecting *Musa* species) was limited, experiments were conducted in association with a UNDP-funded project (through INIBAP to J Thomas, QDPI) to investigate the vector relationships of the two viruses.

Several individuals of each of the aphid species, *Pentalonia nigronervosa*, *Aphis gossipii* and *Rhopalosiphum maydis* were captured from apparently virus-free plants in Lot-18, BPI. These were used to established a culture of each species each in a separate screen cage at the BPI station. Individuals or groups of aphids were used from these cultures in studies on the transmission of BBrMV and AbMV from and between different *Musa* species and other potential hosts of the viruses.

(4) Monitoring *Pentalonia nigronervosa* in the field

Monitoring of aerial movement of *P. nigronervosa* at Lot-18 using a suction trap continued throughout the original project and this follow-on project. Since yellow pan traps had proved unreliable and did not provide much information during the original project, during the follow-on project alternative forms of trap were tested. Also, monthly counting of aphids on a sample of plants in each experimental plot was initiated to try to provide a measure of the total population of aphids in each plot over the season (see annex 2).

(5) Analysis of BBTV incidence records from commercial plantations

Though providing some useful information, the BBTV incidence data collected towards the end of the original project from the two commercial plantations was not considered to cover a suitably long time span to be statistically reliable. The way the data had been collected and recorded from Twin Rivers Research Centre (TRRC) had been changed too often for much meaningful analysis to be done. However, the data from Marsman plantation was suitably consistent and additional records were collected during this project and used in the development of a temporal and spatial model for the spread of BBTV.

(6) Vector relationships and phylogenetic studies on the Bugtok and Moko bacteria

Cultures of *Ralstonia (Pseudomonas) solanacearum* were isolated from the peduncles of banana plants showing symptoms of Bugtok in the Davao region of Mindanao using Engelbrecht selective media (Bacterial wilt newsletter No 10, 1994). Isolations were also made from insects caught on or near the flowers/fruit of infected plants. Cultures derived from Moko infected plants were provided by plant protection staff from TRRC and Marsman plantations. Cultures were tested for pathogenicity on young (2-3 wk) tomato (c.v. Moneymaker) and banana plants. PCR using primer pair OLI1 (Seal *et al*., 1993) and Y2 (Young *et al*., 1991) was used to confirm that the cultures were *R. solanacearum*. All genuine isolates were passed to R Thwaites for inclusion in his PhD studies into the molecular and phylogenetic relationships between the Bugtok and Moko pathogens.

(7) Socio-economic studies

Participatory rural appraisal and other survey techniques continued to be used during this follow-on project to obtain information on farmers' perceptions and practices relating to banana bunchy top virus and other banana diseases to augment that collected during the initial project.

Outputs

(1) Field trials for studying epidemiology of banana viruses

Each of the four field trials set up to study the temporal and spatial spread of BBTV in the Davao city area of Mindanao in the original project, was composed of at least 600 plants of the variety 'Lakatan' produced by tissue culture and indexed for BBTV by ELISA with Dr Thomas' antisera before planting out. Because one of the sites (Kadalian) had a major problem with corm weevils and Panama disease, it was abandoned and only three sites were maintained for the follow-on project.

Lot 18, Bureau of Plant Industry, Bago Oshiro

This site is close to the plant pathology laboratory and the tissue culture facilities of the BPI Davao station (7°04'47"N, 125°29'46"E), and because of this it is where the Burkhard suction trap was set up (see below). Before this trial, the site had been planted to c.v. Lakatan, but all these plants were destroyed before planting the new BBTV indexed plants. The site is at an elevation of about 160m ASL, is on fertile, well-drained soil, and there are numerous stands of desert and cooking banana in fences and gardens near-by (Figure 1)

Only 5 occurrences of BBTV were observed and confirmed by ELISA at Lot-18 during the period November 1994 to March 1996; too few to observe clustering or secondary spread of the virus (A0217/X0285 FTR, 1996). There were 32 occurrences of BBTV and 31 of BBrMV between January 1996 and October 1997 (Figure 2). There was a greater number of infections with BBTV along the southern (bottom of map) border of the plot than would have been expected if distribution had been random across the plot (Figure 3). When all the BBTV occurrences are mapped together as in Figure 3 there appear to be small aggregations. However, when the time dimension is included, these aggregations disappear. The same is true for the occurrences of BBrMV. There continued to be in the region of 100 plants exhibiting mild symptoms of what was suspected of being BBrMV. However, without a reliable and sensitive assay it was not possible to confirm the cause.

Having observed 12 plants infected with CMV during the first phase of the project, only two further cases were recorded in Lot-18 during January 1996-November 1997, and no BSV was confirmed in the trial.

Panama wilt continued to be a major problem in this trial. Typical of a soil/waterborne pathogen, the cases were aggregated along an unofficial footpath through the trial and along a line where surface water tended to pool and flow across and out of the trial. Attempts to eradicate the fungus from the soil using formaline or "Jays Fluid" were not effective. Thus in order to maintain the required population of bananas within the trial, where plants were removed because of Panama wilt, they were replaced with plants of the wilt resistant/tolerant hybrid, FHIA-1 (Gold Finger), from Honduras.

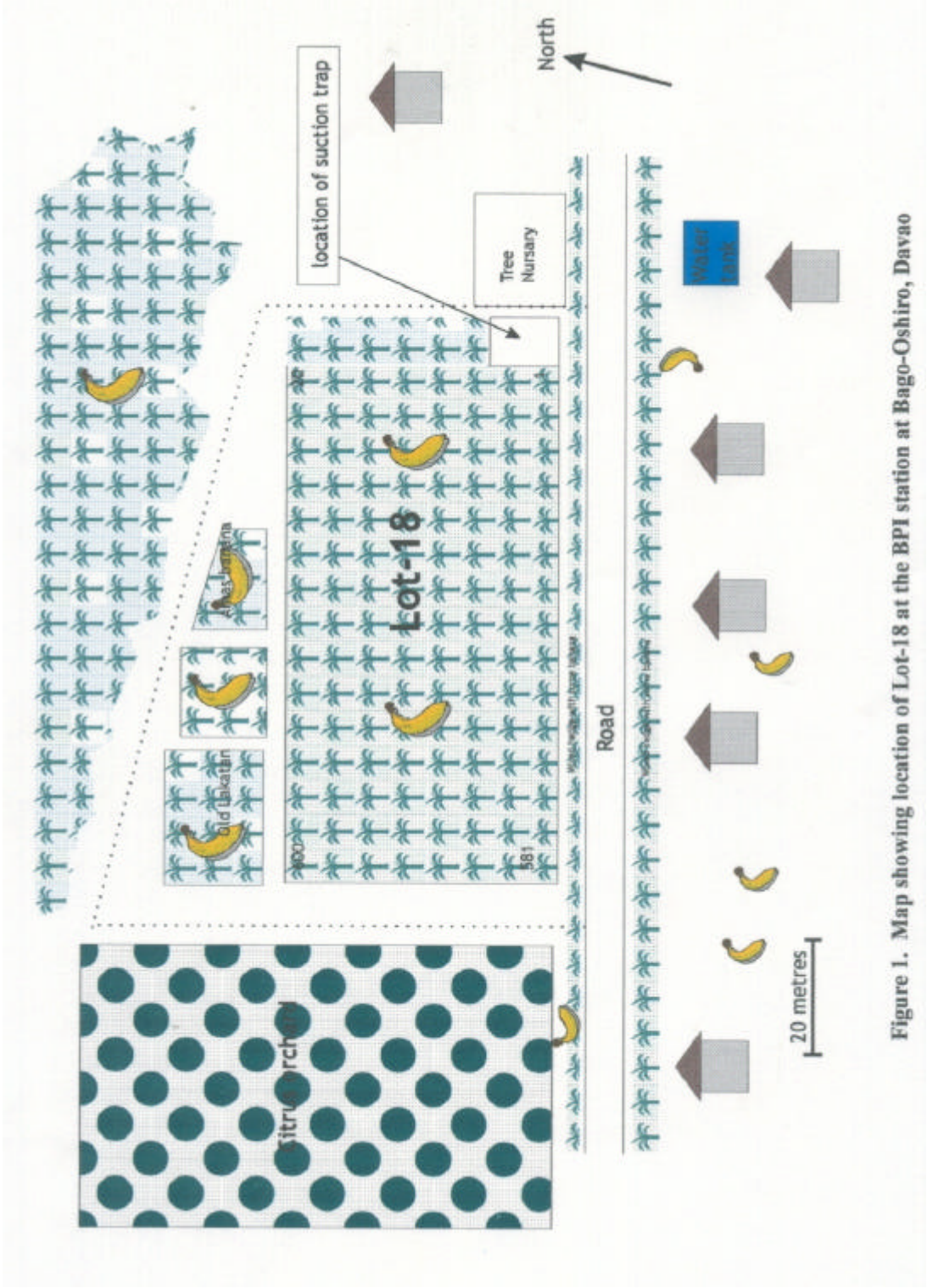

Figure 1. Map showing location of Lot-18 at the BPI station at Bago-Oshiro, Davao

Figure 1.

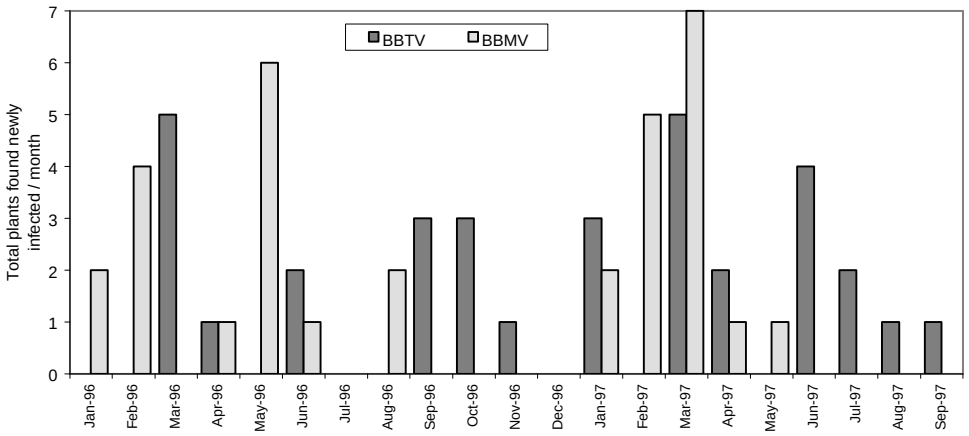

Figure 2 Monthly incidence of BBTV and BBrMV in Lot-18 from January 1996 to September 1997.

