I NEED A DOCTOR!

PLANT HEALTH CAMPS AND PLANT DOCTORS

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Submitted to
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Summary

Mobile plant clinics were set up in 2004 in response to the need to improve primary plant healthcare in communities. Women elected ward officials, training to become plant doctors, now run regular weekly services in their villages in the Amrul district. Links with the DAE have been established through RDA, although closer collaboration needs to be encouraged between the plant doctors and agriculture sub-assistants (ASA's). Shushilan are in regular contact with DAE and Department of Fisheries officials. RDA provides technical backstopping at the plant clinics and, is 'only a mobile phone call away' when the plant doctors are called on for advice outside scheduled hours. The clinics continue to gain in popularity and being a plant doctor is already viewed as a prestigious position. Many farmers make routine visits and plant doctors' report seeing new faces all the time. AAS's version of the mobile plant clinic, the "plant camp", attracted over 89 farmers and over one hundred enquiries were logged. Specialist pathologist and entomologists were on hand to offer advice and diagnoses during the three camps with additional camps planned in the Natore district. The visit to the plant camps and clinics allowed samples to be collected and referred to the GPC laboratory. The GPC plays an important role in providing laboratory and technical support for the partner organisations, when the plant doctors cannot solve enquiries. Datasheets for control of key pests and diseases are currently being developed by RDA and AAS, and should be useful aids for the plant doctors. The datasheets include information on practical control recommendations and are intended to reduce dependency on pesticides. There is a long way to go before farmers will choose other control options in preference to chemicals, but the clinics/plant camps and plant doctors can help in this process. The GPC are committed to providing support to the partner organisations and have already run courses on field methods for learning local knowledge of pests and diseases and trained staff in laboratory isolation techniques and datasheet development. During this visit, a training session called 'stories from the field' was organised to encourage staff to interview farmers in the field and write case studies. Phil Jones also ran training courses on viruses and phytoplasmas for all PHSi staff. Since becoming a PHSi partner in 2005, Sushilan have employed an agriculturist, Dider dedicated to the role of supporting farmers. Director Md. Mostafa Nuruzzaman has the vision of creating a 'one stop shop' where farmers can seek advice about any aspect of production.

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Acronyms

AAS	Agricultural Advisory Society
ASA	Agriculture Sub-Assistant, (formerly ‘block supervisor’).
CABI	CABI
CPC	Crop Protection Compendium
DAE	Department of Agricultural Extension
GPC	Global Plant Clinic
KGUK	Karbala Gram Unayan Kendro
PHSi	Plant Health Services initiative
RDA	Rural Development Academy
RPC	Rural Plant Clinic
UP	Union Parisha
USDA	United States Department of Agriculture

AAS and Plant Camps

Harun and AAS staff attended an orientation workshop on plant clinics and training plant doctors run by RDA on March 7. Harun wants to adapt the RDA model of the mobile plant clinic and try out what he has termed 'plant health camps'. The idea he says comes from 'eye camps' that move from place to place to provide regular eye examinations for the community. Plant camps will be trialed in seven different sites within the Natore district, supported by different service providers. Two schools have been selected, Ramagari high school, and Maidrassa, an Islamic religious school. Ahmedpur College-2 will be supported by the NGO, Karbala Gram Unayan Kendro (KGUK). A local pesticide dealer will also run plant camps supported by the DAE Agriculture Sub-Assistant. The NGO Sopan will run camps in villages where they already have agricultural programmes, and the local Union Parishad will trial the camps supported by the DAE. An independent farmer group, the Deep Bore Water Irrigation Group with assistance from Maidrassa schoolteachers also want to take part in the plant camps. Specialist entomologist and pathologists will attend the plant camps to assist with diagnoses. It is recognised that farmers often want immediate solutions to problems. Specialists must not feel compelled to offer prescriptions when they are perhaps unsure of a diagnosis and need to make farmers aware that some problems may require further investigation. Camps will be promoted by 'miking', where messages are announced over a microphone and amplifier by a rickshaw driver cycling through a village. Groups of farmers have already been informed though selected lead farmers. AAS plan 10-12 camps by the end of June.

Selected AAS staff, school and college teachers, representatives from the various NGO's, and women elected members of the UP, will be trained as plant doctors. Harun wants to produce data or 'fact sheets' for farmers and plant doctors, based on the design and template from the IRRI Rice Knowledge Bank. They will be developed on the basis of plant camps records, which will identify prominent plant crop problems in the Natore district. Specialist pathologists and entomologists have agreed to produce detailed sheets, no longer than two pages to be used by the plant doctors, and Harun intends to distil the information to produce basic fact sheets for farmers.

Regular 'going public' sessions on specific plant health topics will be linked to plant camps for additional impact. We visited a mango garden in Gurudashpur region to take photos and collect samples for the planned going public session. We photographed problems including mango dieback (*Colletotrichum gloeosporiodes*), mango canker, and a destructive parasitic plant belonging to the, Loranthaceae (showy mistletoe) family. Scale insects and symptoms associated with insect damage were also photographed.

Plant Camp 1: Ahmedpur College-2 with the NGO, KGUK

Ahmedpur College-2 boasts over 250 students, aged 16 to 18 years old, studying for their higher secondary certificate. A 'show and tell session' was organised at the collage and Mr M.K.M Borhan Uddin (Additional Deputy Commissioner) was invited to host the inaugural session. This was the first of many similar events designed to encourage students and farmers to participate by bringing in plant samples for diagnosis. Shortly after the preliminary session a line of a more than 30 students and farmers formed to wait patiently to give their preliminary details to teachers, tasked with recording the information.

[Left] Farmers queue to give details to agriculture students and teachers logging the enquiries. They have come to find out what is wrong with their crops.

Farmers were then directed to the room where plant health specialists, Dr Topon Kulnay Dey, senior pathologist, and Md. Zulfikar Haider Prodhan, PhD entomology student, diagnosed samples. Nuruzzaman (Zaman) a lecturer in the colleges agricultural department, and part-time coordinator of KGUK was also present to look at samples. Prescriptions were written according to the specialist's diagnosis on the specially designed forms and given to farmers.

