Draft curriculum for a followup Farmer Field School on NPV Management

Field study on Nuclear Polyhedrosis (NPV)

Pilot study conducted in Ha Tay, Vietnam
Spring-Summer season 1999

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Background

In November 1996, a rearing unit for parasites of *Plutella* and *Pieris* was set up at the Regional Plant Protection Center in Hung Yen. The HY center successfully reared *Diadegma semiclausum*, a parasite of *Plutella*, which was introduced from Malaysia. The HY center carried out small field studies in locations close to the Center. In the following Winter-Spring 1997-1998 season a bigger field study was carried out by farmers in Ha Tay to collect information on the establishment of parasites in their fields. The study was carried out in the same area for the following three seasons from Winter-Spring 1997-1998 to Autumn 1998. The results were not very encouraging, i.e., after the third season no parasites could be recovered or seen. One factor that contributed to this was the extremely high temperature in the summer season and the lack of brassica plants that made it impossible for the parasites to survive.

Instead of being discouraged, the farmers who were involved in the parasite study felt that they should try other options. After hearing from some sources about viruses the farmers, on their own, started collecting diseased insects from the field, crushing them and spraying the solution on other insects. They observed that more insects became sick and died. In Winter-Spring 1998-1999, the trainers helped the farmers seek the assistance of the National Programme in setting up field studies on nuclear polyhedrosis virus (NPV) and in helping farmers access materials and technical backstopping from researchers of a local research institution, the National Institute of Plant Protection (NIPP). This season (Summer 1999) a curriculum based on last season's experience will be tried out in a follow-up activity in order to determine future strategies for the use of biological control agents like NPV in the northern Vietnam.

Objectives of the study:

The study aims at answering the following questions:
1. Can the farmers produce virus for use during the season?
2. Can the virus spread in farmer fields in the North of Vietnam during the Winter-Spring season?
3. Can the farmers mass produce and store virus for use in the next season?
3. What training processes need to be developed to enable farmers to understand how viruses work?

Field set up:

The study will be a follow up FFS with a special focus on Nuclear Polyhedrosis as a biological control agent.

Area identified: The size of the area for field school studies on cabbage and tomato will be $2000m^2$ in Hoai Duc district. Arrangements will be made with owners to use the fields in an area of $3400m^2$ surrounding the $2000m^2$ for trials to initiate the spread of NPV.

A requirement for both fields is that NO CHEMICAL INSECTICIDES will be used for the duration of the study. Only BT can be applied when occurence of *Plutella* is observed. NPV will be applied when occurrence of *Spodoptera exigua* and *Spodoptera litura* on cabbage, and *Heliothis* on tomato is observed. This decision should be based on field observations and in discussion with the farmer group.

Ecosystem analysis will be done on the $2000m^2$. In this area, the farmers will observe crop development, pest and natural enemy populations, etc. throughout the season. The studies will be set up in such a way that the FP field for comparison will be outside the study area, to avoid the use of chemical insecticides within the study area.

The owners of the field can decide on the fertilizer use, water management, and choice of varieties for the study area. With the group of participants, make a map of the study area, and collect weekly information on the practices used for the separate plots. Remember: No chemical insecticides, only BT and NPV when needed! If BT is used, collect information

(timing, brand name, amount etc). If NPV is used, record information on timing, amount etc. If fungicides/herbicides are used, collect this information as well and write it in the map.

Facilitators:

The study will be facilitated by two vegetable IPM trainers of Ha Tay province and one trainer of the Hung Yen center. Mr. Bui Chi Hung and Mr. Nguyen Ba Chuong of Ha Tay province will be facilitating the study, and will be there every week. Ms. Nguyen Thi Hien of the HY Center will arrange time to ensure that she can be there every week, for the duration of the season. Mr. Do Danh Kiem from the PPSD will provide technical backstopping twice a month throughout the season. Ms. Hoang Thi Viet from NIPP will provide technical backstopping for at least 4 weeks of the study, spread over the season and as necessary. Mr. Le Tien Binh from the PPD will visit the study regularly as well.

Participants in the study:

Twenty-five (25) alumni IPM farmers will be selected who have their fields immediately surrounding the study field. Five farmers who were involved in the NPV study in the previous season will be selected to be the trainers for the follow up FFS.
An agreement has to be made with ALL the households that have their plots within the 2,000m^2 study area to ensure that no chemical insecticides will be used throughout the study. One member of the household should join in the study activity and will participate one morning every week in the sessions facilitated by farmer trainers.