20	600	580	560	540	520	500	480	460	440	420	400	380	360	340	320	300	280	260	240	220	200	180	160	140	120	100	80	60	40	20
19	599	579	559	539	519	499	479	459	439	419	399	379	359	339	319	299	279	259	239	219	199	179	159	139	119	99	79	59	39	19
18	598	578	558	538	518	498	478	458	438	418	398	378	358	338	318	298	278	258	238	218	198	178	158	138	118	98	78	58	38	18
17	597	577	557	537	517	497	477	457	437	417	397	377	357	337	317	297	277	257	237	217	197	177	157	137	117	97	77	57	37	17
16	596	576	556	536	516	496	476	456	436	416	396	376	356	336	316	296	276	256	236	216	196	176	156	136	116	96	76	56	36	16
15	595	575	555	535	515	495	475	455	435	415	395	375	355	335	315	295	275	255	235	215	195	175	155	135	115	95	75	55	35	15
14	594	574	554	534	514	494	474	454	434	414	394	374	354	334	314	294	274	254	234	214	194	174	154	134	114	94	74	54	34	14
13	593	573	553	533	513	493	473	453	433	413	393	373	353	333	313	293	273	253	233	213	193	173	153	133	113	93	73	53	33	13
12	592	572	552	532	512	492	472	452	432	412	392	372	352	332	312	292	272	252	232	212	192	172	152	132	112	92	72	52	32	12
11	591	571	551	531	511	491	471	451	431	411	391	371	351	331	311	291	271	251	231	211	191	171	151	131	111	91	71	51	31	11
10	590	570	550	530	510	490	470	450	430	410	390	370	350	330	310	290	270	250	230	210	190	170	150	130	110	90	70	50	30	10
9	589	569	549	529	509	489	469	449	429	409	389	369	349	329	309	289	269	249	229	209	189	169	149	129	109	89	69	49	29	9
8	588	568	548	528	508	488	468	448	428	408	388	368	348	328	308	288	268	248	228	208	188	168	148	128	108	88	68	48	28	8
7	587	567	547	527	507	487	467	447	427	407	387	367	347	327	307	287	267	247	227	207	187	167	147	127	107	87	67	47	27	7
6	586	566	546	526	506	486	466	446	426	406	386	366	346	326	306	286	266	246	226	206	186	166	146	126	106	86	66	46	26	6
5	585	565	545	525	505	485	465	445	425	405	385	365	345	325	305	285	265	245	225	205	185	165	145	125	105	85	65	45	25	5
4	584	564	544	524	504	484	464	444	424	404	384	364	344	324	304	284	264	244	224	204	184	164	144	124	104	84	64	44	24	4
3	583	563	543	523	503	483	463	443	423	403	383	363	343	323	303	283	263	243	223	203	183	163	143	123	103	83	63	43	23	3
2	582	562	542	522	502	482	462	442	422	402	382	362	342	322	302	282	262	242	222	202	182	162	142	122	102	82	62	42	22	2
1	581	561	541	521	501	481	461	441	421	401	381	361	341	321	301	281	261	241	221	201	181	161	141	121	101	81	61	41	21	1
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30

Figure 3 Schematic map of Lot-18 where each cell represents a uniquely numbered plant. Cells filled with horizontal lines indicate those plants were infected with BBrMV, those with vertical lines were infected with BBTV, those with crossed lines were infected with both viruses.

Panabo, Davao del Norte.

The Panabo site (7°18'26"N 125°40'00"E) was within a small local commercial enterprise producing mainly Lakatan for shipment to Manila. Panabo is a warm, humid area close to the coast with good soils and only about 10 metres above sea level. The trial site was surrounded by banana plantation on three sides (Figure 5), where, though a regime of regular scouting and roguing of BBTV infected plants was in force, there were still relatively high levels of this virus present. The trial area received the same management and relatively high inputs as the rest of the plantation, and so produced a higher yield earlier than the other trials.

During the 15 months (Jan 1995 - March 1996) there were 80 incidents of BBTV in the Panabo trial, of which five were of plants that had replaced plants earlier eradicated owing to BBTV infection (A0217/X0285 FTR). The highest number of cases (14) was observed in November and the fewest (1) in July. There was some clumping of incidents in the peak months indicating a possibility of secondary spread within the trial.

Since the first phase of the project, there has been an increasing trend to the monthly incidence of BBTV. (Figure 4). Some of the occurrences appear aggregated (Figure 6).

For the final 5 months of the project, the "vampire technique" was used for eradicating BBTV-infected mats at this site. The declining monthly incidence during this period suggests that this method is more effective than that of digging out the infected mats. However, since this was an un-replicated test of just a few months, no statistical significance can be attached to the observations.

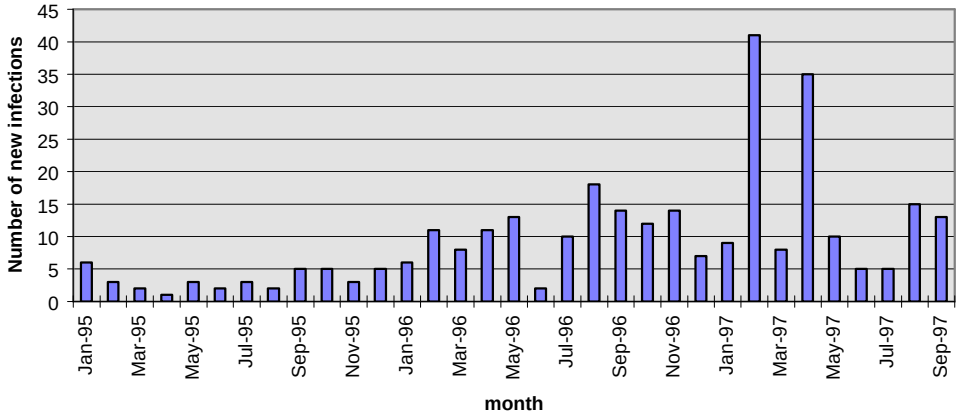

Figure 4. Total new BBTV infections recorded at Panabo each month (The vampire technique was started in April 1997)

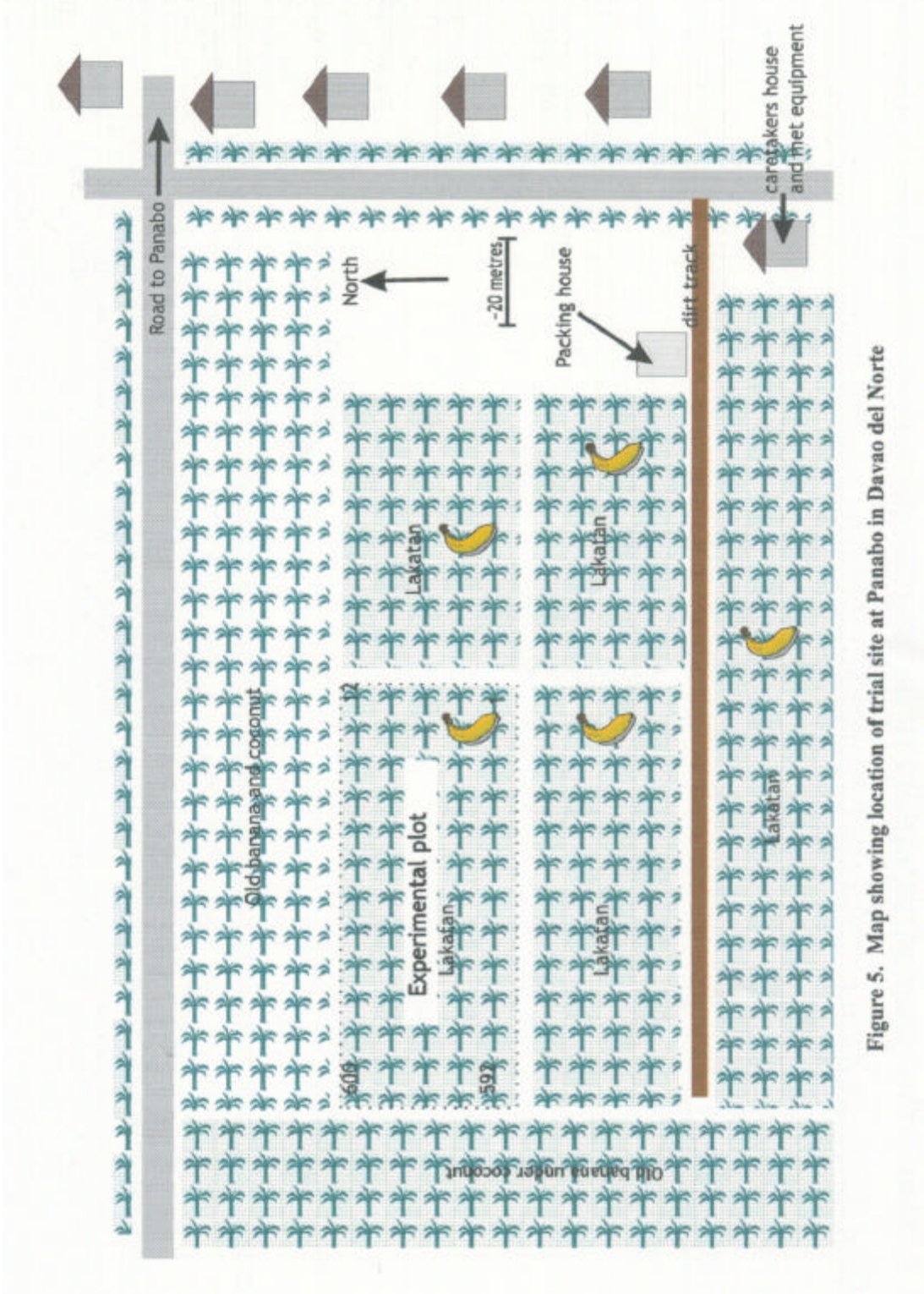

Figure 5. Map showing location of trial site at Panabo in Davao del Norte

Figure 5.

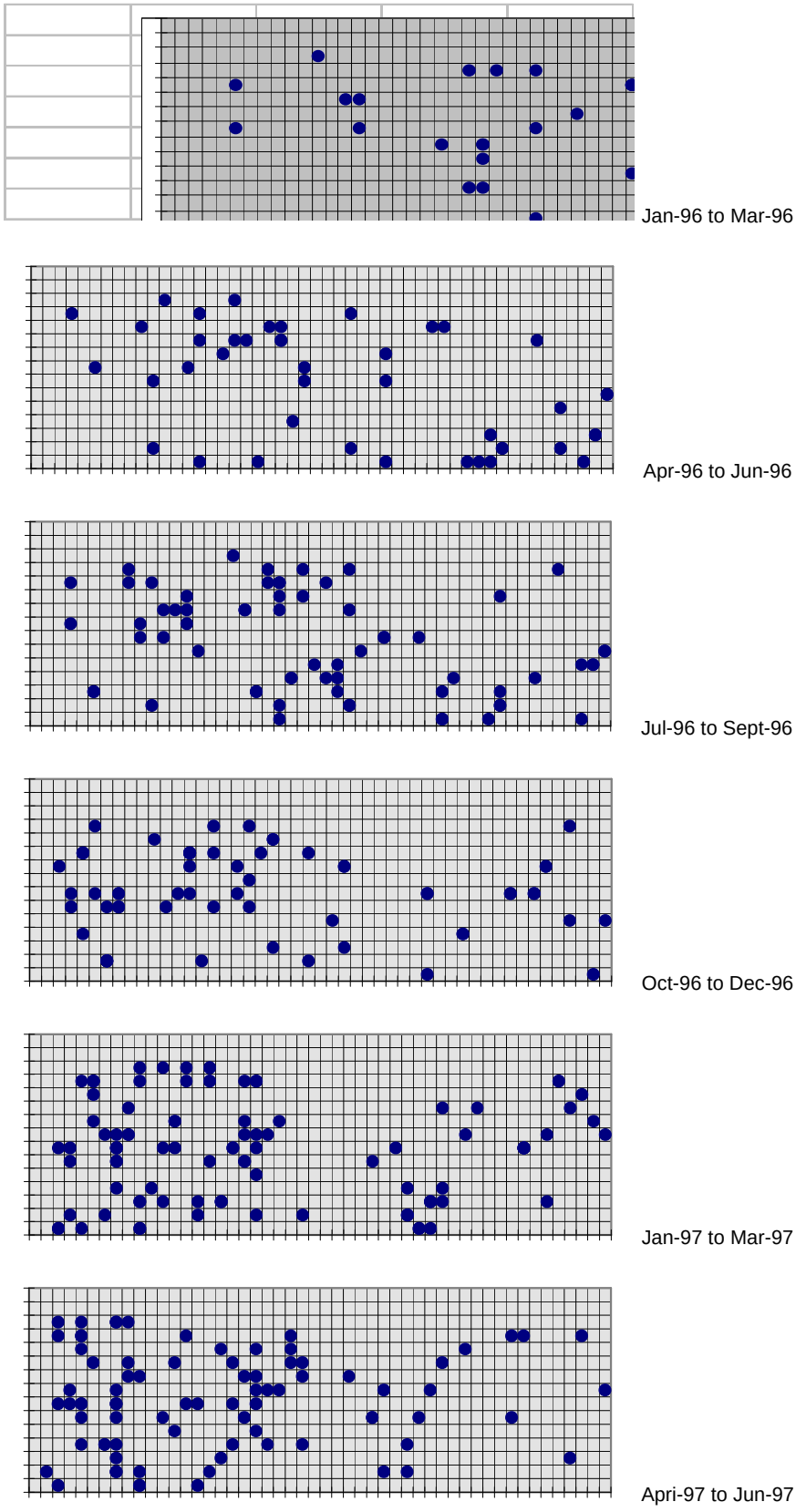

Figure 6. Schematic maps showing location of BBTV infections over various time periods at the Panabo site.