All three people brought garlic plants into the plant camp. [Left] and [centre] Two students delighted to see the plant camp at their college. [Right] The farmer holds his form and waits to see the specialists for a diagnosis. He says that the plants are showing strange symptoms. They have curled leaves and the bulbs are less compact than healthy ones.

One boy attending the college brought in a banana plant 'that just started to die from the middle leaf'. Dr Topon could not come to any firm conclusion about what was causing the problem, although others sat round the table mentioned moko disease and banana bunchy top. As the field was a short walk away from the school, I decided to take a look. Additional extension officer, Babul Kumar Sutradhur came along to translate and the owner of the banana garden joined us. I questioned the farmer about how the symptoms developed. He told us that this was the first time that he had seen this problem and wanted a prescription to save the tree that he had paid 40 Taka (£0.34) for. I found out that eight trees out of an orchard of 110, had the same symptoms which started when the trees were eight to nine months old. The affected plants had failed to flower but healthy trees were already producing flowers. The inner newest leaf had started to dry from the top and become soft, followed by one or two outer leaves drying, then the whole plant had become affected. Babul said that he knew what this was because he worked in the field, so there was no need to ask the farmer further questions. He went on to explain that this was bunchy top and recommended a fungicide. The farmer did not give any details about unusual growth of the plant, so I tried to explain that a detailed symptom description was needed from the farmer to make an accurate field diagnosis.

Plant doctors are being trained to make a diagnosis based on symptoms, so it is essential that they ask farmers the right questions in order to make informed judgements. Unable to question the farmer thoroughly, we went back to the plant camp where I took a closer look at the banana sample. There was no doubt from the smell and the look that the sample contained bacteria, although it wasn't clear if it was the cause or a secondary affect. I suggested that we do a bacterial streaming test, as there were plenty of seemingly healthy inner stem tissues.

[Left] Zulfikar is unfamiliar with the bacterial streaming test and selects lots of plant material which he places directly in the water and filled to the top of the glass.

[Right] A better way to do the test. Care must be taken to select the right part of the plant for testing, remembering that the sap from fleshy plants like banana are milky and may be mistaken for bacterial streaming

Plant camp 2: Ramgari high school

As the second of the camps was on Friday prayer day, we started early to finish in good time. Undeterred by the early start, many farmers had come to the plant camp with precious samples, keen to find a solution to their problem. Farmers lined up to get their prescription from the specialists, rather like an overcrowded doctors waiting room without the magazines or chairs. Farmers continued to arrive throughout the morning, one arriving by bicycle overloaded with a huge banana plant. We were pleased to see another farmer carrying a bundle of samples on his head in typical Bangladeshi style. Acharuddin Pramanik brought in samples of wheat, coconut, jackfruit, banana, betelnut, mango and sugarcane that had been given to him by neighbours and family, too busy to attend the plant camp themselves [Cover photo].

The banana appeared to have the same symptoms as the one seen the previous day. The symptoms appeared after irrigating with urea and potash and also spraying the leaves and crown with Diytham M45 fungicide, a control measure suggested by the agricultural officer.

As the specialists had to speak with farmers in quick succession, and not wanting to slow an efficient process, it was difficult to find out details about samples and recommendations. Md. Zulfikar did stop to say he had suggested a non-chemical solution to protect from the pomegranate fruit borer, by covering ripened fruit. A farmer brought in a coconut palm branch from one affected tree with wildly distorted leaves. He, and everyone else at the plant camp had never seen these symptoms before. Dr Topon didn't know what it was so recommended taking the sample for further analysis.

Looking in reference books with photographs can be very useful. Using the American Phytopathological Society Compendium of Ornamental Palm Diseases and Disorders, we found matching pictures which suggest that the palm leaf [above] was suffering from boron deficiency.

Plant camp 3: Roynaborat, Baraigram, DAE sub-assistant

The final camp was located in a small but permanent covered market place. Thirty or so farmers had come to find out what we were doing. We asked if they would like to collect plant samples for diagnosis. The farmers that came back to the camp commented on the lack of notification for the visit, but waited patiently in turn for the specialists' advice. The plant camp attracted fewer farmers than the previous camps, but allowed specialists to question the farmers in greater detail. One farmer brought in small coconut buds fallen prematurely from the tree. Dr Topon suggested that it was most likely a nutritional problem and recommend applying manure and regularly watering the tree. One farmer brought in papaya seedlings that had wilted and died off. Seedlings can suffer from a range of problems making diagnosis difficult without taking samples for confirmation. Despite the lack of advertising, many farmers came back to the plant camp with many crops and trees including eggplant, jackfruit, sweet gourds and other vegetables.

[Above] Lots of people are attracted by the sign painted on silk which advertises the plant camp. Photo Harun-Ar-Rashid

[Above] Nuran Nabe has encouraged a group of farmers to return to the market with samples for the plant camp. He is busy recording preliminary information, which will help when the farmers visit the specialists for the diagnosis. Photo Harun-Ar-Rashid

[Above] Dr Topon writes out a prescription for the farmer. The farmer had returned to his field to collect samples, he realised that the specialist could offer him free advice about his lychee tree.

Problems emerging from the field

Plant camps are a good place to find out what new plant problems are emerging in the region and what farmers are doing about them. Farmers brought in samples of garlic plants with unusually curled leaves. Harun and Nuran Nabe were both aware of a garlic problem new to the Natore region. We wanted to find out more about this problem so visited the field with the DAE sub assistant.

Garlic curling

'The leaves look like they are praying'. This is the observation that block supervisor, Mohammad Melidi Hassar has made about the sick garlic plants that he has observed in one of the farmers fields. This is the first year that he has seen anything like this and he attributes this to the late planting of the garlic due to extended rains. Normal planting time is during October, but garlic was planted in mid November and again early December. Plants were growing normally a few weeks after planting, then all of a sudden about six to seven weeks after they had emerged, the younger leaves were prevented from emerging, then began to twist and curl and bend downwards, Hassar tells us. The plants started to die in small round patches in the field, which then gradually spread to the rest of the field. The garlic planted in December showed the symptoms first, although gradually this extended to those planted in January. I asked how the ground was prepared for planting. Nematacides, pesticides and nutrients are added to the ploughed field first, because their application is less effective when the ground is covered with straw mulch. The garlic is then transplanted in the field and covered with mulch. After four weeks of growth the plants began to show the symptoms. We pulled up some of the bulbs and Nuran Nabe tells us that the bulb is softer and smaller than healthier ones.