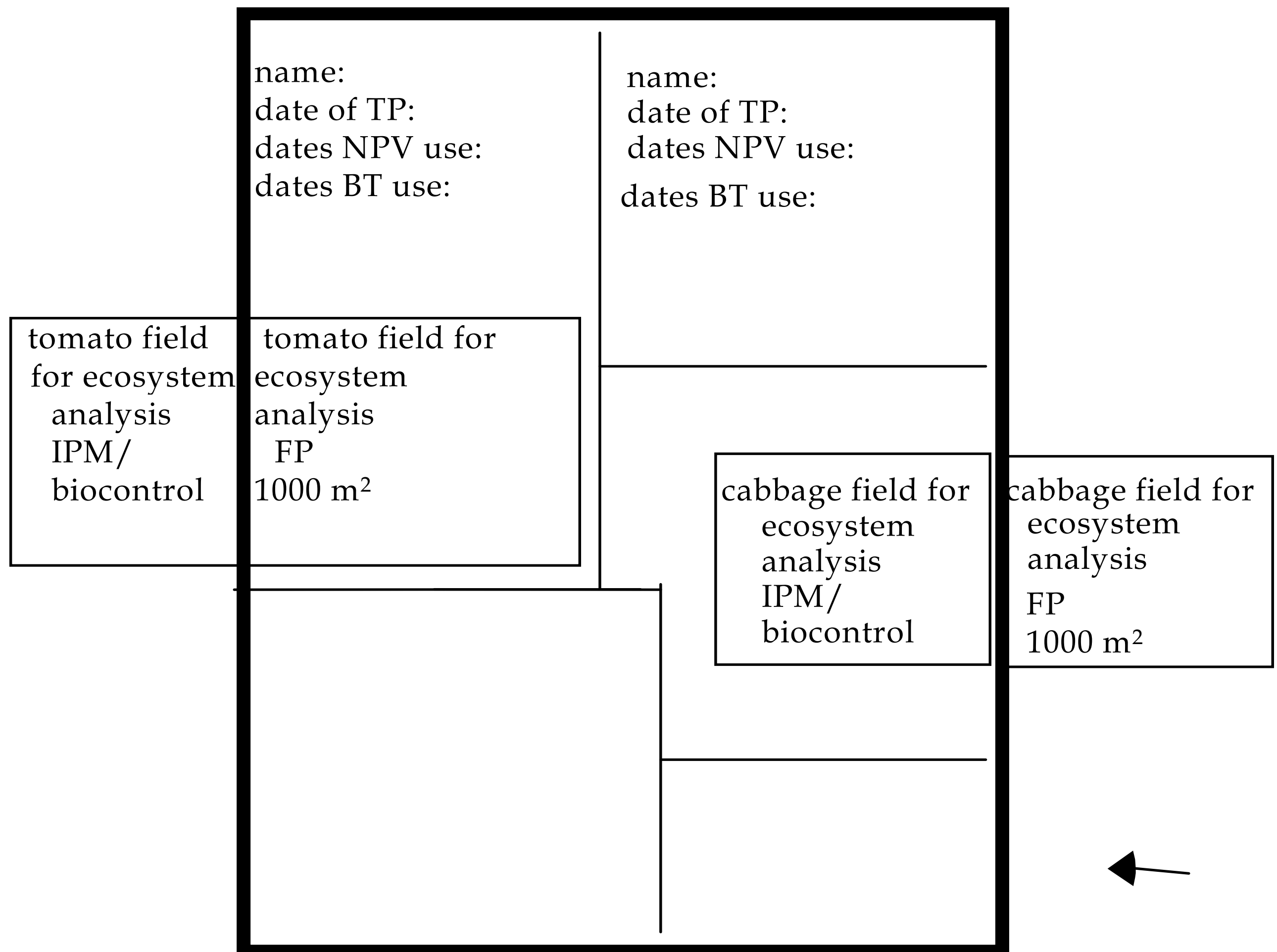
Introduction of NPV in the field:

NPV will be introduced as soon as the occurrence of *Spodoptera/Heliothis* is observed to avoid the build up of population of the insect. When there are 1.5-2 young *Spodoptera* larvae per plant or when there are 3 young *Heliothis* larvae per plant, introduce NPV.

Initially, the biocontrol agent may be brought in from NIPP. Once it has been introduced to the field and some larvae in the field show signs of infection, production may be done using infected insects from the field. The process of collection, production and using in the field may be repeated as often as necessary. Keep records of the number of NPV sprays made.

Observations of the study:

These observations will be carried out every week as part of the FFS/study group.



I. The FFS field- field for ecosystem analysis

The FFS field will consist of 2000m^2 plot inside the study area (IPM-biological control field) and a field using the regular FP in the area that will be outside the study field, but very close to it. The 2000m^2 will be divided into two for cabbage and tomato IPM plots. The area for FP will also be divided into two plots for cabbage and tomato. Regular observation procedures will be followed in both IPM and FP plots for both cabbage and tomato. Observations should cover plant development, pests, natural enemies, diseases, weeds, weather conditions, etc. Ask the groups of farmers to make drawings of the ecosystem, discuss the questions in the ecosystem analysis, present and discuss with the whole group.

II. The study area

The total study area will be 15 sau. Divide the participants into 5 groups, and ask each group to make observations in the area assigned to that group. These observations will concentrate more on the populations of *Spodoptera exigua* and *S. litura* on cabbage; *Heliothis* on tomato. The observations will also consider the diseased/deceased (by virus infection) populations of the same insects.

Each week, each group should do the following:

1. Observe 30 sample plants in their sub-field where NPV has been sprayed. Do not remove the plants from the field.

2. Count carefully:

Spodoptera:
- number of larvae per plant
- number of pupae per plant
- if possible, the number of egg mass per plant (though it is quite difficult to see.) If too difficult, do not observe.
- number of diseased/deceased larvae (by virus infection)
- number of diseased/deceased larvae (by other factors)

Heliothis:
- number of larvae per plant
- number of pupae per plant
- if possible, the number of eggs per plant (though it is quite difficult to see) If too difficult, do not observe.
- number of diseased/deceased larvae (by virus infection)
- number of diseased/deceased larvae (by other factors)

3. As the area was used before for releases of *Diadegma*, observe also for this parasite. Record the following:

Diadegma: number of parasitoid cocoons by *Diadegma*

Plutella: Another parasite, *Cotesia plutellae* is present in Vietnam already. If you observe it, also count the number of parasitoid cocoons of *Cotesia plutellae*.

If you see adults of *Diadegma* or *Cotesia* in the field, record as well.