Catalunan Grande

This site was on a small farm (7°06'20"N, 125°31'10"E) with similar soils and at a similar altitude (140m ASL) to Lot-18. The site was newly cleared for the trial which was immediately surrounded by rough grassland. Another small plot of bananas (with regular occurrence of BBTV) was 50m to the south east. A dry spell at the time of planting meant that initial establishment of the trial was poor, and animals escaping into the trial also caused some losses. Twenty nine plants were observed to become infected with BBTV during the course of the first study (A0217/X0285 FTR). Two thirds of these were within the northern third of the trial and were in 2-3 loose aggregations.

As in the Panabo plot, incidence of BBTV has been steadily increasing (Figure 7), and there is some indication of secondary spread within the trial. (Figure 8)

Between May and August 1995 there were 12 occurrences of CMV. Banana streak badnavirus (BSV) symptoms were observed in four plants during the year, and in February 1996 BBrMV was confirmed in two plants.

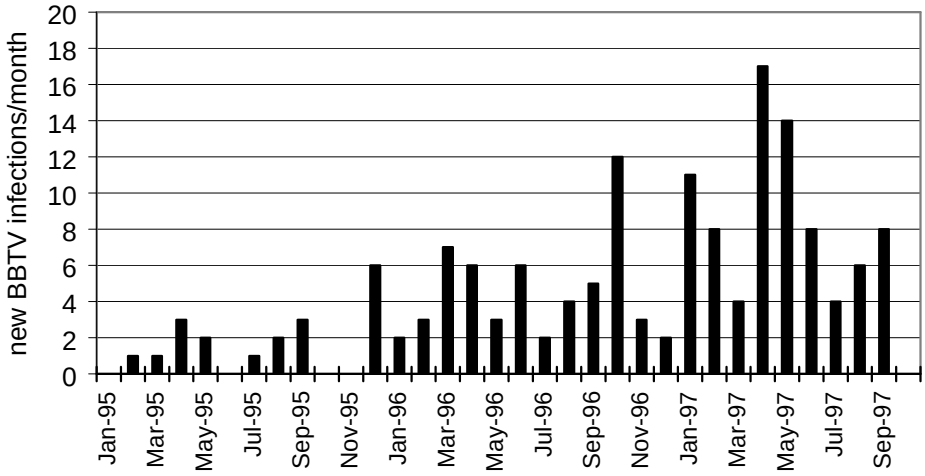

Figure 7. Monthly incidence of BBTV at Catalunan Grande site.

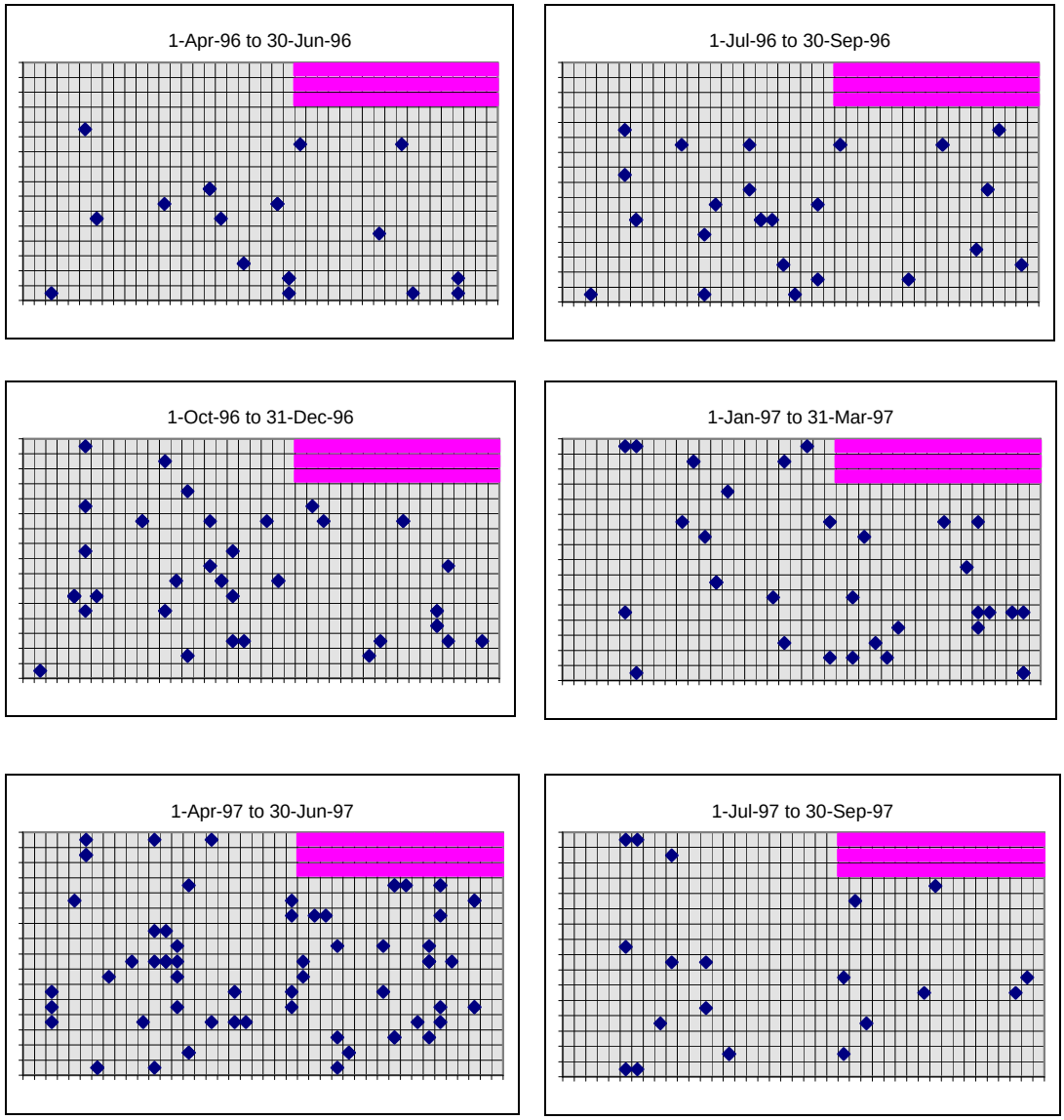

Figure 8. Maps showing locations of BBTV infected plants over various time periods at the Catalunan Grande site (each cell represents an individual mat; those filled with a dark diamond are infected).

Analysis of the data from the trial plots

Attempts have been made to analyse the data from these trials using "2D-Class" and "ST-Class" (SC Nelson, 1994), a pair of programmes for the PC designed to assess the degree of clumping/aggregation of infections in space, and space and time respectively. However, because the programmes are designed for annual crops where there is no roguing and replacing of the infected plants with healthy ones, they require some modification, or alternative procedures have to be found to analyse this banana data.

(2) Tests for detecting and identifying viruses of banana

As reported in the final report of the first phase of this work, at the start of the project the methods generally available for detecting and identifying viruses infecting banana were:

- field symptoms
- symptoms when transmitted to indicator plants, and host range
- electron microscope study of plant sap for virus particles (usually with antibody capture/ labelling-ISEM)
- serological detection (ELISA)

However, during that first phase several other workers were investigating the viruses of banana using the then rapidly developing array of molecular biology tools. The nucleic acid sequences of components of several of the viruses were determined, and these allowed the design of oligonucleotide primers for use in the polymerase chain reaction (PCR) amplification of specific viral sequences. For this project, the emerging techniques were regularly reviewed, and where feasible and applicable were tried out at NRI.

BBTV.

TAS-ELISA using the polyclonal and monoclonal antisera developed by J Thomas (QDPI) (Thomas & Dietzgen, 1991) remained the standard assay for the routine detection of BBTV in samples collected from the field sites in the Philippines since equipment and reagents for this had been installed at the BPI in the first phase of the project. During Autumn 1995, samples of banana with BBTV symptoms were received from P Khonje in Malawi. These tested positive for BBTV using the TAS ELISA, and were used to try out a PCR assay based on oligonucleotide primers BBT-1 and BBT-2 (Thomson and Dietzgen, 1995). A PCR amplification product of the right size (349 bp, see figure 9) was obtained from the Malawi samples. When this product was digested using restriction enzymes *Aci I* and *Rsa I* the restriction patters obtained were those expected for BBTV isolates of the South Pacific group (Figure 10). Product from a Philippine (Asian group) sample was not cut by these enzymes. This was the first report of BBTV in Malawi (annex 1).

```
   1 AGATGTCCCG AGTTAGTGCG CCACGTAAGC GCTGGGGCTT ATTATTACCC CCAGCGCTCG

  61 GGACGGGACA TTTGCATCTA TAAATAGACC TCCCCCCTCT CCATTACAAG ATCATCATCG

 121 ACGACAGAAT GGCGCGATAT GTGGTATGCT GGATGTTCAC CATCAACAAT CCCACAACAC

 181 TACCAGTGAT GAGGGATGAG ATAAAATATA TGGTATATCA AGTGGAGAGG GGACAGGAGG

 241 GTACTCGTCA TGTGCAAGGT TATGTCGAGA TGAAGAGACG AAGCTCTCTG AAGCAGATGA
                (BBT-1)
 301 GAGGCTTCTT CCCAGGCGCA CACCTTGAGA AACGAAAGGG AAGCCAAGAA GAAGCGCGGT
                  Cf0 I                                        Aci I
 361 CATACTGTAT GAAGGAAGAT ACAAGAATCG AAGGTCCCTT CGAGTTTGGT TCATTTAAAT

 421 TGTCATGTAA TGATAATTTA TTTGATGTCA TACAGGATAT GCGTGAAACG CACAAAAGGC

 481 CTTTGGAGTA TTTATATGAT TGTCCTAACA CCTTCGATAG AAGTAAGGAT ACATTATACA

 541 GAGTACAAGC AGAGATGAAT AAAACGAAGG CGATGAATAG CTGGAGAACT TCTTTCAGTG
      Rsa I                              (BBT-2)
 601 CTTGGACATC AGAGGTGGAG AATATCATGG CGCAGCCATG TCATCGGAGA ATAATTTGGG

 661 TCTATGGCCC AAATGGAGGA GAAGGAAAGA CAACGTATGC AAAACATCTA ATGAAGACGA

 721 GAAATGCGTT TTATTCTCCA GGAGGAAAAT CATTGGATAT ATGTAGACTG TATAATTACG

 781 AGGATATTGT TATATTTGAT ATTCCAAGAT GCAAAGAGGA TTATTTAAAT TATGGGTTAT

 841 TAGAGGAATT TAAGAATGGA ATAATTCAAA GCGGGAAATA TGAACCCGTT TTGAAGATAG

 901 TAGAATATGT CGAAGTCATT GTAATGGCTA ACTTCCTTCC GAAGGAAGGA ATCTTTTCTG

 961 AAGATCGAAT AAAGTTGGTT TCTTGCTGAA CAAGTAATGA CTTTACAGCG CACGCTCCGA

1021 CAAAAGCACA CTATGACAAA AGTACGGGTA TCTGATTGGG TTATCTTAAC GATCTAGGGC

1081 CGTAGGCCCG TGAGCAATGA ACGGCGAGAT C
```

Figure 9. DNA sequence of BBTV component 1 of an Australian Isolate (Harding et al, 1994) showing in red the region amplified using primers BBT-1 and BBT-2 (underlined) and restriction enzyme cutting sites (underlined green) specific to South Pacific isolates.