Above] Nuran Nabe holds up an affected plant on the left nd an apparently healthy plant on the right. Garlic and other llium spp. are prone to curling when they are sick. We find ut later from the CPC that mite damage, nematodes, utritional deficiencies and a disease called 'seven curls isease' caused by *Colletotrichum* all cause similar symptoms n garlic. We collected samples for analysis.

A number of different remedies have been tried. Firstly Melidi thought it was boron deficiency so suggested that farmers add this to their soil. When this had no effect, he suggested spraying a range of fungicides, and bactericides at seven-day intervals. This also had no effect so suggested applying a mixture of several different nutrients. The high addition of the fertilizers has begun to show other effects. Garlic usually form tight clove bunches but had begun to separate and splay. Another plot is affected about a half kilometre away, although other plots of garlic in the area appear healthy. The seed is produced locally so Melidi does not think that the problem is seed borne. 'I don't know what to tell the farmers when they ask what they can do', Melidi adds.

Phil Jones finds out from the farmers that the variety Italy is affected more than the local variety Pabna. The farmers describe that they first saw the symptoms in a few plants a couple of years ago, although this year they report the problem has escalated. Phil explains that if seed is kept and propagated from plants that show symptoms, over time inoculum can build up causing the problems seen in the fields today.

Onion insect

Farmers have noticed numerous aggregations of tiny insect larvae burrowing into their onion flowers in the vegetable growing area of Natore. The cooler microclimate in Natore, Rangpur, and Dineypur is suitable for growing vegetables and seed production, and Natore is famed for its onions. Thrips, usually a problem for onion growers have been superseded this year by this unknown insect problem. AAS staff collected larvae for identification, unfortunately the farmer had recently sprayed for control. The larvae were dead when they reached Dr. G.P. Das, an entomologist and ex BRRI colleague of Harun's who agreed to identify the pest. Das suspected that the larvae were *Spodoptera*, of the lepidopteron order due to the two small dots on the head, although requested live larvae for rearing adult moths for a definitive identification.

The insect is persistent year round, as there are many alternate hosts for the female moth to lay eggs. Das recommended a contact spray as soon as small larvae are observed and suggested handpicking to control larger larvae. Instead of being destroyed, Das recommended placing the larger larvae away from the crop in containers to attract and encourage the development of natural enemies.

[Above] Small holes are seen on the head of the onion flower where the insect larvae burrow. The flowers do not form properly and are smaller.

RDA and Plant Doctors

RDA staffs are enthusiastic about the new phase of the PHSi. They have designed and written 15 pest and disease datasheets in the local language intended for the plant doctors. They aim to do 30 in total. They select plant health problems important to farmers, and in season so that good photographs can be taken. Datasheets are peer reviewed with 20 farmers. Farmers then suggest how the sheets can be improved. One farmer recommended measuring pesticides in matchbox-sized amounts (equal to 25 grams), as most farmers don't own scales. Information was sourced from local books and the Crop Protection Compendium. The sheets also contain information on pesticide residues and when to harvest the crop, as human health has to be considered too.

Zakaria has plans to develop more general datasheets including one on organic methods for reducing pests and pathogens, and the 'Ten Commandments of Correct Pesticides Use'. It would be useful if the datasheet were also in English and so that GPC advisors can assist in developing recommendations and so that sheets can be posted on the website.

Babu and Parvin, both full time project staff, continue to support the plant doctors at the clinics.

Visit to the Union Parisha

[Above] The plant doctors and their assistants are beginning to feel like valued members of the Union Parishad. Babu and Parvin are a constant presence supporting the plant doctors.

Changes have happened in the clinics. All plant doctors have been allotted helpers. Plant doctor Piraya Begum, is assisted by helper Aquima at rural plant clinic (RPC) 1 in Wards 1, 2 and 3. Anjuara and her assistant Tsalima run RPC-2 in Wards 4, 5 and 6 and plant doctor Firka, is assisted by Rosetta at RPC-3 in Wards 7, 8 and 9.

The Saturday clinic now runs on Tuesdays in alignment with government changes that have made Saturday a rest day. Clinic 3 has moved location from Polipalash village to Rajagampur so that Firka can be near her official duties. The plant doctors are busy people and have official duties to perform and meetings to attend. After official meetings at the parishad building, they meet in a room set-aside especially for them. It's good that they have a place to sit and talk about plant health problems that they have seen in the clinics. The plant doctors, and their helpers were waiting to talk with us at the Union Parishad in Amrool. They all recognised me and said that I should visit more frequently.

Rosetta (Rose) often takes over duties when Firka is away on official business. She has received training at RDA and is learning fast. Rosetta told us that lots of people attending the clinic were interested in knowing about animal health, and wanted her to solve these problems. The concept of training people in communities responsible for primary-level veterinary care is not new (Cately *et. al* 2002), although combining the two services is innovative. Rose requested help from RDA, who have made it possible for her to attend a 45-day training course on basic treatment of livestock. In future, Rose will be able to offer advice on livestock during clinic hours too.

Phil had never met the plant doctors and interviewed them about their clinic work.

Do you keep records of the type of problems that come to the clinic?
Yes we keep registers, the name of the farmer, where they come from and what the plant sample is. These are kept in the prescription book.

In cases where you can't answer the farmer's questions what do you do?
If farmers are coming on non-clinic days and there is no RDA to help then we call RDA on our mobiles, then we can give the solution straightaway or tell Babu that he should come to the village.

How many plant samples do you get per day?
Approximately 7-12 per clinic day.

Do farmers come along regularly to the clinics?
Yes even on non-clinic days. We have started to see new faces at the plant clinics, which is very welcome. Where we are performing our official duties, granting passes for moving animals for example, people who know we are the plant doctors also ask us questions then.

What are the top three problems that are brought to the clinics?
Mainly bean, fruit, whatever the women are growing they will bring in samples.