After observations by each subgroup in the field, ask each group to summarize the following information:

number of larvae of *Spodoptera litura* or *exigua* per plant

number of larvae of *Heliothis* per plant

number of virus diseased/deceased larvae of *Spodoptera litura* or *exigua* per plant

number of virus diseased/deceased larvae of *Heliothis* per plant

$$\% \text{ of infection by NPV} = \frac{\text{\# of virus diseased or deceased larvae}}{\text{Total number of larvae}}$$

(Note: The total number of larvae = healthy larvae + virus diseased or deceased larvae + diseased or deceased larvae due to other factors)

If *Diadegma* was observed, groups should also summarize:

% of *Plutella* parasitized by *Diadegma semiclausum and Cotesia*

Ask each subgroup to summarize and present their information to the whole group. Calculate the average populations of insect pests and virus infected insects for the whole study area. Summarize the information in two ways:

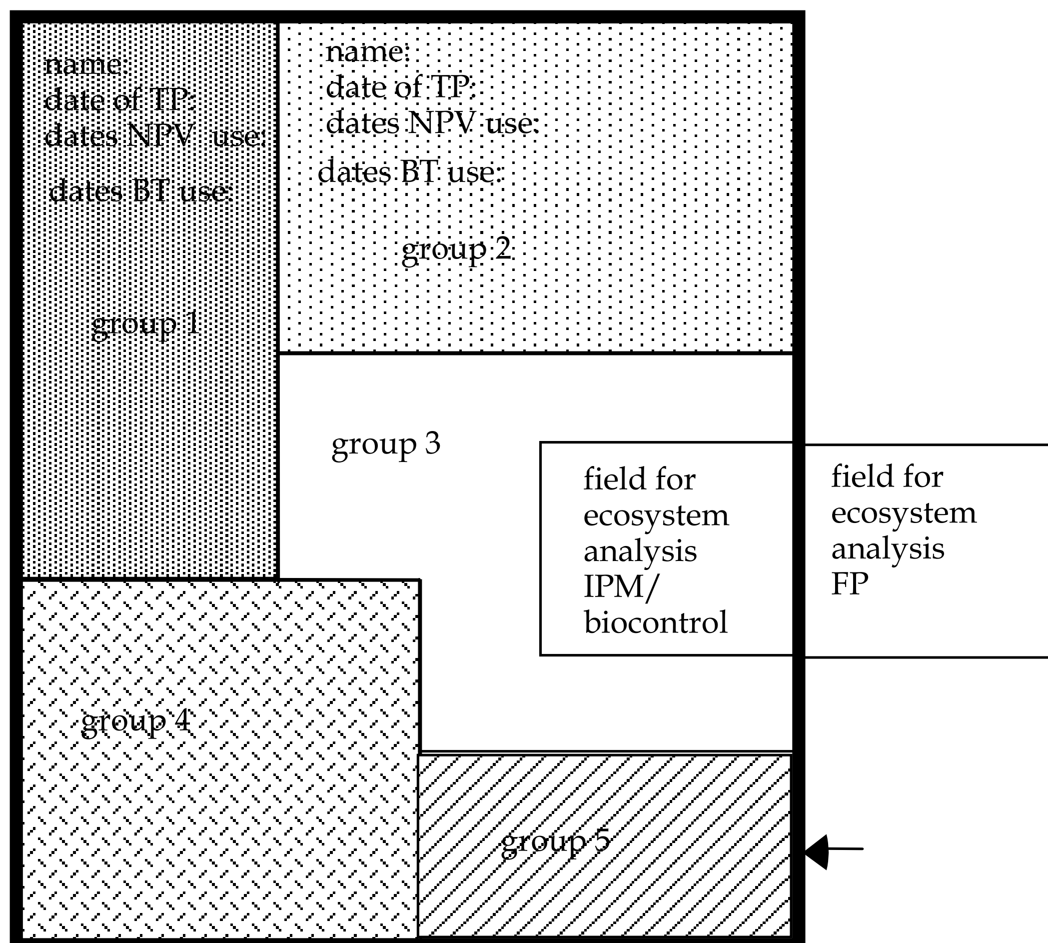
a. Make a map of the study area (see next page)

Draw each individual plot in it, and put the name of the owner, date of transplanting, use of NPV, use of BT.

In the beginning, decide which subgroup observes which part of the field.

When you spray the bioagent/s on the field put the date next to it.

Every week, each group records in tables that are drawn on big sheets of paper their observations on the insect pests and the virus infected populations.



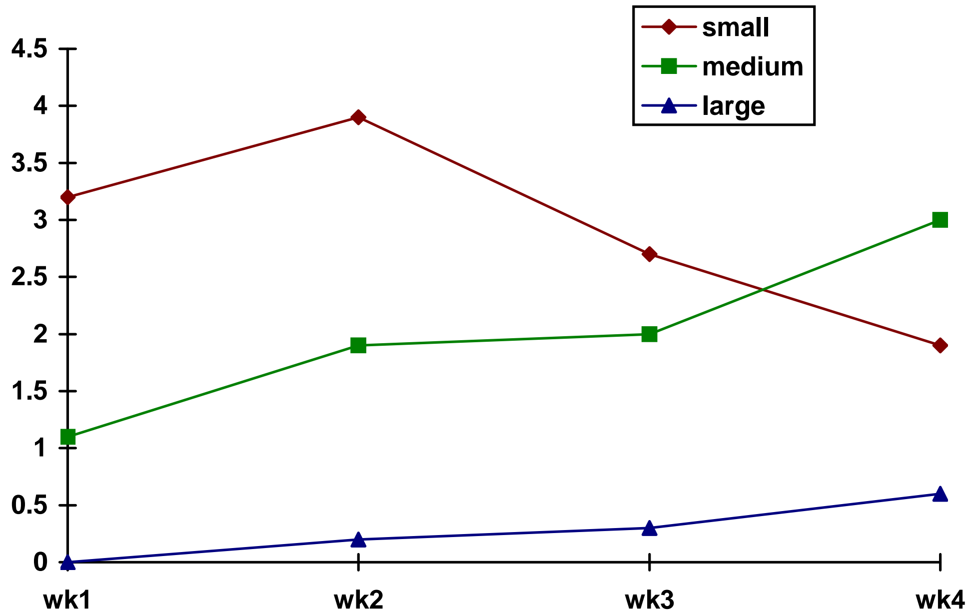
Tables:

Group 1

Date of transplanting subfield ....