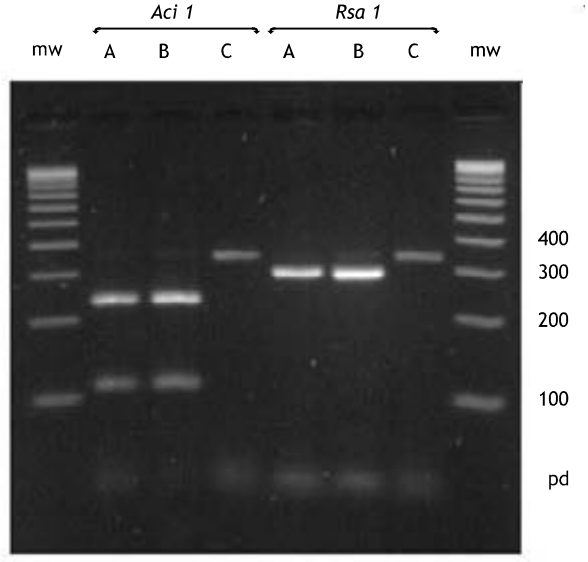

Figure 10. Ethidium bromide-stained agarose gel of restriction digests of PCR amplification products. A = sample from Malawi, B = sample from India (South Pacific group), C = sample from Philippines (Asian group; not cut with these enzymes), mw = molecular weight marker (bp - ladder), pd = primer-dimer.

BBrMV.
Though BBrMV was first recognised as a potyvirus in late 1980s (Magnaye & Espino, 1990), the attempts since then to make a reliable, specific antiserum against it for diagnostic use generally have not met much success. Polyclonal and monoclonal antisera developed against the virus particles by both J. Thomas (QDPI) and M-L Iscra-Caruana were tested in various ELISA formats at the BPI lab in Davao, but all suffered with very high background readings. To overcome this, facilities were established at BPI to use these antisera in immuno-"western" blots where non-specific binding of the antisera is not such a problem. A reliable test for BBrMV was required in order to be more confident about the results of the BBrMV transmission studies (see below). Meanwhile, as with BBTV, other workers were sequencing the genome (RNA in this case) of BBrMV and developing specific reverse transcriptase-PCR tests for it. Primers bract-1 and bract-2 (Bateson and Dale, 1995) were obtained, but there has been little opportunity to do more than simply show they worked at NRI when tested with a preserved BBrMV sample from the Philippines.

BSV
Though symptoms associated with BSV were encountered in the Philippines (mainly in the Musa gene banks at Los Banos and Davao), rarely did they appear to be causing significant loss in production there. The losses caused by the virus do appear to be greater in some locations in India and Sri Lanka, and some effort is being made by other workers to investigate the incidence and dynamics of this virus in Africa. Since others were working on the molecular biology of BSV, and with the complexity of its ecology within the host (encapsulated, episomal naked DNA and integrated form) making it beyond the scope of this project to develop diagnostic tools, the project sought only to maintain links with those other workers. PCR tests/primer sequences are now available, but these have to be used in combination with ISEM and immunocapture PCR to distinguish between the different forms of the virus.

CMV
Several different antisera against different strains of CMV are available, but not all detect banana-infecting strains which may also be location-specific. Sequences for PCR primers for several different CMV strains, including banana infecting strains have been published over recent years (e.g. Hu *et al*. 1995). CMV appears relatively uncommon and a rather transient disease in banana in the Philippines and else where, and so little effort was made in assessing diagnostic techniques for it in this project. Where a reliable diagnostic test is required is in the tissue-culture business to avoid multiplying the virus and then distributing it to different areas/countries in tissue cultured material.

(3) Transmission studies on BBrMV and AbaMV

Table 1 lists the mechanical (sap) transmission experiments conducted with the BBrMV isolate originally found in an old stand of cooking banana c.v. Cardaba near the Entomology Laboratory of BPI, Bago Oshiro. Since a reliable diagnostic test for BBrMV was not available at the BPI station where these experiments were conducted, there is a risk that the symptoms seen in the test plants might not have been caused by BBrMV. Bearing this in mind, it appears that BBrMV is relatively easily transmitted from banana to *N. glutinosa* or *N. tabacum* (3/8) or between the Nicotianas (23/28) by mechanical transmission. No success was achieved in trying to transmit from Nicotiana back to banana (0/17) or from banana to

abaca (0/4), and there was only low transmission from banana to banana (1/23). Mechanical transmission from abaca to abaca appeared easy (1/4) and from abaca to banana even more so (1/1), though with such small numbers involved it is difficult to determine how reproducible these findings are.

The results of transmission experiments with BBrMV using *Aphis gossipii* and *P. nigronervosa* are presented in Table 2 and Table 3. Transmission with this aphid was most efficient when abaca was the receiving plant, though it was also possible to transmit from banana (either Lakatan or Grand Naine) to banana using this aphid. *P. nigronervosa* was the most efficient of the aphid species tested at transmitting BBrMV.

Experiments on the transmission of AbMV using these aphid species are still in progress at BPI in Davao.

Exercise No.	**Date started**	**Virus source**	**source host for ex.**	**Test plant**	**No. plants inoculated**	**No. with symptoms (@3 wks pi)**	
100	09-Jul-96	Field c	Field c	Abaca	3	0	0/4
156	05-Aug-96	Ex 67	G.naine	Abaca	1	0	
99	09-Jul-96	Field c	Field c	Lakatan	6	0	
227	08-Nov-96	Ex 23	G. naine	Lakatan	4	1	
131	24-Jul-96	Ex 67	G.naine	Lakatan	2	0	
157	05-Aug-96	Ex 67	G.naine	Lakatan	2	0	1/23
160	05-Aug-96	Ex 67	G.naine	Lakatan	2	0	
170	06-Aug-96	Ex 67	G.naine	Lakatan	3	0	
185	09-Aug-96	Ex 67	G.naine	Lakatan	4	0	
333	10-Dec-96	Ex 221	Lakatan	*N.tabacum*	2	2	3/8
77	26-Jun-96	Field c	Field c	*N.glutinosa*	6	1	
115	16-Jul-96	Ex 77	*N.glutinosa*	Lakatan	2	0	
207	02-Sep-96	Ex 77	*N.glutinosa*	Lakatan	2	0	
209	02-Sep-96	Ex 77	*N.glutinosa*	Lakatan	2	0	0/17
200	19-Aug-96	Ex 114	*N.tabacum*	Lakatan	9	0	
204	02-Sep-96	Ex 114	*N.tabacum*	Lakatan	2	0	
113	16-Jul-96	Ex.77	*N.glutinosa*	*N.glutinosa*	2	2	
208	02-Sep-96	Ex 77	*N.glutinosa*	*N.glutinosa*	2	1	
114	16-Jul-96	Ex. 77	*N.glutinosa*	*N.tabacum*	2	1	
201	19-Aug-96	Ex 158	*N.tabacum*	*N. tabacum*	5	5	
215	02-Sep-96	Ex 158	*N.tabacum*	*N.glutinosa*	2	2	
289	31-Oct-96	Ex 158	*N.tabacum*	*N.glutinosa*	2	2	23/28
158	05-Aug-96	Ex 114	*N.tabacum*	*N.tabacum*	2	1	
203	02-Sep-96	Ex 114	*N.tabacum*	*N.tabacum*	2	1	
214	02-Sep-96	Ex 158	*N.tabacum*	*N.tabacum*	2	2	
241	01-Oct-96	Ex 214	*N.tabacum*	*N.tabacum*	3	3	
266	08-Nov-96	Ex 214	*N.tabacum*	*N.tabacum*	2	1	
302	08-Nov-96	Ex 214	*N.tabacum*	*N.tabacum*	2	2	
344	18-Dec-96	Ex 151	Abaca	Abaca	4	1	1/4
286	23-Oct-96	Ex 151	Abaca	Lakatan	1	1	1/1

Table 1 Results of mechanical transmission experiments with BBrMV isolate from c.v. Cardaba (Field c).

Experiment No.	Date	Starve time	AF	IF	No. Aphids	Virus source	Source host	Test Host	No. Plants inoc.	No. Plants w/ sympt	Proport
236	01-Oct-96	3h	30m	48h	20	Ex 146	Lakatan	Lakatan	4	0	
246	02-Oct-96	3h	30m	48h	20	Ex 150	Lakatan	Lakatan	2	0	
258.1	11-Oct-96	3h	30m	48h	20	Ex 108	Lakatan	Lakatan	3	1	
298	07-Nov-96	3h	30m	48h	10	Ex 150	Lakatan	Lakatan	5	0	1/33
299	07-Nov-96	3h	30m	48h	10	Ex 150	Lakatan	Lakatan	6	0	
300	07-Nov-96	3h	30m	48h	10	Ex 150	Lakatan	Lakatan	4	0	
307	14-Nov-96	3h	30m	48h	10	Ex 150	Lakatan	Lakatan	3	0	
322	06-Dec-96	3h	15m	24h	10	Ex 221	Lakatan	Lakatan	6	0	
247	02-Oct-96	3h	30m	48h	20	Ex 150	Lakatan	Abaca	2	0	
247	07-Nov-96	3h	30m	48h	10	Ex 150	Lakatan	Abaca	2	1	
308	15-Nov-96	3h	30m	48h	10	Ex 97	Lakatan	Abaca	3	2	6/16
323	06-Dec-96	3h	15m	24h	10	Ex 221	Lakatan	Abaca	5	2	
339	17-Dec-96	3h	15m	24h	10	Ex 222	Lakatan	Abaca	4	1	
108	15-Jul-96	3h	30m	48h	20	Ex 67	G. naine	Lakatan	2	1	
142	26-Jul-96	3-4h	30m	48h	20	Ex 67	G. naine	Lakatan	4	0	2/9
152	05-Aug-96	3h	30m	48h	20	Ex 68	G. naine	Lakatan	3	1	
44	10-Jun-96	3h	20m	12h	20	Ex 67	G. naine	G. naine	2	0	0/2
143	26-Jul-96	3-4h	30m	48h	20	Ex 67	G. naine	Abaca	2	0	1/6
301	07-Nov-96	3h	30m	48h	10	Ex 23	G. naine	Abaca	4	1	
262	14-Oct-96	3h	30m	48h	20	Butuhan	Butuhan	Lakatan	6	0	0/9
276	16-Oct-96	3h	30m	48h	10	Butuhan	Butuhan	Lakatan	3	0	
281	22-Oct-96	3h	30m	48h	20	Ex 151	Abaca	Lakatan	4	0	0/10
341	17-Dec-96	3h	15m	24h	10	Ex 151	Abaca	Lakatan	6	0	
375	08-Jan-97	3h	30m	48h	20	Ex 151	Abaca	Butuhan	6	0	0/6
280	22-Oct-96	3h	30m	48h	20	Ex 151	Abaca	Abaca	5	1	
342	17-Dec-96	3h	15m	24h	10	Ex 151	Abaca	Abaca	5	0	
372	07-Jan-97	3h	30m	24h	20	Ex 151	Abaca	Abaca	6	0	1/28
373	07-Jan-97	3h	30m	24h	20	Ex 151	Abaca	Abaca	6	0	
379	09-Jan-97	3h	30m	24h	20	Ex 151	Abaca	Abaca	6	0	