When you are looking at the plant samples can you say if insect, disease or virus causes it for example?
Zakaria replies: *They have only had basic training, but they are fast learners and are keen to know more.*

How do you deal with samples, what process do you follow?
We examine the sample and ask the farmer questions, such as what stage of growth is the crop, about field conditions etc.

Do you ever consult each other about what is going on at the other clinics?
Yes, here in this room we talk about the common problems in the local area

What plant problems can you recognise?
Aphid (Menda poka), Larvae (Kirra poka); Fruit borer of jackfruit ;White scale on guava; Banana fruit beetle (Daud rog); Dieback of lemon, and *rot of pointed gourd*

What contact have you had with the DAE?
Zakaria replies: There hasn't been much interaction because the DAE are interested to be elected as resource persons because honorariums are available

Is there anything you would change about the plant clinics better?
After a prompt from Zakaria, the plant doctors stand up one by one and begin to list their demands

[Above] The plant doctors want to improve the service. They tell us that they want the clinics to be more visible within the village, signs updating and the clinics to look like a clinic, with posters of pests and diseases on the walls

The plant doctors also want identity cards with their picture, and visiting cards to hand out to farmers. The prestigious position of being a plant doctor they tell us will also help with re-election campaigns. The plant doctors also want more datasheets. The plant doctors mentioned microscopes and soil testing kits, although these are expensive items for a community clinic, and better suited to a central laboratory with a constant, regular supply of electricity. They want a collection of reference samples and a locked cupboard to keep their equipment. 'We need more training' said Piraya 'so that maybe in a few years we won't need you' she said pointing to Zakaria. These are ambitious plans, but reinforce the plant doctors' eagerness to learn about field diagnosis and become better at their job. Clinics operating in isolation of RDA would however, fail to benefit from advisory support and the network of laboratory services.

Zakaria has drawn up plans for the conversion of one of the rooms into an agricultural laboratory. This will be primarily for the seed health project supported by Cornell University although Zakaria wants to set an area aside for the plant doctors. He wants to equip the lab with microscopes and plant specimens showing disease symptoms for training the plant doctors. Training on datasheets would also be based at the laboratory. Phil thinks the laboratory has great potential for being a central place for the routine screening of viruses and phytoplasmas, although this would require additional training and equipment (see Annex 1).

Training at RDA

Babu, Parvin (RDA), Nuran Nabe, Nuruzzaman (AAS), and Shahriar Zaman Dider (Dider) from Suchilon were brought together at RDA for training. Training involved morning visits to the rural plant clinics to observe plant health symptoms, combined with afternoon classroom-based lectures and discussion with Professor Phil Jones. Phil is a plant pathologist and has many years experience in the field diagnosis of viruses and phytoplasmas. Phil explained why it was important for agronomists to be able to recognise symptoms of viruses and phytoplasmas and collect samples for confirmation. Firstly, insects are vectors for transmitting viruses and phytoplasmas and have the ability to prevail year round in the warm tropics, this means there is potential for severe crop losses if managed incorrectly. Secondly, Babu has told us that farmers call plant health problems they don't know, virus (vairus) and spray insecticides indiscriminately. Only when the right diagnosis is made can appropriate advice and training be given to the plant doctors, which in turn can be shared with the farmers visiting the clinics.

The trainees all mentioned that they enjoyed visiting the rural plant clinics and meeting with staff outside their own organisation, to share knowledge and experience of plant problems and recommendations. Another aspect of the training consisted of writing case studies about specific plant health problems. Writing reports and documenting activities is sometimes difficult. It is important to write down notes when visiting the field so that facts and snippets of information don't get lost or forgotten. With that in mind, staff were asked to interview farmers at RPC-3, take notes and write up 'stories from the field'. As a guideline, Zakaria read out 'my leaves are sleeping', written from the perspective of a farmer describing his observations about rotting pointed gourd. Case studies written by staff will be available from the plant clinic when translations are complete, and the PowerPoint presentation on phytoplasmas by request. Virus lecture notes are given in Annex 2.

[Above] Dider reads his story to the rest of the group so that they can give their valued comments.

Rural plant clinics

Rural plant clinics 2 and 3 were visited on scheduled clinic days so that we could observe them with little disruption. With staff from the other organisations eager to look at samples and the presence of Phil and myself, the first of the clinics we visited (RPC-2,) became a popular event. We sat with the plant doctor Anjuara and her assistant, Tsalima while they efficiently logged each farmer's plant problem. The plant doctors were calm and in control, seemingly ignoring the crowd. Anjuara and Tsalima dealt with the samples together, talking between themselves, occasionally looking to Babu for help. The equipment box was missing a knife to cut open samples, but everything else appeared in order.

[Above] The mobile plant clinics are run in the villages and are a welcome service to the people using them.

[Above] Plant doctors ask farmers detailed questions about their samples and about field conditions, to eliminate certain causes and pinpoint others.

Lots of plant problems came into the plant clinic including mango inflorescence dieback, various problems on gourds, and an insect problem on beans. Phil and Babu stayed with the clinic, and the rest of the staff went to collect samples in the field and talk with farmers. One farmer Dudu, started to explain about the problem he had in bitter gourd (corolla). He had observed a 'vairus' in the crop, which had been seen in the area for a few years, although never as bad as now. He explained that in the vegetative state, leaves started to become speckled (mottled) after two months of planting the seeds. Leaves near the growing point had become affected first, spreading to other leaves lower in the vine. In the field, the symptoms occurred in small patches. Affected flowers and leaves were smaller than normal ones, and fruits were small and discoloured, turning yellow when they should be green. Some of the vines also had flattened stems. This was the first time that he had grown the hybrid variety 'Laltir' and tried many types of medicine for control although nothing had worked.

[Above] Dider, Nuruzzaman, Parvin, Nuran Nabe and I are all keen to find out what is happening in the field, although it is important to remember to let the farmer speak and find out what they know about the etiology of the problem.

Photo Babu Haque

[Above] Affected chilli plant with small leaves [Insert] Curled chilli pepper

Farmers are quick to detect new problems in their field. Farmer Ashraf Ali, was eager to show us a problem affecting his entire chilli crop, intercropped with brinjal. The chilli plants showed a 'little leaf' symptom usually associated with phytoplasmas and abnormal fruits, curled like pigs tails. We took samples and photos to show Phil, who had never before seen symptoms like these on chilli plants.