Date of Observa-tion	DAT	Number *Spodop-tera* larvae (see note above)	Number *Spodop-tera* larvae diseased/ deceased due to other factors	Number *Spodop-tera* larvae diseased/ deceased due to virus	% of infection by NPV	Number *Heliothis* larvae (see note above)	Number *Heliothis* larvae diseased/ deceased due to other factors	Number *Heliothis* larvae diseased/ deceased due to virus	% of infection by NPV
Week 1									
Week 2									
Etc.									

b. Make a graphic of insect pests and virus infected/deceased populations for the whole study field. Update this weekly together with all participants. Make a separate graphic for *Spodoptera* and *Heliothis*. Record the hosts by size of the larvae. Example:



Discussions:

1. What is the population of *Spodoptera litura/exigua*? How many larvae per plant? How many are small, medium and large? How many are diseased/deceased due to virus?
2. What is the population of *Heliothis*? How many larvae per plant? How many are small, medium and large? How many are diseased/deceased due to virus?
3. Did the populations of the insect pests increase compared to last week? Did the populations of the virus diseased/deceased insects increase compared to last week? Why or why not?
4. What factors affect NPV? Consider factors like host species, host populations, insecticide use, weather etc.
5. Can parasitized cocoons of Plutella still be found? Why or why not?
6. What is the recommendation on the use of biocontrol agents especially NPV, for next week?

Think of more questions that are useful to discuss. Record them, so they can be included in future studies on biocontrol agents.

Special topics:

Base yourself on exercises on special topics that you would normally include in a FFS.
Include group dynamics and team building exercises.

Here are some suggestions for special topics that will focus more on the parasites, and will be suitable to be carried out:

1. Insect Zoo
   - predation studies (see field guide cabbage)
   - life cycles of *Spodoptera litura/exigua*; *Heliothis* and other important pests (field guide and additional)
   - life cycles of predators (field guide cabbage)
   - life cycles of parasites (additional exercises)

2. Food Webs for *Spodoptera* and *Heliothis* (cabbage field guide)

3. NPV studies (annnexed)

4. Effect of pesticides on Natural Enemies (field guide)

5. Effect of fungicides on NPV (additional exercise)

5. Production and storage of NPV in the room (additional exercise)

6. Viability of NPV after storage (additional exercise)

7. Spread of viruses to surrounding fields (annexed)

7. Crop development (field guide)

8. Discussions on “clean vegetables”

9. Plans for the future

Identification of Virus-infected Insects (Classroom Exercise)

Introduction

Today, natural viruses are used to manage insect pests and to reduce the use of chemical insecticides that are harmful for man and the environment. Natural viruses as a biocontrol agent have become a component of the IPM system. The Biological Control Research Center, National Institute of Plant Protection (NIPP) has done some work on methods of production and application of viruses in controlling insect pests. The methods have been simplified to allow farmers to work with the agent with minimum support from the research institute and trainers. This exercise will provide farmers first hand experience in the classroom in observing and comparing symptoms of virus-infected insect pests and insects which died because of other reasons, e.g., pesticides, etc. which is the first step in the procedure for producing the material for biocontrol.

Objective: Describe symptoms of virus-infected insects

Materials:

Specimen, i.e., virus-infected insects from the laboratory (enough material for all five subgroups)
Paper and markers

Procedure:

Use this exercise at the start of the season, when infected insects or insects which have died because of virus are not yet readily seen in the field. Ask each subgroup of 5 farmers to recall their experiences on seeing dead or infected insects in the field. Each subgroup should then draw their observations on big paper for presentation.

After all subgroups have presented their outputs, introduce the specimens of virus-infected insects from the laboratory. Ask each subgroup to describe symptoms of the specimens from the laboratory.

(Note: Insects infected by viruses become weak and activity is slowed down; the body color is changed; the cuticle becomes fragile and ruptures easily when touched, releasing the body content which has become liquefied. Dead larvae may be found hanging from or lying on leaf or plant surfaces with no filamentous structure on the cuticle.)

Discussions:

1. Based on experience, describe different appearance or symptoms exhibited by dead insects in the field? Describe the field conditions at the time the observations were made. Discuss about host populations, insecticide use, weather etc.
2. Describe appearance and characteristics of specimen from the laboratory. Have insects with such appearance and characteristics been observed in the field? What could have caused such symptoms? What does this mean for management of insect pests?

Identification of Virus-infected Insects (Field Exercise)

Introduction

Today, natural viruses are used to manage insect pests and to reduce the use of chemical insecticides harmful for man and the environment. Natural viruses as a biocontrol agent have become a component of the IPM system. The Biological Control Research Center, National Institute of Plant Protection (NIPP) has done some work on methods of production and application of viruses in controlling insect pests. The methods have been simplified to allow farmers to work with the agent with minimum support from the research institute and trainers. This exercise will provide farmers first hand experience in the field in observing and comparing symptoms of virus-infected insect pests and insects which died because of other reasons, e.g., pesticides, etc. which is the first step in the procedure for producing the material for biocontrol.

Objective: Describe symptoms of virus-infected insects

Materials:

Specimens collected from fields
Paper and markers

Procedure:

Do this activity once viruses are seen to spread in the FFS area. (Initially, inoculum may be introduced from the laboratory.) Ask each subgroup of five farmers to recall the classroom exercise done earlier, i.e., the discussion re: experiences on seeing dead or infected insects in the field and introduction of specimen from the laboratory.

Go to the field. Ask each subgroup to collect all dead insects that they see in the field.

In the classroom, subgroups should sort the dead insects based on appearance and symptoms exhibited. Then, using the group's drawing from the earlier exercise, subgroups present their field observations, i.e., their collection of insects from the field.

(Note: Insects infected by viruses become weak and activity is slowed down; the body color is changed; the cuticle becomes fragile and ruptures easily when touched, releasing the body content which has become liquefied. Dead larvae may be found hanging from or lying on leaf or plant surfaces with no filamentous structure on the cuticle.)

Discussions:

1. Based on experience, describe different appearance or symptoms exhibited by dead insects in the field? Describe the field conditions at the time the observations were made. Discuss about host populations, insecticide use, weather etc.
2. Were there insects with the same appearance and characteristics as the specimen from the laboratory? What does this mean for management of insect pests?