Table 2 Results of transmission experiments with BBrMV using *Aphis gossipii.*

Experiment No.	Date	Starve time	AF	IF	No. Aphids	Virus source	Source host	Test Host	No. Plants inoc.	No. Plants w/ sympt	Proport
222	27-Sep-96	3h	30m	48h		Ex 146	Lakatan	Lakatan	3	1	
223	27-Sep-96	3h	30m	48h		Ex 148	Lakatan	Lakatan	3	1	
248	02-Oct-96	3h	30m	48h	20	Ex 150	Lakatan	Lakatan	4	1	6/30
257	10-Oct-96	3h	30m	48h	20	Ex 108	Lakatan	Lakatan	10	1	
309	15-Nov-96	3h	15m	24h	10	Ex 97	Lakatan	Lakatan	4	1	
320	05-Dec-96	3h	15m	24h	10	Ex 221	Lakatan	Lakatan	6	1	
310	15-Nov-96	3h	15m	24h	10	Ex 97	Lakatan	Abaca	3	2	5/7
321	05-Dec-96	3h	15m	24h	10	Ex 221	Lakatan	Abaca	4	3	
21	24-May-96	2-3h	15-20m	24h		Ex 67	G. naine	Lakatan	4	0	
97	09-Jul-96	3h	30m	48h	20	Ex 67	G. naine	Lakatan	6	1	
109	16-Jul-96	3h	30m	48h		Ex 67	G. naine	Lakatan	3	0	4/25
146	30-Jul-96	3h	30m	48h	20	Ex 67	G. naine	Lakatan	6	2	
148	31-Jul-96	3h	30m	48h	20	Ex 67	G. naine	Lakatan	3	0	
150	05-Aug-96	3h	30m	48h	20	Ex 67	G. naine	Lakatan	3	1	
23	12-Aug-94		10-15m	48h		Ex 67	G. naine	G. naine	2	1	1/2
98	09-Jul-96	3h	30m	48h		Ex 67	G. naine	Abaca	5	0	
149	31-Jul-96	3h	30m	48h	20	Ex 67	G. naine	Abaca	3	0	1/11
151	05-Aug-96	3h	30m	48h	20	Ex 67	G. naine	Abaca	3	1	
73	26-Jun-96		dir		20	Field c	Field c	Lakatan	9	0	0/9
67	09-Aug-93			48h		Field c	Field c	G. naine	6	2	2/6
69	10-Aug-96		dir	48h	?	Field c	Field c	Butuhan	6	3	3/6
374	08-Jan-97	3h	30m	48h	20	Ex 151	Abaca	Butuhan	6	3	3/6
378	09-Jan-97	3h	30m	24h	20	Ex 151	Abaca	Abaca	6	0	0/6

Table 3 Results of transmission experiments with BBrMV using *Pentalonia nigronervosa*.

(4) Monitoring *Pentalonia nigronervosa* populations in the field

A variety of methods for assessing the populations of aphids associated with the epidemiology plots were explored. The main results of this part of the study are presented in annex 2 (Chancellor & Araño).

(5)Analysis of BBTV incidence records from commercial plantations

Additional disease incidence records to those collected in the original project for Marsman plantation were collected, and the entire data set was used in statistical analysis at NRI. The results and conclusions of this analysis were published (Annex 3; Smith *et. al.* 1996).

(6) Vector relationships and phylogenetic studies on the Bugtok and Moko bacteria

BPI staff are continuing this work. A total of 581 insects were collected from June 1996 to July 1997 from which 43 isolates of typical morphology for *R. solanacearum* were obtained on Engelbrecht selective media. Of these 43, only 14 continued to look like *R. solanacearum* on subculturing. The isolates were obtained from insects belonging to the Orders Hymenoptera and Thysanoptera, and were obtained from washing the insect in phosphate buffer (1 isolate), maceration in phosphate buffer (11 isolates) and by maceration in distilled water (2 isolates). The isolates were gram negative, catalase positive and those tested for melanin showed positive results. Positive reactions were also obtained on those isolates tested for hypersensitive reaction (HR) on tobacco.
When these isolates were inoculated by injection into the emerging flower bud of c.v. Cardaba plants, all eventually gave rise to the typical Bugtok discoloration to the vascular bundles (Table 4.).Tests are still underway to test the reaction on each isolate on inoculation into a range of alternative hosts (tomato, eggplant, heliconia, turmeric, ginger and other banana cultivars). PCR with primers OLI1 and Y2 confirmed that the isolates were *R. solanacearum.*

Date of Inoculation	Isolate	Date of Debudding	Date of Harvest	Bugtok symptoms
17/04/97	T5.3.1(thrips)	14/07/97	14/07/97	positive
29/07/97	P9.2.1(bee)	03/12/97	04/01/98	positive
29/07/97	W1.2.1 (wasp)	03/12/97		positive
09/09/97	control (water)	03/12/97	07/01/98	negative
09/09/97	R11.3.1(bee)	03/12/97	07/01/98	positive
09/09/97	P12.2.1 (bee)	03/12/97	04/01/98	positive
09/09/97	P12.2.1 (bee)	03/12/97	04/01/98	positive
09/09/97	R11.3.1(bee)	03/12/97	07/01/98	positive
09/09/97	W2.2.1(wasp)	03/12/97	07/01/98	positive
09/09/97	P11.2.1(bee)	03/12/97	07/01/98	positive
12/09/97	R11.3.1(bee)	03/12/97	04/01/98	positive
12/09/97	H4.2.1(bee)	03/12/97	04/01/98	positive
12/09/97	control (water)	03/12/97		negative
12/09/97	R11.3.1(bee)	03/12/97	14/01/98	positive
12/09/97	P11.2.1 (bee)	03/12/97	14/01/98	positive

Table 4. Results of inoculation into emerging banana flower bud of bacterial isolates obtained from insects.

(7) Socio-economic studies

Preliminary results of the socio-economic study indicated that farmers were concerned about banana diseases, but that there were differences in farmers' and researchers' perceptions of diseases and that farmers' control measures against banana bunchy top virus (BBTV) were generally ineffective. Most farmers had heard of BBTV but did not recognise it until the disease was at an advanced stage; young infected suckers were completely overlooked. Other less damaging viruses (BBrMV & CMV) generally were not recognised. There was confusion amongst farmers over different control measures used for different diseases and a lack of clear extension messages. Recommended control measures of complete eradication of BBTV-infected plants were not adhered to, even when farmers were aware of them, as they tended to leave part of the infected root mats and young suckers in the ground after the infected mother plant had been removed. Farmers were unaware that this provided a source of disease inoculum which could then be spread to other plants. Even the farmers who knew that BBTV was a virus spread by aphids, and who had good contact with extension staff, did not appear to be aware of the risks of leaving infected suckers in the farm.

New planting material came from the farmer's existing plants where possible, or was bought from other farmers. Tissue cultured material was not generally available to individual farmers. Where it was available it was often planted in amongst infected plants, and hence most of the benefits of clean planting material were immediately lost. An article describing the methods used and the results obtained during the socio-economic study is in preparation (H. Warburton *et al* 1998)

Contribution of Outputs

(a) Further market studies needed.

At the start of this project it was generally assumed that BBTV was the most serious of the virus diseases of banana in the Philippines, and so socio-economic and market studies tended to concentrate on this disease. However, during the course of the project it became more apparent that for the smallholder or small-scale grower, one can not limit ones study to this disease alone, but the other diseases such as BBrMV, Moko, Bugtok and *Fusarium* wilt also have to be taken into consideration. Thus, any future market studies should probably be more general, looking at farmers knowledge, perceptions and understanding of all the major constraints to banana production in the field. Factors such as what type of planting material a former uses, where he gets it, and how much he is prepared to pay for it should also be determined along with obtaining more information of farmer' knowledge about specific diseases and pests of banana. With such information, it will be more straight-forward to determine what sorts of integrated measures for controlling the major diseases are likely to be effective and taken up by the farmers.

(b) How to make the outputs available to the intended users.

Once the information from the epidemiology plots has been reliably analysed it is intended to publish another paper to follow-on from the one based on the records from the commercial plantations (annex 3). A workshop was held in conjunction with ASPNET/INIBAP at the BPI station at the end of August 1997 to publicise the findings and to allow small-scale growers in the region to see the diseases in the field and be shown how they spread and what strategies can be used to control that spread. The workshop was well received and there were requests that it should be held in other areas of the Philippines. The workshop also highlighted the need for a simple publication describing BBTV and its means of spread and control that could be distributed widely to small-holder/subsistence growers and extension agents. To address this, a small "comic book" is currently being prepared by project staff in Davao.

(c) Further stages needed to develop and test improved control strategies.

This project identified BBTV and BBrMV as the most damaging virus diseases of banana in smallholder and commercial plantings in the Philippines. The epidemiology indicated that for BBTV aphid transmission is generally over relatively short distances, and the main sources of inoculum are probably abandoned or poorly managed stands in smallholder plantings, re-growth of suckers from incompletely destroyed infected mats in larger plantations and unrecognised infection of suckers or tissue-culture derived plants used to plant new areas. It is anticipated that once these results are fully analysed, it will be possible to make some more positive/ substantial recommendations for control strategies applicable to the smallholder or small-scale banana producer. Continued epidemiology and socio-economic studies are required to further substantiate the findings and test if they are applicable to other countries.. Once a set of potentially useful integrated control measures are identified, then it will be necessary to develop methods to test those practices in the field.

(d) How and who will carry out and pay for the next stages.

More time needs to be spent analysing the data collected during this project, and then preparing the results and conclusions for publication/dissemination. It is anticipated that a proposal will then be drawn up and submitted to DFID for funding for a project to determine if the factors observed in the Philippines to be influencing the spread and increase of banana virus and bacterial diseases are also active in India and/or East Africa (DFID target countries), and if the control practices advocated based on the Philippine experience are also pertinent to these other areas. Though the Philippines are no longer a target for DFID funding, any future work on this subject should maintain link with them because of the great experience and knowledge base there. The BPI at Davao has the mandate to work on banana production and crop protection in the Philippines. Thus work will continue there on this crop, though this will be mainly in connection with the INIBAP International Musa Testing Program (IMTP).

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A small database (PC) of references relating to banana diseases (mainly virus) has been compiled and is available from L. Kenyon. The following are a few key references relating to this report:

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Annex 1. Publication (Kenyon et al. 1997)

Kenyon, L., Brown, M.; Khonje, P. (1997) First report of Banana Bunchy top Virus in Malawi. *Plant Disease* **81:** 1096.

First Report of Banana Bunchy Top Virus in Malawi.

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Plant Dis. D-1997-0724-02N, 1997 (on-line). Accepted for publication 23 July 1997.

Banana plants of the Cavendish subgroup (Musa AAA, locally known as "Kabuthu") with classical banana bunchy top virus (BBTV) symptoms were observed to be widespread in Thiwi Valley, Salima Agricultural Development Division, Malawi. The symptoms included marginal yellowing of the younger leaves, dark-green dot-dash streaks along the veins, petioles and midribs, and shortened internodes. The aphid vector of this virus, *Pentalonia nigronervosa* was abundant on bananas in this area (H. Thindwa, pers com.). Young leaf and midrib samples from apparently healthy plants and plants with symptoms were transported to the United Kingdom for testing. In a triple antibody sandwich ELISA with poly- and monoclonal antibodies specific for BBTV (1), the samples from symptomatic plants gave positive reactions ($OD_{Infected} \geq OD_{Healthy}+3SE_{Healthy}$). Polymerase chain reaction (PCR) amplification tests were performed for confirmation using oligonucleotide primers BBT1 and BBT2, which are homologous to conserved regions in BBTV DNA component 1. All ELISA positive samples produced a PCR amplification product of about 349 bp, whereas the healthy control samples did not. The sizes of the DNA fragments produced following restriction enzyme digest of the PCR product suggests that the Malawi virus falls within the South Pacific group of BBTV isolates (2). The presence of both the virus and it's vector has the potential for causing great economic damage to this important banana growing region, and recommendations have been made to eradicate all plants with symptoms.