It is important to find out what is causing problems like these so that Bangladesh's' inventory of pests and diseases are up-to-date.

The next morning, we spent some time discussing the case study training exercise with staff and decided to practise it at RPC-3. We watched the plant doctors run the clinic first, and then staff selected some problems from the clinic for their detailed case studies.

Above] Women crowd round to bring their samples

[Above] Firka and Rosetta get on with the normal business of running the plant clinic

Nuran Nabe talked with a woman farmer about her dieing banana tree, which she said was due to mazra poka. Nuran Nabe took samples of the insect for further diagnosis.

[Above] Florescence appear as small leaves

Nuruzzaman talked with a farmer about a problem on mango. The farmer explained that he saw small leaves on the ends of some branches, where flowers should be forming. The symptom that the farmer described is called phyllody and usually associated with phytoplasmas, however in mango, this symptom is associated with the fungi *Fusarium*. This example highlights two things; that diagnosing samples from symptoms is complex and requires experience and, that samples should be taken to confirm causes.

We visited RPC-1 in Maria on an unscheduled clinic day, prompting a spontaneous session in the village yard with Piraya and her assistant Aquima. A few people, mainly women, attended the session possibly because farmers were attending the local market that was being held at the main road nearby. This gave us the opportunity to ask the plant doctors a few questions about their role, and the samples that had amassed on the table.

We asked how many people attended the last clinic and Piraya told us that eight people had come with lots of vegetable problems. We asked what sort of problems had come into the clinic. 'One women bought in a bitter gourd that had started to turn yellow but should have been green, the fruit was small and sometimes dropped off the vine'. The fruit symptom sounded very much like the problem that Dudu had described to us caused by virus. Pireya said that a kirra (larvae) poka had been found in the fruit. It is important to identify primary causes of plant problems and be aware of secondary attack, which, on inspecting samples, may appear more significant than they actually are. The plant doctors told us that people who found the advice helpful come back to the clinics again.

[Above] Rustam Ali, a local pesticide dealer (white shirt) also wants to be associated with the plant clinic

[Above] A local woman is keen to observe insects running around on the leaf

Phil asks Piyera what the white powdery stuff is on the leaf on the table. ' I know that this is poka, she explains, and you treat it by spraying it with a soap solution'. It is good to see that the plant doctors are not over prescribing pesticides, and they are aware of alternatives. She picks up the leaf and examines it with the magnifying glass that is part of the plant doctor kit. The women round the table look unconvinced, so I ask Piyera to show them. One women looks under the magnifying glass and tells the others, 'yes, I can see it is an insect, it is running'.

Pointed gourd with the same symptoms observed last year are still being brought to the plant doctors. Fruits dry on the vine, and a rot is observed near the junction with the stem, although other fruits show stem end rot symptoms. Isolations were done from fruit samples back at RDA and the UK, although the problem remains unsolved. We ask the plant doctors to show us the pointed gourd problem and other things in the field.

[Above] Piraya holds and affected pointed gourd fruit that is showing the early signs of the problem.

Piraya is concerned that she does not know the cause of the problem on the pointed gourd. It could be due to natural fruit shedding, although it is difficult to assess by a single visit to the field. It is not apparent how many times the plant doctors go to the field to look at problems first hand, although this should be an important part of their duties.

Rustam Ali, a local pesticide dealer, also attended the clinic session, and wants to be associated with them. He is under strict instruction from Zakaria not to interfere with what the plant doctors are doing. The plant doctors themselves requested more training and information about pesticides, so that they will be able to recommend more effective ones in the future. We are trying to encourage the plant doctors to recommend alternatives to chemical control, although it is not our intention to force pesticide dealers like Rustam out of business. The association with the pesticide dealer could prove beneficial, as this could be an ideal way to provide farmers with bio-pesticides and organic products which could potentially sold at his shop. Plant doctors could be the driving force behind re-educating farmers and promoting alternatives to pesticides through the plant clinics. There is a currently a great dependency on chemical pesticides in Bangladesh.

Phil spoke with Zakaria about one example that he knew about chemical alternatives from Cuba. Professor Brian Kerry of Rothamsted has been collaborating with scientists at the National Centre for Animal and Plant Health (CENSA) in Havana Cuba on the biological control of nematode pests. This collaboration has resulted in the successful development of a bio control system whish is widely used in Cuba. Recently Rothamsted and CENSA set up a joint company in Nairobi (Dudutech) to produce the agent under licence and to train African farmers in its use. Dr Kerry will be happy to provide details (email brian.kerry@bbsrc.ac.uk).

Shushilan and the Community Plant Health Clinic Project

We were unable to visit Kaligonj, but director Md. Mostafa Nuruzzaman, and project manager Ripon Kumar Ghase came to Dhaka to talk about Shushilan's achievements since becoming a PHSi partner. Nuruzzaman has a vision to create a 'one stop shop' or central place where farmers can come to get advice on everything. This is difficult he adds because the district where Sushilan is based contains over 200,000 people and communicating with that number of farmers is complex. The first stage of the process has been to employ a new laboratory assistant, Shahriar Zaman Dider who has been working with local farmer groups in the surrounding area. Dider has been employed since November '05 and continued his training by attending the viruses and phytoplasmas course held at RDA. With the addition of Internet and new software on pests and diseases in Bangla, enquiries that have been coming into the laboratory have been solved with greater ease. Nuruzzaman also indicated that he would like to visit the GPC and Rothamsted when he comes to UK in May.

Following is a summary of the progress report submitted by Nuruzzaman.