Method of production and application of virus in controlling insect pests on vegetables

Introduction

In the natural setting, insect pests are infected by many microorganisms like viruses, bacteria, fungi, protozoa, etc. In a number of cases, viruses have been recognized as a biocontrol agent in checking insect pest population. Every year, during months when the climatic condition is hot and sunny and the humidity is high, several insect pests on vegetables, e.g., *Heliothis armigera* in tomato, armyworm (*Spodoptera litura*) and diamondback moth (*Plutella xylostella*) on cabbage, and armyworm (*Spodoptera exigua*) on onion are infected by viruses. This helps reduce the caterpillar population.

Today, natural viruses are used to manage insect pests and to reduce the use of chemical insecticides which are harmful for man and the environment. Natural viruses as a biocontrol agent have become a component of the IPM system. The Biological Control Research Center, National Institute of Plant Protection has done some work on methods of production and application of viruses in controlling insect pests. The methods have been simplified to allow farmers to work with the agent with minimum support from the research institute and trainers. This exercise will provide farmers first hand experience in producing virus for use in controlling insect pests in vegetables.

Objective: Experience producing virus for use in controlling insect pests in vegetables

Materials:

Small rectangular plastic containers/penicillin vials and wood rack (depending on species of larvae)
Muslin cloth for filtering virus solutions
Paper to cover the plastic containers
Mortar and pestle
Glass bottle with cover 0.5 liter capacity
Pincers
Dark plastic can or glass bottle to keep virus suspension
A large number of small larvae
Natural diet: cabbage leaves, etc.
Jaggery or vegetable oil
Boiled water (boil for 20-30 minutes and let it cool)

Procedure:

I. Producing the virus solution
There are two procedures for producing virus solutions. Virus products are prepared using infected larvae collected from the field, or larvae infected in the laboratory.

Procedure A: Using infected larvae collected from the field
1. Look out for the time of appearance and the development of disease in natural conditions. As soon as they are observed, collect dead larvae from the field and store them in a covered glass bottle to produce the virus products.
2. Putrefy for two – three days.
3. Macerate in mortar.
4. Add a little boiled water at a time, stir and filter through muslin cloth to discard tissue debris. Repeat this step until the extract obtained is clear.
5. To extract from 500 – 1000 large-sized larvae, add one liter boiled water. Do not use unboiled water because it can ruin products.
6. After filtering, store the mixture in colored bottles or dark cans. Store in a cool dark place.
7. Add 0.5% jaggery before using to spray in the evening.

Procedure B-1: Using small-sized larvae infected in the laboratory

1. Mass rearing of insects

A large number of larvae is needed to produce viruses. Rear the larvae in clean plastic containers with natural diet, i.e., cabbage leaves, etc. Replace leaves every day. Leaves should be fresh and not too old. Cover the containers with cloth or paper to keep larvae from escaping**.** When the larvae have grown up to 10 - 15 mm long, they can be infected with the inoculum.

2. Preparing the inoculum

To create an initial disease source or inoculum, collect larvae from the field exhibiting symptoms of virus infection. Use one (1) rather big larva (about 30mm long) to 100ml clean boiled water. With the mortar and pestle, grind the diseased dead larvae. Add a little clean boiled water at a time, stir and filter through muslin cloth to discard tissue debris. Repeat this step until the extract obtained is clear. Do not use unboiled water because it can ruin products. Soak food plant leaves in the liquid, air dry them, and then feed them to the healthy larvae in the containers.

3. Preparation of virus product/solution

Observe the larvae every day. Three to four days after the symptoms of disease have appeared and larvae begin dying, use pincers to remove all the dead larvae and transfer them into a glass bottle with lid/cover. Discard all the dead larvae not infected by virus.

4. Putrefy for 2 - 3 days.
5. Macerate in mortar. Add a little clean boiled water at a time, stir and filter through muslin cloth to discard tissue debris. Repeat this step until the extract obtained is clear.
6. To extract from 500 – 1000 large-sized larvae, add one liter boiled water. Do not use unboiled water because it can ruin products.
7. After filtering, store the mixture in colored bottles or dark cans. Store in a cool dark place.

Procedure B-2: Using medium-sized larvae infected in the laboratory

1. Collect medium-sized, healthy larvae from the field.
2. Inoculate larvae by feeding virus treated leaves for two days. To prepare the leaves, soak them in the inoculum and air dry before feeding to the healthy larvae. (For details, refer to Procedure B-2 step 2.)
3. Infected larvae will turn white and die in seven days. Collect diseased larvae in clean boiled water.
4. Putrefy for 2 - 3 days.
5. Macerate in mortar. Add a little clean water at a time, stir and filter through muslin cloth to discard tissue debris. Repeat this step until the extract obtained is clear.
6. To extract from 500 – 1000 large-sized larvae, add one liter clean boiled water. Do not use unboiled water because it can ruin products.
7. After filtering, store products in colored bottles or dark cans. Store in a cool, dark place.

II. Applying the virus solution

Larvae in the final instar are resistant to the virus. However, the virus will efficiently control earlier instar larvae if applied as follows:

1. Use a dosage of extract from 500 – 1000 large-sized, diseased larvae to 600 - 800 liters of water per hectare.
2. Add 0.5% jaggery or vegetable oil.
3. Spray 2-3 times at intervals of 7-10 days.
4. Spray in the evening hours to prevent destruction of the virus by the UV fraction of sunlight.

Avoid:

1. Brackish water for storing as well as spraying the virus
2. Grown up caterpillars for virus inoculation
3. Spraying in hot, sunny conditions.

Discussions:

1. How are larvae infected by the virus?
2. Were viruses found in the FFS fields? Describe conditions in the field at the time when the viruses were observed. What factors influence whether or not viruses can spread? Discuss about host populations, insecticide use, weather etc.
3. What action should the farmer group take if it wants more farmers in the area to make use of viruses? How can this be done?