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Annex 2.

REPORT ON THE VECTOR ECOLOGY COMPONENT OF THE PROJECT ON BANANA DISEASES IN THE PHILIPPINES (A0507)

Tim Chancellor and Ben Araño

Background

This report describes research on the ecology of the banana aphid *Pentalonia nigronervosa* Coq. in relation to the epidemiology of banana bunchy top disease. The work formed part of a project on banana diseases (A0217) and a follow-on project (A0507) and was conducted in collaboration with the Bureau of Plant Industry in Davao City, Philippines from December 1994 to September 1997. The activities on aphid ecology contributed to the following outputs designated under A0507:

1. Some characteristics of the epidemiology of principal virus and bacterial diseases of banana established for the Davao region of the Philippines.
3. Information on the vector relationships for BBrMV and on methods for assessing the population dynamics of aphids associated with banana.
4. Information on, or a model of, the interactions between the factors affecting the incidence and spread of banana virus diseases.

Introduction

Banana bunchy top disease (BBTV) may be spread through infected planting material or by an aphid vector *Pentalonia nigronervosa* Coq. There have been few studies on the role of *P. nigronervosa* in the epidemiology of BBTV in the tropics, particularly with regard to its importance as a vector of primary inoculum and the extent to which it is responsible for secondary plant-to-plant spread within banana plantings.

Aerial trapping was conducted in order to examine the seasonal abundance of alate *P. nigronervosa* and the potential for the introduction of primary inoculum into new plantings. Previous workers used sticky traps (Viswanathan and Regupathy, 1991) and yellow water pan traps (Gavarra and Eastop, 1976) to measure aerial abundance of *P. nigronervosa*. In our study, we used sticky traps, yellow water pan traps and a suction trap in order to estimate aerial abundance and to examine dispersal characteristics. Direct counts on banana plants were carried out in order to investigate the population development of *P. nigronervosa* in relation to season and crop phenology. The relationship between aphid numbers and BBTV incidence was also examined.

The potential for ants to interfere with the biological control of *P. nigronervosa* has been reported in Tonga (Stechmann *et al*., 1996). We designed an experiment to determine the potential impact of ant attendance on survival of *P. nigronervosa* through protection from parasitism and predation. The experiment was also designed to test the hypothesis that a

direct benefit may accrue to the aphid through lower contamination with honeydew due to regular collection of droplets by ants.

Materials and Methods

Aerial trapping of aphid populations

A Johnson-Taylor 30cm Vent-axia segregating suction trap (Taylor, 1951) was used to measure aerial density of aphids at a height of 1.5m above ground level in Lot 18 in the Bureau of Plant Industry Experimental Station in Davao City. The suction trap was located at one corner of the experimental plot. The trap was fitted with a disc-dropping device which segregated the catch at 50-minute intervals into a cylindrical magazine. Twenty-three samples were collected between 0730 hours and 0240 hours the following morning and a single collection was made for the period between 0240 hours and 0730 hours. Muslin, impregnated with a proprietary synthetic pyrethroid insecticide, was glued to the metal discs to knock down and kill insects landing on them quickly. Aphids were placed in 70% ethanol in glass vials and sorted and counted in the laboratory.

From December 1994 to August 1996, yellow water pan traps were placed near the four corners and at the centre of each of the experimental plots in Catalunan Grande, Panabo and Lot 18. Each trap was placed at a height of 1.5m above the ground. Aphids were removed daily by farmer-co-operators and stored in 70% ethanol in glass vials for weekly collection and subsequent identification in the laboratory.

From April to August 1996, sticky traps were placed on bamboo poles at heights of 1m, 2m, 3m and 4m above the ground, respectively, in Lot 18. Four colours of unknown spectral absorbance were used for the traps; yellow, brown, dark green and pale green. The glue was a proprietary product, Tanglefoot, designed for trapping aerial insects. Eight poles were used, so that there were two traps of each colour at each height. Aphids were removed weekly for identification and counting and additional glue applied periodically, as required.

From August 1996, sticky yellow traps were placed on bamboo poles at heights of 0.3m and 1.0m above the ground, respectively, in the experimental plots at Lot 18, Panabo and Catalunan Grande. Poles were located near the four corners and at the centre of each plot. Aphid collection and counting was done as described above for the earlier sticky trap recording in April to August 1996.

Population counts of P. nigronervosa *on banana plants*

From January to November 1995, numbers of *P. nigronervosa* were recorded on banana plants which were rogued after the appearance of BBTV symptoms in the experimental plots at Catalunan Grande, Panabo and Lot 18. The method used was direct counting *in situ* and areas within roots, leaf whorls and bracts were examined as well as external parts of the plant. From November 1995 to April 1996, counts of *P. nigronervosa* were conducted on all 600 banana plants in each of the plots. A non-destructive method of sampling was used in which only aphids visible on external plant parts, and those concealed under leaf bracts, were

counted. Counts were restricted to the above-ground portion of banana plants to a height of *c*. 1.75 metres and thus represent a relative measure of aphid numbers. From April 1996 to September 1997, aphid counts were conducted on a sample of 180 banana plants at each site. Throughout the recording period, counts were categorised as alatae and apterae. The apterae classification included both nymphs and adults as these were counted together.

Interactions between P. nigronervosa, natural enemies and attendant ants

A survey of sugar-feeding ants present in Lot 18 was conducted in January 1997. Baits of filter paper soaked in sugar solution inside 9mm diameter plastic petri dishes were placed on the ground at eight locations within the trial area in Lot 18, BPI. After 15 minutes the dishes were examined and ants feeding on the sugar solution were collected and transferred to 70% ethanol in glass vials for subsequent identification. Subsequent surveys of natural enemies of *P nigronervosa* in banana plantations, including pathogenic fungi, were planned but were not conducted systematically due to time constraints.

A field trial was conducted in June 1997 in Lot 18 to examine the effect of attendant ants on the population development of *P. nigronervosa*. A colony of *P. nigronervosa* was reared on virus-free plants of abaca in the screenhouse. Plants at the 4-5 leaf stage were placed in plastic pots with carbonised rice hulls to provide apterous viviparous aphids of known age. The trial was located in an open area of Lot 18 where a number of banana plants affected by Panama disease had been rogued out. A total of twenty-four potted banana plants at the 4-5 leaf stage were placed in holes dug at the trial sites so that the rim of the pot was at ground level. There were three rows of eight plants at a spacing of c. 75cm within and between rows. The perimeter of the trial site was enclosed with a barbed-wire fence to exclude animals.

A randomised complete block design was used with three treatments and eight replicates. The three treatments were: $T_{1,}$ exclude ants; T_2, exclude predators and parasitoids and T_3, control. Tanglefoot glue was applied around the base of the pseudostem to plants in $T_{1,}$ and more glue was added when required to ensure ants could not climb up the plants. Each plant was infested with 15 viviparous apterae and covered with a wooden-framed nylon mesh cage for four days. After four days, the adult aphids were removed and the cages from T_1 and T_3 were taken away. Plants were inspected daily to record the arrival of ants and to remove any natural enemies that may have entered the cages.

Preparations were made to observe and record the behaviour of the ants towards the aphids and towards natural enemies after the cages have been removed from T_1 and T_3. Plans were drawn up to count the number of adults and nymphs on each of the plants after twenty days and to record any predators, mummified aphids and corpses of aphids killed by pathogenic fungi. A protocol was devised for placing surviving aphids in vials and rear on suckers of the selected host plant to detect the presence of any parasitoids.

Results and Discussion

Aerial trapping of aphid populations

The seasonal abundance of *P. nigronervosa* caught in suction traps between November 1994 and August 1997 is shown in Fig. 1. Trap catches remained low throughout the recording period and no clear and consistent patterns were detected. There was a tendency for relatively high trap catches to occur between the months of June and August and November to February, with variation between years in the monthly peaks. Trap catches in the hottest months between March and May were generally low. The suction trap will continue to be operated until November 1997 after which time series analysis will be conducted on the complete three-year data set. An attempt was made to correlate trap catches with temperature, rainfall and relative humidity but it was considered that the weather data were insufficiently reliable to proceed with the analysis.

The periodicity of catches of *P. nigronervosa* is shown in Fig. 2. Most of the aphids were trapped during daylight hours and the distribution of these catches was fairly even throughout the day. The largest number of aphids was trapped during the first sampling period between 0730h and 0820h.

Yellow pan catches were extremely small at all sites and only 33 individuals were caught throughout the whole recording period. Because these traps were not providing useful information, it was decided to discontinue this activity and to examine catches from sticky pole traps. Results of the initial evaluation of trap colour and height conducted from April to August 1996 in Lot 18 showed that the largest numbers of *P. nigronervosa* were caught on yellow sticky traps and at the lowest height of 1m (Table 1). The sticky yellow trap catches of *P. nigronervosa* at 0.3m and 1.0m in Panabo, Catalunan Grande and Lot 18 from August 1996 to August 1997 are shown in Fig. 3. At all three sites catches were larger at 0.3m than at 1.0m and an analysis for homogeneity of ratio on the pooled data indicated that this was a statistically significant effect ($\chi^2 = 22.4$, $P<0.01$). These results indicate that most of the dispersal by winged aphids occurred close to ground level and they suggest that much of the movement was over short distances.

Table 1. Numbers of *Pentalonia nigronervosa* caught on different coloured sticky traps mounted at on bamboo poles[1] at heights of 1m, 2m, 3m, and 4m, respectively at Lot 18 from April to August 1996.

Trap colour	*Trap height (metres)*				
	1.0	2.0	3.0	4.0	Total
Yellow	10	1	3	2	16
Pale green	8	4	0	0	12
Dark green	2	0	2	0	4
Brown	2	0	0	0	2
Total	22	5	5	2	34

[1] Total number of individuals caught on 2 replicates of each treatment

At Panabo and Catalunan Grande, the largest numbers of aphids were caught on traps in the south and west corners of the plots, suggesting that there were directional effects. By contrast, more aphids were caught on traps in the north and east corners of the plot in Lot 18. Sticky trap catches were not well correlated with suction trap catches in Lot 18.

Table 2. Combined totals of *Pentalonia nigronervosa* caught on yellow sticky traps mounted on bamboo poles at heights of 0.3m and 1.0m at Lot 18, Catalunan Grande and Panabo from September 1996 to July 1997.

Site	*Trap location*					*Total*
	East	South	West	North	Centre	
Lot 18	22	13	5	19	10	69
Catalunan Grande	5	12	11	1	7	36
Panabo	4	11	10	3	6	34

An attempt was made to detect the presence of BBTV in trapped aphids using the polymerase chain reaction. This work was conducted in collaboration with the Bureau of Plant Industries in Brisbane, Australia. BBTV was not detected in any of the aphids. However, no virus was detected in the positive control aphids reared on BBTV-infected plants so that the results are difficult to interpret.

Other aphid species which were collected from suction trap and yellow water pan catches were identified as *Aphis craccivora* Koch, *Aphis citricola* van der Goot, *Aphis gossypii* Glover, *Rhopalosiphum maidis* (Fitch), *Rhopalosiphum padi* (L.), *Schizaphis rotundiventris* (Signoret), *Tetraneura nigriabdominalis* (Sasaki). *A. craccivora* and *A. gossypii* were the two most abundant aphid species caught in Moericke yellow trays in a previous study conducted at the Bureau of Plant Industry Experimental Station in Davao (Gavarra and Eastop, 1976).