Objectives

The major objectives that Shushilan have set are to increase crop and shrimp production in the project area and to-

- Create awareness about soil and plant health among the farmers
- Help farmers identify pests and diseases and recommend control
- Provide laboratory facilities to identify nutrient status of soil, salinity, pH of soils and water for fertilizer management
- Increase library facilities for an improved service to farmers

Completed activities

- Hiring a laboratory assistant, Shahriar Zaman Dider.
- Eleven existing agricultural groups, 9 new groups of farmers, and 3 ward 'Susamaj committees' have been selected in the project implementation area to benefit from the plant health clinic project. Dider has been instrumental in orientation and building up working relationships with the groups.
- Building links with the Department of Agricultural Extension and the Department of Fisheries to provide better a better flow of information for farmers
- IPM module development and training with farmers
- Improved communications with the GPC by installing internet connection
- Necessary consumables identified and bought, including laboratory reagents and pest management software in Bangla
- Extension type activities.
    - Video shows shown on different technologies (Table 1)

Table 1. Number and type of videos shown

Subject	# show	Total attendees	Females	Males
Beef fattening	4	38	20	18
Seed preservation	6	57	29	28
Climate change	2	21	10	11
Total	**12**	**116**	**57**	**59**

- Pot show song- Over 1500 people attended the three shows shown on rice production technologies.

- To date there have been 115 pest and insect enquiries submitted to the laboratory. With DAE and support 113 have been solved (Table 2). Soil and water have also been tested at the laboratory (Table 3)

Table 2. Numbers of Insect and pest enquiries

Host	Solved	Under investigation	Total
Rice	29	0	29
Vegetables	38	1	39
Tree	46	1	47
Total	**113**	**2**	**115**

Table 3. Numbers of soil and water tests

Parameter tested	Number of samples
Soil pH	7
Water pH	12
Nutrient status (N,P,K,S and OM)	5
Total	**24**

Problems and limitations

Plant problems may remain unsolved because soil and water testing is not a free service and farmers are reluctant to pay. Lack of Internet facilities delayed communications with GPC advisors, consequentially affecting the response time to farmers. The project area is large and numbers of staff on the project is limited.

Annex 1. Setting up a small diagnostics laboratory for virus diseases

There are a number of ways that you can diagnose viruses

1. Host range by sap inoculation to a range of test species

Serological methods:

2. Gel diffusion tests
3. Enzyme-Linked Serological Assays (ELISA)
4. Dot Immuno-Binding Assay
5. Dip sticks or lateral flow tests

HOST RANGE INOCULATION

This requires a stock of healthy test plants to which the sap from your test plant can be inoculated usually by grinding an infected leaf in buffer and then rubbing (gently) the resulting solution onto the leaves of the test plants. Each virus needs a specific range of test plants but generally we use members of the Nicotiana genus as a first step. There are lots of published information about which test plants are needed for a particular virus (see www.dpvweb.net) and it generally takes between 7 and 14 days for symptoms to begin to show, so it's not a quick test. However, it is a good way to show that you have a virus in your sample. It is important to know that not all plant viruses can be transmitted in this way so you may get false negatives.

SEROLOGICAL TESTS

These are tests that use antibodies which have been raised to the virus you are testing for. They are highly specific so if you test for VIRUS A and your sample happens to be infected with VIRUS B you will not detect it. There are simple serological tests (gel diffusion) and more complex (ELISA), each type of test has its place in the diagnostics laboratory. The gel diffusion test will often detect viruses that are closely related (strains of cucumber mosaic virus for example) while the ELSA is generally more specific. There are advantages and disadvantages to both types of test but generally the ELISA test is regarded as the standard diagnostic test these days, however set up costs are high as you will need to buy virus test kits and an ELISA plate reader.

A variation of the ELISA test is the DOT IMMUNOBINDING ASSAY (DIBA) which can use the same kits but has a lower set up cost. All serological tests with the exception of the Dip stick and lateral flow test require a minimum of good laboratory practice to be used.

	Gel diffusion	DIBA	ELISA	Dip Stick / LFT
Use of antibody	High	Low	Low	N/A Antibody included
Equipment needed	Glass slides, agarose, Pasteur pipettes, incubator	Antibody kits, calibrated pipettes, plastic dishes, membrane to bind sample, incubator	Antibody kits, calibrated pipettes, incubator, microtitre plates, optical reader	You buy the test kit which contains everything you need.
High through put	No	Yes	Yes	No
Sensitivity	Low	Medium - high	High	Medium – high
How is test evaluated?	Visual	Visual	Visual or optical reader	Visual
Cost per unit test	Low	Low - medium	Low - medium	High
Set up cost	Very low	Medium	High	None
Specificity	Depends on antibody	High	High	High
Usage	A single sample per test	Many samples per sheet	Up to 70 samples per plate	Single sample per test
Field use	No	No	No	Yes

Here are some protocols so you can see what is involved:

SAP TRANSMISSION

1. Place infected leaf material in cooled mortar add buffer (1:1) and grind with pestle to a fine slurry.
2. Filter through muslin into test tube, place in ice bucket until needed.

   Dust test plants with a fine cover of carborundum (600 grade), leaves to be inoculated should be marked, pots should be labelled with treatment.
3. Make pad with muslin, moisten with slurry and squeeze out any excess, gently stroke upper leaf surface with pad, ensure whole leaf is covered. Alternatively you can use a fingertip to inoculate leaves but remember to wash hands between treatments.
4. Place inoculated plants in glasshouse and cover with newspaper overnight.
5. Next day wash leaf surface with a fine spray of water.
6. Use sap from healthy plant or phosphate buffer to inoculate control plant.
7. Observe plants daily for expression of symptoms especially from 4/5 days after inoculation.

EQUIPMENT AND BUFFER

0.01M potassium phosphate buffer pH 7 - 7.5

17.4g potassium phosphate monobasic	1000ml
3.4g potassium phosphate dibasic	250ml
mix to required pH	

mortar and pestle (cooled)
5 x 5cm muslin
test tube
ice tray /bucket
carborundum powder - 600mesh
newspaper
test plants and pot labels

ELISA – PLATE COATED WITH ANTIGEN

1. Prepare samples by grinding in coating buffer and making appropriate dilutions.
2. Add 100µl aliquots of sample under test, together with positive and
3. negative controls. to duplicate wells. Incubate 4h at 37 C or overnight at 4 C.
4. Wash plate.
5. Add 100µl of specific antibody (in extraction buffer, usually diluted 1:1000) to wells, incubate 37 C 4h.
6. Wash plate.
7. Add 100µl of anti-rabbit enzyme conjugate (diluted in conjugate buffer) to each well, incubate 2-4h at 37 C
8. Wash plate
9. Add 100ul of enzyme substrate to each well and incubate at room temperature for only 20-30min (peroxidase), or as long as necessary to observe reaction (up to 24h with alkaline phosphatase).
10. Assess results by visual observation or measurement of absorbance.