Spread of Viruses to Surrounding Fields

Introduction

In the FFS field, viruses (NPV) have been sprayed and farmer groups have made weekly observations in the study area. If you start finding diseased/deceased insects (insects that are sick or have died because of virus) regularly in the FFS area, it will be good to find out whether the viruses have also spread to surrounding fields. Carry this activity out as a special topic, once you start to see the virus spread in the FFS area. It can also be repeated several times during the season to determine the extent of spread of the virus.

Objective: Find out if the viruses can spread to other fields surrounding the FFS area

Materials:

Fields surrounding the FFS area
Paper and markers

Procedure:

Ask each subgroup of five farmers to select three fields close to the FFS area. Each group will observe 30 plants in each field following the same methods as in the FFS area:

Spodoptera: number of larvae per plant
number of pupae per plant
if possible, the number of egg mass per plant (though it is quite difficult to see) If too difficult, do not observe.
number of diseased/deceased larvae (by virus infection)

Heliothis: number of larvae per plant
number of pupae per plant
if possible, also the number of eggs per plant (though it is quite difficult to see) If too difficult, do not observe.
number of diseased/deceased larvae (by virus infection)

As the area was used before for releases of *Diadegma* and *Cotesia*, observe also for these parasites. Record the following:
Diadegma: number of parasitoid cocoons by *Diadegma*
Plutella: Another parasite, *Cotesia plutellae* is present in Vietnam already. If you observe it, also count the number of parasitoid cocoons of *Cotesia plutellae*.
Cotesia: number of parasitoid cocoons
If you see adults of *Diadegma* or *Cotesia* in the field, record as well.

After observations by each subgroup in the field, ask each group to summarize the following information:
number of larvae of *Spodoptera litura* or *exigua* per plant
number of larvae of *Heliothis* per plant
number of virus diseased/deceased larvae of *Spodoptera litura* or *exigua* per plant
number of virus diseased/deceased larvae of *Heliothis* per plant

$$\% \text{ of infection by NPV} = \frac{\text{\# of virus diseased or deceased larvae}}{\text{Total number of larvae}}$$

(Note: The total number of larvae = healthy larvae + virus diseased or deceased larvae + diseased or deceased larvae due to other factors)

If *Diadegma* was observed, groups should also summarize:
% of *Plutella* parasitized by *Diadegma semiclausum*

Ask each group also to record the stage of crop development.

Make one map indicating the fields each subgroup observed. Write down on the map the number of insect pests and how many of these are sick or died due to virus and the % of infection in each field.

Discussions:

1. Were viruses found in the surrounding fields?
2. What factors influence whether or not viruses can spread? Discuss about host species, host populations, insecticide use, weather etc.
3. If activity was repeated at a later stage: How does the situation of surrounding fields compare with the previous observation? Did the number of virus-infected insects increase or decrease?
4. What action should the farmer group take if it wants more farmers in the area to make use of viruses? How can this be done?

Assessment of viability of NPV

This exercise will use living organisms to determine if NPV has maintained its toxicity in storage or whether the NPV purchased from a store is still useful for application in the field.

Introduction

Cabbage/tomato caterpillar pests such as *Spodoptera* and *Heliothis* have become resistant to a wide range of chemical insecticides. From different research studies around the world, NPV has been shown to effectively control *Spodoptera* and *Heliothis* and other caterpillar pests. However, since it is a sensitive biological agent, it is subject to rapid breakdown and loses its killing power. Use of NPV is part of an IPM programme which works with other natural enemies to control cabbage/tomato pests. Chemical insecticides do not do this. Therefore, this exercise is to discover the toxicity of NPV as well as to determine if the NPV bought from the local shop is still useful.

Objective: Answer the common question: Did I buy good NPV and will it be effective against the caterpillar pest in my field?

Materials:

1 teaspoon
Marker for labelling cup
Pincers for dipping leaf sections
1 unsprayed cabbage/tomato plant
2 camel or fine hair brushes
1 pair of scissors
10 plastic cups with plastic/organdie sheets used to cover the cup, and rubber bands
1 packet of NPV bought from a local shop (use a different brand of NPV for each group)
1 litre of clean water
16 or more *Spodoptera* or fewer *Heliothis* larvae - preferably small ones
1 roll of tissue paper

Procedure:

1. Fill 2 plastic cups with water.
2. Mix 1/4 teaspoon NPV into water in one cup. Label the cup "NPV" and the other cup "Water".
3. Collect fresh leaves from the unsprayed cabbage/tomato plant. Cut the leaves into sections of 1" diameter.
4. Dip one leaf section into the "NPV" cup and continue for three other sections. Similarly, dip four leaf sections into "Water" cup.
5. After removing the leaf sections from the solution, the leaf sections should be allowed to dry in a cool, shaded place.
6. Place one in each cup and label according to the treatment used. There should be four cups with leaf sections treated with "NPV" and four more with leaf sections treated with "Water". Each cup should be lined with tissue paper.
7. Using the brush, transfer two caterpillars onto each of the leaf sections. Avoid damaging the caterpillars. Quicker results are obtained if smaller caterpillars are used. If using *Heliothis*, use one caterpillar per leaf section as they may be cannibalistic.
8. Each cup should be covered with either the plastic or organdie sheet held securely with rubber bands.

Observations:

Check the cups every 10-12 hours and observe for frass (caterpillar droppings) and larval death. Usually, obvious differences can be seen within 1.5 day.

Discussions:

1. What happened to the larvae in the two treatments?
2. Is there any difference in the amount of frass produced by the caterpillars? If yes, why so?
3. Did I buy good NPV?
4. Why were cups lined with tissue paper?
5. Why were cups placed in the shade?
6. Why was a comparison with water included?
7. Did any of the NPV products perform better than others?

Inhibition of larval feeding by NPV

Introduction

This study will show how NPV inhibits larval feeding. Many farmers spray NPV without seeing immediate kill of the target insect. This is because NPV acts more slowly than conventional chemical insecticides, thought it is no less effective. Before actual death occurs, feeding by larvae stops. This slow action often causes farmers to think that NPV is not effective. However, the benefits of NPV (conservation of parasitoids and predators, overall minimal health risk to farmers and consumers, minimal adverse effects on the environment) far outweighs the benefit of speedy killing of caterpillars using chemical insecticides. Moreover, resistance (in the target caterpillars) to chemical insecticides has rendered insecticides less effective than NPV.