Population counts of P. nigronervosa *on banana plants*

Mean numbers of alate and apterous *P. nigronervosa* per banana plant in the plots at Catalunan Grande, Panabo and Lot 18 for the period November 1995 to July 1997 are shown in Fig. 4. Aphid numbers were greatest in Lot 18 (Fig 4b) and smallest in Panabo (Fig. 4c) throughout the recording period. The low number of aphids in Panabo was probably due to the management practice of detrashing, or stripping back and removing old leaf bracts. This practice was not carried out at the other trial locations, so that there were more sites on banana plants for aphids to colonise and multiply.

At all three sites, numbers of *P. nigronervosa* were greatest between the months of November and February and tended to be lowest during March to June when mean

temperatures were higher. High temperatures were found to be unfavourable for population development of *P. nigronervosa* in Tamil Nadu, India (Viswanathan and Regupathy, 1991). Similar numbers of alatae and apterae were counted on banana plants throughout much of the recording period in Panabo. By contrast, numbers of apterae were much greater than those of alatae in Lot 18 and Catalunan Grande, except for a few months at the latter site when aphid populations were very low. There was no indication of a seasonal effect in the production of alatae. There was a poor statistical correlation between both total numbers of aphids and numbers of alatae on banana plants in Lot 18 and numbers of alatae caught in suction traps. The general pattern of suction trap catches was similar to that of numbers of alatae on banana plants (Fig. 5a), although the relative magnitude of the peaks and the troughs differed. There appeared to be a lag of one or two months in the increase in total numbers of *P. nigronervosa* on banana plants following an increase in suction trap catches (Fig. 5b). However, this was not a statistically significant effect.

Numbers of P. nigronervosa *and BBTV incidence*

The number of new BBTV infections in Lot 18 peaked in the month of March in both 1996 and 1997. In 1996, a clear peak in numbers of alatae in suction traps (Fig. 6a) and in total numbers of *P. nigronervosa* on banana plants (Fig. 6b) occurred in the month of February suggesting a relationship with increased BBTV incidence in the following month. In 1997, the March peak in new BBTV infections was again preceded by an increase in aphid numbers on banana plants, although the relationship was less obvious with suction trap catches. However, there were no statistically significant correlations between numbers of aphids and new BBTV infections in the same month of for any lag periods at any of the sites. Consequently, it is considered that neither aerial abundance nor population counts of *P. nigronervosa* alone can be used as a predictor for BBTV incidence.

Interactions between P. nigronervosa, natural enemies and attendant ants

A total of seven species of ants was recorded in the survey conducted in Lot 18; *Paratrechina longicornis* (Latreille, 1802); *Paratrechina bourbonica* Forel, 1886; *Tapinoma indicum* Forel, 1895; *Solenopsis geminata* (Fabricius, 1804); *Odontoponera transversa* (Smith, 1857); *Dolichoderus thoracius* (Smith, 1860); *Anoplolepis gracilipes* (Smith, 1857). All of the ant species collected are known to feed on aphid honeydew, except for the Ponnerine *Odontoponera transversa*. However, only *Dolichoderus thoracius* is an intimate attender of honeydew producing insects (M.J. Way, personal communication). *D. thoracius* will transport Hompotera over short distances, but it is not clear whether it moves *P. nigronervosa* from one banana plant to another. *Solenopsis geminata* is frequently found collecting honeydew from aphid colonies and was found to be associated with *P. nigronervosa* on banana in Tonga (Stechmann and Völkl, 1990).

The following aphidophagous insects associated with *P. nigronervosa* were observed in Lot 18 during the survey; Dermaptera, Syrphidae and Coccinellidae. It was not possible to determine whether numbers of aphidophagous insects were greater in unattended compared with ant-attended colonies. Stechmann *et al*. (1996) reported that in Tonga syrphids and coccinellids were frequently found in unattended colonies of *P. nigronervosa* on banana and tarotoga but were almost entirely absent in ant-attended colonies. No aphid mummies were seen during our survey and no attempted parasitisation of aphids was recorded.

The pot experiment on ant-aphid interactions was abandoned after one week due to disturbance to the site by livestock and dogs. In spite of the precautions taken, it did not prove to be possible to protect the trial area and there was insufficient time to repeat the experiment.

Conclusions

Numbers of alatae caught in yellow water pan traps, sticky yellow traps and a suction trap were consistently low indicating that *P. nigronervosa* is not an active migrant. Spatial patterns of BBTV incidence in commercial plantations (Smith *et al*., in preparation) showed that immigration of infective alatae from adjacent smallholder plantings occurred over short distances. Sticky trap catches suggested that dispersal of alatae within banana plantings occurred at low heights above the ground and that such movement was localised. The aggregated pattern of BBTV incidence that was observed in the trial plots supports this analysis.

There were seasonal changes in aerial abundance and in population development of *P. nigronervosa*, with greatest numbers in aerial traps and on banana plants recorded between the months of November and February. Although the number of new infections of BBTV was greatest in the month of March at all sites, there was no consistent relationship between aphid numbers and disease increase. The possibility that the increase in banana plants showing BBTV symptoms in March was due to a temperature effect should also be considered. It was expected that valuable information would have been gained from infectivity testing conducted on trapped aphids, but difficulties were experienced in developing a suitable protocol and no data on aphid infectivity were available.

The possibility that attendant ants might protect aphids from natural enemy action was explored during this study. No mummified aphids were recorded during the population sampling or field surveys. No native parasitoids have been reported for *P. nigronervosa* in Southeast Asia and throughout the Pacific region (Carver *et al*., 1993; Wellings *et al*., 1994), although mummies have been collected recently in northern Luzon, Philippines (V.J. Calilung, personal communication). Because of problems associated with the pot trial, it was not possible to quantify the effect of ant attendance on aphid predation. However, predators of *P. nigronervosa* and attendant ants were commonly observed in the trial plots and the findings of Stechmann *et al*. (1996) showed that ant attendance can have a major effect in reducing predation. The hypothesis that attendant ants might move aphid colonies between banana plants was not tested during this study and further research is needed to investigate this possibility.

The results of this study suggest that, by utilising virus-free planting material and minimising nearby sources of infection, BBTV incidence in new banana plantings can be reduced to very low levels. As *P. nigronervosa* does not disperse actively, it might be expected that secondary plant-to-plant spread within plantings would be limited by timely and efficient roguing. The wider adoption of recommended roguing procedures, including the application of an appropriate insecticide to kill aphids on affected plants, is likely to have a major effect in reducing the incidence of BBTV.

Acknowledgements

We thank Dr Venus Calilung for identifying and mounting on slides representative aphids from aerial trap catches and Dr John Thomas for conducting the infectivity tests. We are grateful to Dr Cedric Collingwood for identifying the ant species.

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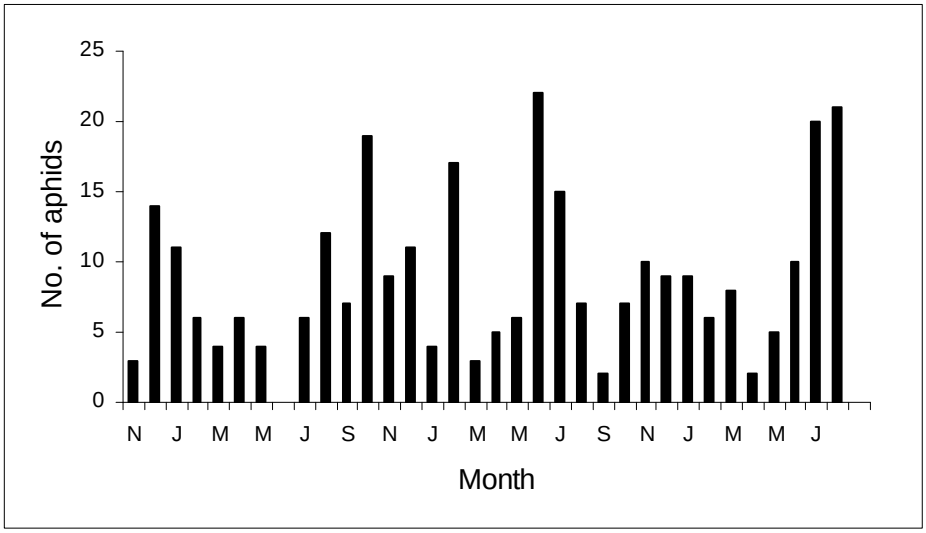


Fig. 1 Monthly catches of *Pentalonia nigronervosa* in a suction trap at a height of 1.5m located at the edge of a banana planting in Lot 18, Davao from November 1994 to July 1997.

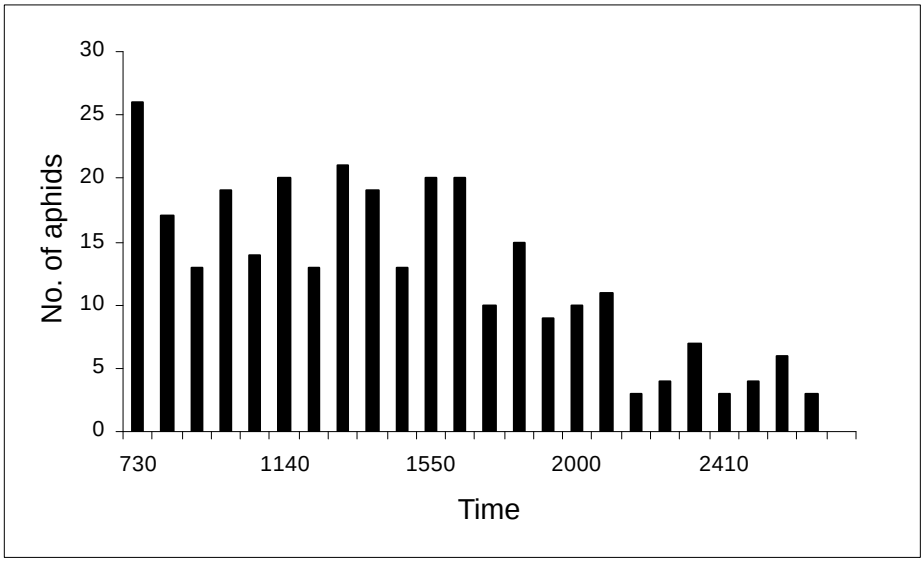


Fig. 2 Periodicity of flight of *Pentalonia nigronervosa* as assessed by suction trapping at 1.5m in Lot 18, Davao from November 1994 to July 1997.

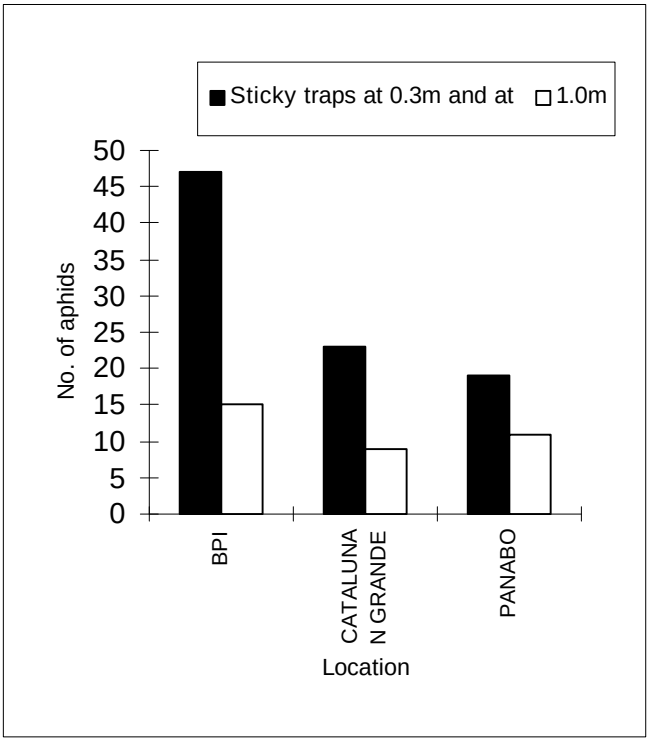


Fig. 3 Numbers of *Pentalonia nigronervosa* caught on yellow sticky traps at heights of 0.3m and 1m at three locations in Davao City from September 1996 to July 1997.