MATERIALS

Coating Buffer - 0.2M sodium carbonate pH 9.6

6.63g Na_2CO^3
11.72g $NaHCO^3$
+distilled water to 1000ml

Phosphate buffered saline (PBS) pH 7.4

this makes a 10x concentrated stock:
80g NaCl
2g KH^2PO^4
29g $Na^2HPO^4.12H^2O$ or 14.2g $Na^2HPO^4.2H^2O$
2g KCl
+distilled water to 1000ml

PBS - Tween
100ml 10x PBS stock
5ml 10% Tween 20
+distilled water to 1000ml

Extraction buffer
10ml 10x PBS stock
2g PVP 44 000
0.5ml 10% Tween 20
0.5% full cream milk powder (we use NIDO)
+distilled water to 100ml.

ELISA Peroxidase substrate:
Solution A: 1M Sodium acetate adjusted to pH5.8 with citric acid.
Solution B: Dissolve 2 TMB (Sigma) tablets in 5ml DMSO.
Solution C: 6% hydrogen peroxide (store at 4 C)

You need 10ml of substrate per plate so just before use mix:
9ml distilled water
1ml solution A
100ul solution B
10ul solution C
Stop reaction by adding 25ul 3N H^2SO^4 per well.
If you have a spectrophotometer read absorption peak at 450nm, turbidity at 650nm

ELISA Alkaline phosphatase substrate
97ml of diethanolamine
800ml distilled water
add HCl to give pH 9.8
make up to 1000ml
Stop reaction by adding 25ul 3M NaoH per well
Measure absorbance at 405nm

DIP STICK / LATERAL FLOW TESTS

These are single shot tests that come as a complete package. The test leaf is mixed with the buffer *supplied) and then either a drop placed in the well of a lateral flow test or the test stick is dipped into the solution. The result is read as a colour change on the stick or lateral flow plate. The result appears within 2 -3 minutes and positive and negative controls are built into the tests. You do not have to supply any extras.

COMPARATIVE COSTS (excluding labour)

Dip stick and lateral flow tests cost between $3-$8 per test.

ELISA kits can be purchased from a variety of sources they cost about $500 but you do need to provide some additional chemicals, kits are usually sold for 250 /500 tests and cost about $1 per sample.

You can als0 buy the antibody and conjugate and provide your own microtitre plates. This reduces your costs slightly.

DIBA the costs are the same as for ELISA but you use a membrane instead of a microtitre plate. This is a lot cheaper than buying plates but the cost of the antibodies is the same.

SOME USEFUL WEB SITES

For information about plant viruses
www.dpvweb.net - Descriptions of Plant viruses. This site is also available on CD ROM for those with slow or no Internet access. Email Phil for details
www.image.fs.uidaho.edu/vide/ - Plant Viruses Online. A site that lists all known plant hosts in alphabetical order and the viruses that infect them, use it in conjunction with dove.
www.ncbi.nhm.nih.gov -This site has a database of all the genomic details of plant viruses. You can also find out if anyone has recorded your virus in another country.

Diagnostic tests
www.bioreba.com -this Swiss company sells many antibody test kits and equipment.
www.agdia.com - as does this US company
www.dmsz.de -This German company often has very competitively priced antiserum.
www.pocketdiagnostics.co.uk -This company produces many lateral flow tests for testing plant samples in the field.
www1.amershambiosciences.com -Amersham bioscience now called GE healthcare sells nylon membrane for the BIBA tests.

Professor Phil Jones

Annex 2. Viruses and Phytoplasmas

Professor Phil Jones and Paula Nash, 28th March 2006

What is a virus?

Put simply a virus is a very small microorganism with a protein coat and nucleic acid (RNA or DNA). The protein coat has the same function as the coat of a fungal spore i.e. it is protective.

Plant viruses

Plant viruses lose their protein coat when they first infect plant cells and their nucleic acid genome 'hijacks' the biosynthetic processes of the cell and begins to replicate. It is this alteration of the plants normal metabolism that causes the characteristic symptoms we see in the plant. There are over 1000 plant viruses which have been described. Viruses are classified into families, genera and species according to the type and organisation of t heir genome (DNA, RNA etc) and their morphology / structure (shape of particle). Viruses belonging to the same family or genus often share many similar biological properties (symptoms produced in host, type of vector and manner of transmission).

What is a vector?

A vector can be an insect, fungus, nematode, parasitic plant or the actions of man that passes the virus from plant to plant. Parasitic plants pass the virus from plant to plant when they attach themselves to the phloem of other plants. Vectors can be temporary carriers of the virus, or permanent carriers of the virus. The relationships are described below.

Relationship between plant viruses and insect vectors

- **Non-persistent** (temporary)– Viruses can stick themselves to the mouthparts of insect vectors when they probe or feed on the plant. When the vector probes or feeds another plant the virus detaches from the mouthparts and the plant becomes infected with the virus. The vector looses the virus and is no longer infective.
- **Semi-persistent** (temporary) – Vectors become infected with viruses when they feed on virus-infected plants, but don't loose the virus straight away when they feed/probe other plants. Vectors only loose the virus when they moult.
- **Persistent** (permanent) – Vectors feed on virus infected plants and become infected with the virus for life. The virus has to be passed through the gut wall, into the haemoceol and then salivary glands of the vector and then reproduce so that it becomes permanent in the body of the vector. Even if the vector moults it does not loose the virus. iSome viruses can be passed from vector parent to offspring.