Objective: Understand how fast NPV kills the target caterpillar and to realize that NPV makes the pest stop feeding hence there is less damage caused.

Materials:

1 unsprayed cabbage plant
2 camel or fine hair brushes
1 pair of scissors
8 plastic cups with plastic/organdie sheets and rubber bands
1 roll of tissue paper
1 packet of NPV
2 litres of clean water
1 plastic pail/container
1 long wooden stirrer
Pincers for dipping leaves
1 set of paper and pencil
Marker for labelling cups
Caterpillars

Procedure:

1. Collect fresh leaves from the upper part of the cabbage plant. Cut leaves into 1" diameter sections.
2. Using a pail or containers, pour a litre of water and mix the recommended dose of NPV on the label. Mix well using a long stirrer.
3. Dip four leaf sections into the pail with NPV solution.
4. Place one section in each of four cups lined with tissue paper and label "NPV".
5. Dip another four leaf sections into a cup with only water, and place each of these into a separate cup labelled "Water".
6. Place caterpillar in each cup with camel hairbrush.
7. The next morning, check for feeding and/or larval death.
8. Replace the leaf sections (NPV treated ones in the "NPV" cup and water treated ones in "Water" cup).
9. At noon, check again on feeding.
10. Using paper and pencil, trace the area of the leaf section from the "NPV" cup and the "Water" cup.
11. Replace the leaves removed for drawing.
12. Compare the leaf tracings from "NPV" and "Water" cups.
13. Observe the amount of frass, caterpillar droppings in both treatments.
14. Repeat the above observations in the late afternoon, and continue up to three days.

Discussions:

1. Were there any differences in feeding/and caterpillar deaths between NPV-treated and water-treated leaf sections?
2. When did these differences occur?
3. Were there any differences in amount of frass produced?
4. What do these differences indicate?
5. Why did the larvae stop feeding, if they did?
6. What does this mean for crop damage after NPV is applied?

Sensitivity of NPV to sunlight

Introduction

Since NPV is a biological agent, it is sensitive to sunlight. In bright sunlight, it loses its effectiveness and strength to kill caterpillars This study will show that sunlight breaks NPV down.

Objective: Make appropriate decisions on how to apply NPV

Materials:

2 rows of cabbage/tomato plants (about 15-30 days after planting), untreated with any pesticide
1 potted cabbage/tomato plant (about 15-30 days after planting), untreated with any pesticide
16 *Spodoptera* or fewer *Heliothis* (or similar caterpillars) (small and of similar size)
1 hand sprayer (1 litre size will suffice)
1 packet of NPV
3 camel or fine hair brushes
1 pair of scissors
1 pail of clean water
12 plastic cups with plastic/organdie sheets and rubber bands

Procedure:

1. Mix NPV at recommended rate in a pail of water and spray one row of cabbage/tomato plants at midday. Use about four plants.
2. In the evening just before sunset, spray another row of cabbage/tomato plants (another 4 plants).
3. An hour after the last spray, collect leaves from the canopy of both rows, cut out 4 leaf sections of 1" diameter, and ensure that these are labelled.
4. Prepare four similar sections from a potted plant free of insecticides.
5. Keep each of the leaf sections in a separate plastic cup lined with tissue paper and label as "NPV-sunlight", "NPV-no sun" and "No NPV".
6. Collect caterpillars from the field (preferably smaller ones, as these react faster than older caterpillars) for the study.
7. Using a camel hairbrush, drop two caterpillars (one if using *Heliothis*) onto each leaf section.
8. Store the cups in a cool, shady place.
9. Observe signs of feeding (size of holes made in the leaf section, as well as amount of frass produced).
10. Record the number of living larvae.
11. Continue the study for up to 3 days.

NOTE: Use this same method at two-day intervals to determine the effectiveness of NPV on cabbage/tomato in the field.

Discussions:

1. Did the larvae feed on the leaves?
2. Did any of the larvae die? In which treatment?
3. What was the effect of sunlight on NPV?
4. Why should the study be repeated?
5. When is the best time of the day to apply NPV?

Effect of NPV on predators and parasitoids

Introduction

This exercise will attempt to show the impact of spraying NPV on both predators (insects or spiders that eat other insects, particularly pests) and parasitoids (insects that lay eggs in or on its host so that the host provides food for the young stages of the parasitoid). A danger in using chemical insecticides is that it kills friendly insects that help farmers control pest organisms. Since NPV is applied as a spray, this exercise will help farmers to discover the impact of NPV on these beneficial insects.

Objective: Describe the effect of NPV on a natural enemy and better appreciate the role of NPV in an IPM programme

Materials:

2 cabbage/tomato plants (15-30 days after planting)
2 hand sprayers (1 litre)
1 pail of clean water
1 packet of NPV
4 large plastic cups with organdie cloth sheet and rubber bands
2 camel or fine hair brushes
10 parasitoid cocoons
10 common predators from cabbage/tomato field
10 clear plastic film containers
1 small bottle of honey
1 roll of cotton wool
1 roll of tissue paper

Procedure:

1. Place one parasitoid cocoon collected from the field into each of the film containers.
2. Store the containers in a cool shaded place until the adult parasitoids emerge.
3. Feed the adult parasitoids with a diluted honey solution (on a moist cotton wool).
4. When there are sufficient adult parasitoids, mix NPV at the recommended rate and spray a cabbage/tomato plant with it. Allow the plant to dry for an hour.
5. Collect leaves from the upper part of the plant and cut out leaf sections of 1" diameter and place these into each of two large plastic cups with cover. Label each cup.
6. Two cups with leaves from an unsprayed plant should be similarly prepared.
7. Place a solution of diluted honey in each plastic cup.
8. Introduce parasitoids into each of the cups and secure the cover with rubber bands.
9. Store the cups in a cool shaded place and observe every day.
10. Record the number of dead parasitoids in each situation.
11. A similar study is conducted with field collected predators (e.g. spiders, syrphid larvae etc.). With predators there is no need for honey solution.