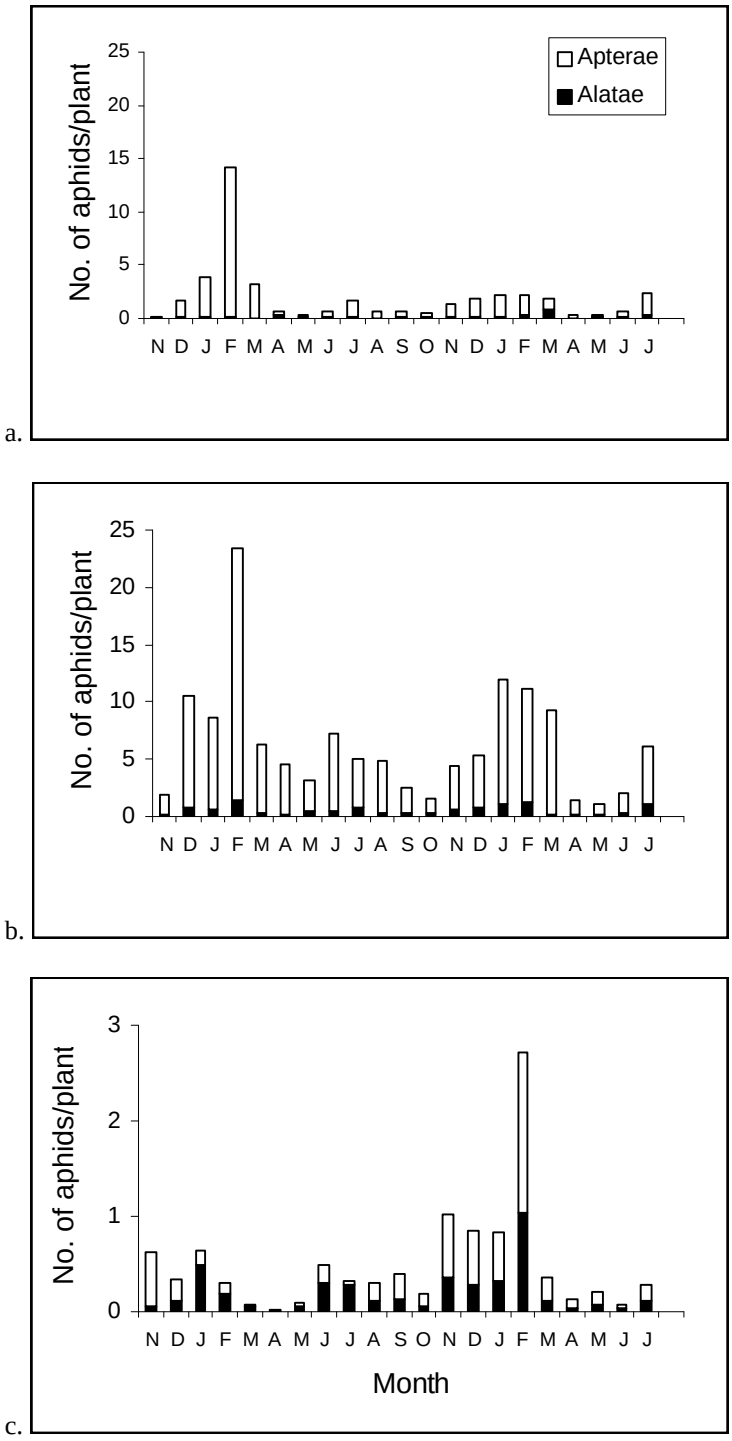
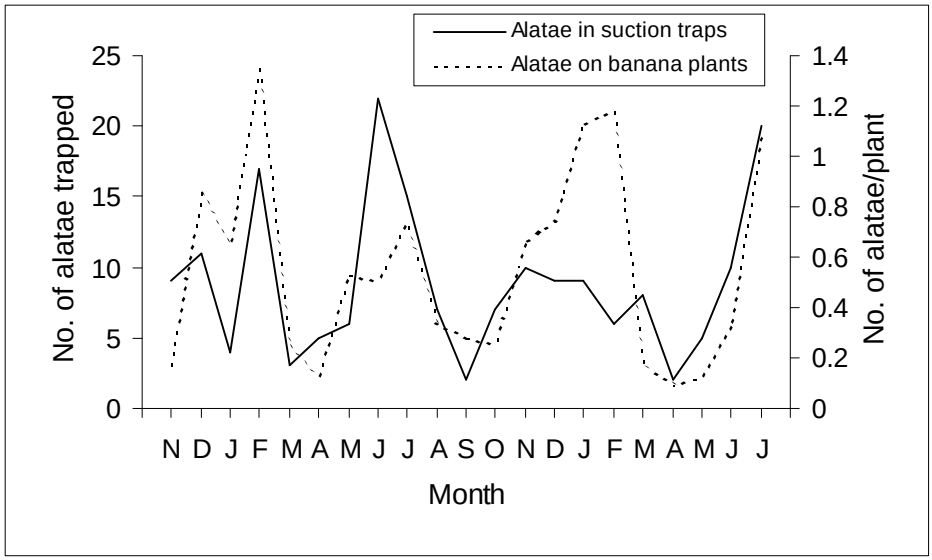


Fig 4. Numbers of apterae (nymphs and adults) and alatae of *Pentalonia nigronervosa* recorded on banana plants at (a) Catalunan Grande (b) Lot 18 and (c) Panabo from November 1995 to July 1997.

a.



b.

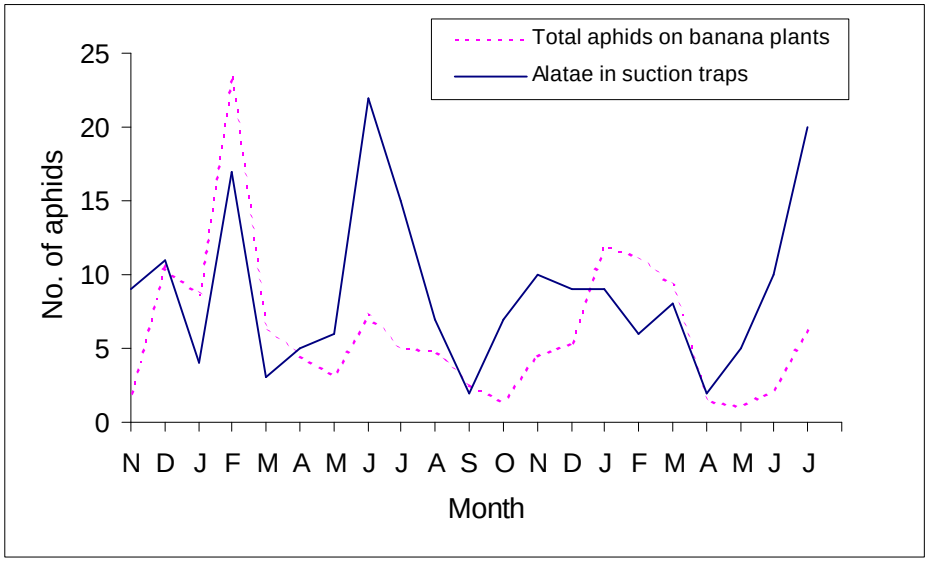
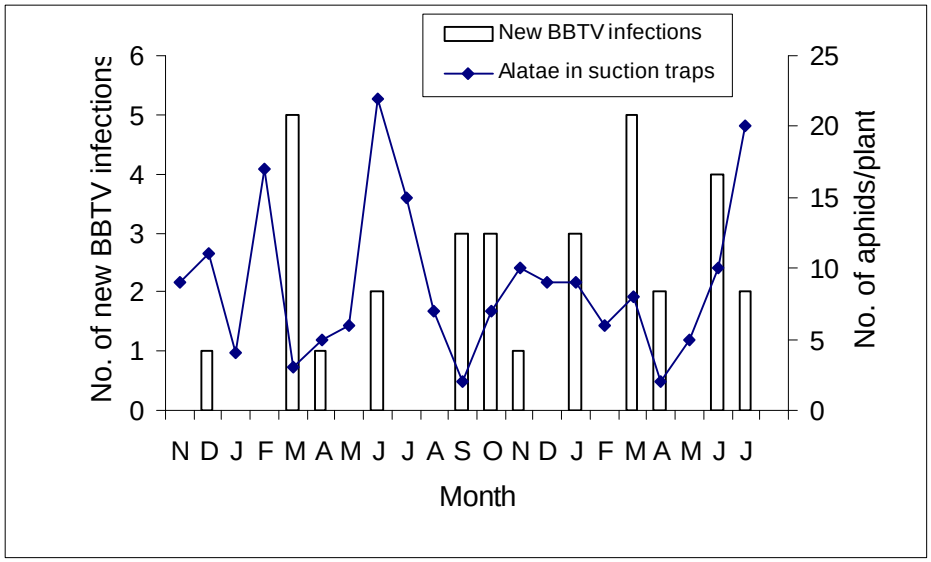


Fig. 5 Numbers of alatae of *Pentalonia nigronervosa* caught in a suction trap at a height of 1.5m plotted against (a) numbers of alatae on banana plants and (b) numbers of apterae and alatae on banana plants in Lot 18, Davao from November 1995 to July 1997.

a.



b.

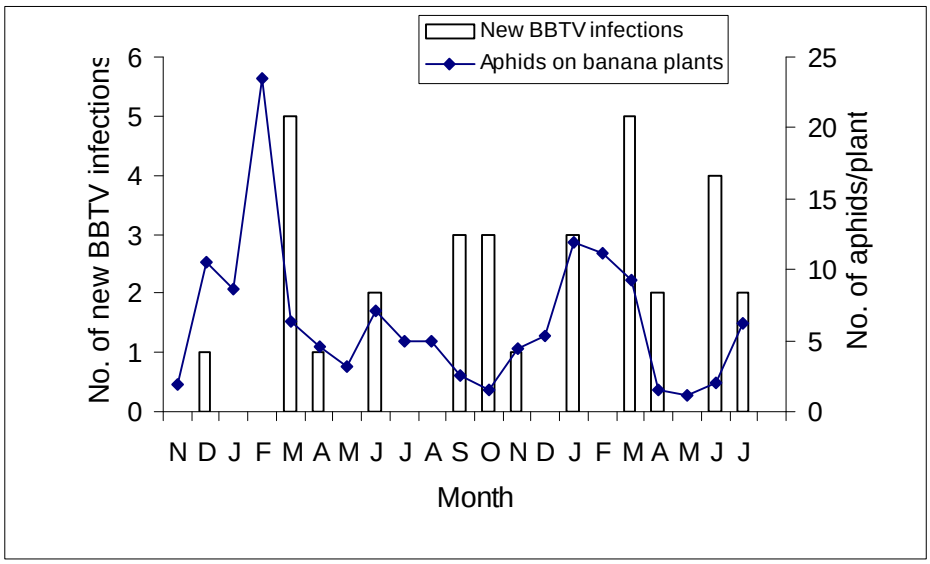


Fig. 6 Number of new monthly infections of banana bunchy top disease (BBTV) plotted against (a) numbers of alatae of *Pentalonia nigronervosa* caught in a suction trap at a height of 1.5m and (b) numbers of apterae and alatae on banana plants in Lot 18, Davao from November 1995 to July 1997.

Annex 3. Publication (Smith *et al.* 1998)

Smith, M.C., Holt, J., Kenyon, L., Foot, C. Quantitative epidemiology of banana bunchy top virus disease and its control. *Plant Pathology* **47**: 177-187.