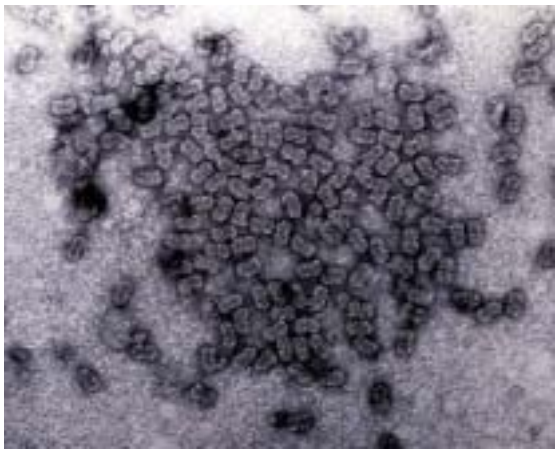
It is important to know how the virus is transmitted, as this will determine the control methods

The shapes of plant viruses

The advent of electron microscopy and its use by biologists gave virologists their first opportunity to see the structure of virus particles. During the 1950s and 70s the structure of virus particles was studied in great detail and plant viruses were classified according to their structure and properties. It soon became apparent that viruses of a similar shape often shared biological properties and so using electron microscopy as a diagnostic tool became routine. Unfortunately electron microscopes are expensive, costly to run and require expert technician backup so very few laboratories had one. The present day classification of plant viruses is still largely based on the shape of the particle. Here are some examples:

Rod-shaped viruses:
These can be long flexible (filamentous) example *Potyviridae* or more stiff, often short and fat rod shaped viruses example: *Tobamoviridae*. You will often see ring spot symptoms on papaya these are caused by the papaya ringspot virus a member of the potyviridae. Papaya Ringspot virus is transmitted by aphids in a non-persistent manner. When the virus is injected into the plant cell by its vector it takes over the metabolism of the cell to replicate itself.

Bipartitie virus: example Geminiviridae
These are twinned particles, angular in appearance with a genome of DNA. This family are only transmitted by hoppers, which need to feed on the infected plant to become infected. E.g. Maize leaf streak. There is a delay in the hopper becoming permanently infected with the virus, as this is a persistent virus.



Isometric (spherical) viruses: example Luteoviridae
These are 'ball' shaped viruses. Once an aphid has this virus it has it for life. This family causes symptoms of leaf rolling. Leaf rolling occurs when the leaf rolls up.

Virus symptoms

If you hold leaves up to the light the symptoms of virus are easier to observe. There are many including:-

- Colour changes – yellowing / reddening
- Rolling. Leaf roll occurs when the side of leaf rolls downwards and meet in the middle
- Cupping. Sides of the leaf roll upwards slightly, like a cupped hand
- Mosaic. Distinct patches of colour on a leaf
- Mottle. Leaf has a speckled 'dappled' appearance.
- Necrosis: along veins, or streaks on stem, ringspot on fruit.
- Distortion of leaves and fruits
- Stunting – a feature of some viruses, although mainly associated with phytoplasmas
- Differences in age of leaves- The same virus may show different symptoms in different ages of leaves, so it is important to look at all ages of leaves

Examples

Left. A mosaic caused by tobacco mosaic virus on tobacco. The dark green patches look like islands against the yellow of the leaf. The leaf has a crumpled appearance we call rugosity (roughness).

Right. Yellow net on *Malvastrum sp.*
The veins stand out as dark green against the bright yellow of the leaf lamina,

Left. Leaf yellowing and mosaic on M*alvastrum spp.*

Right. This virus infection of a cucurbit gives the leaf a rugose appearance and its edges become pale green and yellow

Tomato

Leaf curl - leaves curl up like the sides of a spoon. Spread by a whitefly vector *Bermissia tobaccae* which multiply quickly at high temperatures.

[Above] E.g. *Tomato spotted wilt*. The leaves look as if they have been sprayed with a browm / purple paint giving them a spotted appearance.

[Above] E.g. *Tomato yellow leaf curl*- A persistent virus so once a vector is infected it is infected for life. A few resistant varieties have been developed in Latin America, although people complain that the taste is poor. Prevent the crop getting the virus by growing under 'fleece' which allows water and air to the crop, but prevents whitefly entering.

Passion fruit [Left]

Passion fruit woodiness virus - leaves show mosaic, and are crinkled or rugose in appearance, may be mis-shapen the outside of the fruit gets bumpy and also gets localised areas of tissue death, known as necrosis, or necrotic areas. The fruits are hard and woody. Inside the fruit, the area surrounding the passion fruit seeds enlarges and so fruits contain less juicy flesh for pulping.

Grasses [Right]

Veins in grasses run along the length of the leaf, so mosaics and mottles tend to follow the area between the virus, giving streaking or lining.

Phytoplasmas (See power point presentation 'Life without walls')

These are bacteria without a cell wall. They are obligate pathogens which means they can't live outside of the host plant or insect vector. Phytoplasmas cannot be cultured *in vitro* and so research on them has been very difficult. As we begin to understand the genetic make up pathologists have devised rapid detection methods based on the amplification of the phytoplasma DNA. This has led to the classification of phytoplasmas into a number (17 at the time of writing) species. Phytoplasmas can affect lots of different hosts including vegetable crops, ornamentals trees and fruit crops. Only leafhoppers, planthoppers and psyllids members of the Auchenorrhyncha transmit them. The range of hosts is determined by the feeding preferences of the vectors.

Symptoms of phytoplasmas

- Stunting
- Yellowing
- Phyllody, in which the flowers become leaf-like.
- Virescence in which the flowers retain their shape but are green in colour
- Proliferation of tissues (distorted additional growth) or witch's broom
- Colour changes in flowers – Break up the normal colour of the flower, but tend not to cause a colour change in the leaves, as they exist only in the phloem. Viruses are systemic so can cause a colour change in the leaves.

Field Diagnosis for virus and phytoplasma

Some symptoms shown by virus and phytoplasma can also be caused by other pathogens. It is important to rule out other causes e.g. fungi (for vascular pathogens check for staining in the tissues) and bacteria (do the bacterial streaming test with plants in their early stages of symptom expression).

- Stem bleeding – can be caused by both virus and fungi
- Canker manly associated with bacteria, but can be caused by fungi. Not usually associated with virus
- Gummosis-
- Galls- mainly associated with bacteria if symptom occurs on the tree, but if found on the leaves can be caused by insects of viruses. On the roots usually associated with nematodes
- Distortion- twisting and leaf rolling can be caused by
- Wilting- the broad bean wilt viruses can cause wilting, although this is mainly associated with fungi, bacteria and lack of water!