Discussions:

1. Why was diluted honey solution placed in cups with the parasitoids?
2. Was there any dead parasitoid or predator in the cups? Why?
3. Does NPV kills parasitoids and predators? Why or why not?
4. What role could NPV play in an IPM programme?

Effect of Fungicides on the Viability of NPV

Introduction

We have seen how insect pests can be infected by microorganisms like viruses that are now recognized as a biological control agent in checking insect pest populations. We also know from experience that farmers still have problems managing crop diseases, and use fungicides to control disease. NPV is a sensitive biological agent. Since NPV is a causal organism for disease in insect pests and farmers use fungicides to control disease in the crop, there is a danger that fungicides may inhibit the action of NPV. This exercise will attempt to show the effect of fungicide sprays on the viability of NPV.

Objective: Explain the effect of fungicides on the action of NPV

Materials:

Method A:
20 plastic cups covered with plastic/muslin cloth and secured with rubber bands
32 or more *Spodoptera* or 16 or more *Heliothis* larvae - preferably small ones

Method B:
20 plastic cups covered with plastic/muslin cloth and secured with rubber bands
32 or more *Spodoptera* or 16 or more *Heliothis* larvae - preferably small ones
Four small hand sprayers (0.5 liter capacity)

For both methods:
1 unsprayed cabbage/tomato plant
2 camel or fine hair brushes
1 pair of scissors
NPV preparation
Fungicide
1 litre of clean water
1 roll of tissue paper
Labels
Paper and pen

Method A: Using leaf sections

1. Fill 4 plastic cups with water. Prepare solutions for 4 treatments:

- Mix 1/4 teaspoon NPV into water in one cup and label the cup "NPV"
- Mix fungicide based on recommended dose into water in one cup and label the cup "fungicide"
- Mix 1/4 teaspoon NPV and fungicide based on recommended dose into water in one cup and label the cup "NPV + fungicide"
- Keep one cup only with water, and label the cup "Control"

2. Collect fresh leaves from the unsprayed cabbage/tomato plant. Cut a total of sixteen leaf-sections, each section measuring 1" in diameter.
3. Dip four leaf sections into the "NPV" cup. Similarly, dip four leaf sections into the "Fungicide" cup. Do the same for the "NPV + fungicide" and the "Water" cups.
4. After removing the leaf sections from the solutions, allow them to dry in a cool, shaded place.
5. Line each cup with tissue paper.
6. When the leaf sections are fairly dry, place one section in each cup. Label cups according to the treatment used. There should be four cups for each treatment.
7. Using the brush, transfer two caterpillars onto each of the leaf sections. Avoid damaging the caterpillars.
8. To obtain quicker results use smaller caterpillars. If using *Heliothis*, use one caterpillar per leaf section, as they may be cannibalistic.

9. Cover each cup with the plastic/muslin cloth and secure with rubber bands.

Method B: Spraying directly on insects

1. Prepare four hand sprayers before the practical. If a sprayer has been used before, wash it thoroughly with detergent.
2. Prepare solutions according to the four treatments below. Put one solution each of the hand sprayers and correspondingly. The four preparations are:

- "NPV": Mix 1/4 teaspoon NPV with one cup of water
- "Fungicide": Mix fungicide with one cup of water based on recommended dose
- "NPV + Fungicide": Mix 1/4 teaspoon NPV and fungicide based on recommended dose with one cup of water
- "Control": Put only water

3. Spray four pieces of muslin cloth with one of each treatment and air-dry the pieces of cloth.
4. Collect fresh leaves from the unsprayed cabbage/tomato plant. Cut a total of sixteen leaf-sections, each section measuring 1" in diameter.
5. Line each cup with tissue paper and place one leaf section in each.
6. Collect several caterpillars from the field. Avoid damaging the caterpillars. Quicker results are obtained if smaller caterpillars are used.
7. Using the brush, transfer two caterpillars onto each of the leaf sections. If using *Heliothis*, use one caterpillar per leaf section as they may be cannibalistic.
8. Cover each cup with the muslin cloth and secure with rubber bands.
9. Label cups according to the treatment used. There should be four cups for each treatment.
10. Check the cups every 10-12 hours and look for frass (droppings of caterpillars) and larval death. Usually, obvious differences can be seen within 3- 4 days.

Discussions:

1. What happened to the larvae in the four treatments?
2. Is there any difference in the amount of frass produced by the caterpillars? If yes, why so?
3. How many days did it take to observe symptoms of disease on the caterpillars? Describe the symptoms and time of occurrence of disease on caterpillars in the different treatments. Do caterpillars in one treatment exhibit more symptoms than those in other treatments? Did they get sick sooner? What are the possible reasons for these differences?
4. What do these observations mean for the use of NPV in managing insect pests?
5. What do these observations mean for the use of fungicides in managing crop diseases?

Inputs for exercises re safety precautions with Bt and NPV insecticides, based on info from

1. WHO Recommended Classification of pesticides by Hazard
2. BioPesticide Manual, 1998, British Crop Protection Council

NPV: Considered to have low mammalian toxicity, with no evidence of allergic or other adverse effects reported by users, so safety prcautions are much less than for chemical pesticides. However, the detailed precautions necessary for use of a pesticide product depend on the nature of the formulation and the pattern of use; therefore, users should refer to precautions given on the product label. No pre-harvest interval required.

Bt: Considered non-toxic to people, with no allergic reactions or other health problems reported by users, so safety precautions are much less than for chemical pesticides. However, the detailed precautions necessary for use of a pesticide product depend on the nature of the formulation and the pattern of use; therefore, users should refer to precautions given on the product label. No pre-harvest interval required.