

Rearing of diamondback moth parasites



Asian Vegetable Research and Development Center

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Preface

Diamondback moth, *Plutella xylostella* (L.), an insect of European origin, is now widespread throughout the world, causing enormous damage to all economically important cruciferous vegetables, such as cabbage, cauliflower, broccoli, radish, mustard, kale, and others. The pest's damage is especially serious in the tropics where host plants and ideal temperatures for insect development and multiplication exist throughout the year.

In Europe, the diamondback moth causes little damage and control measures are rarely required. This is because about 40 species of insect parasites attack diamondback moth larvae and pupae keeping the pest population well below the level where it can cause damage. In the tropics, none of these parasites occur naturally. As a result, diamondback moth populations multiply fast, forcing farmers to use insecticides, the quickest way to check the pest population.

Now, as a result of frequent application, the diamondback moth has developed resistance to practically all insecticides, including some strains of the latest biological insecticides, *Bacillus thuringiensis*. In addition, insecticide use has increased the cost of production, has destroyed non-target arthropod predators—thereby reducing biodiversity, has increased the chances of consumers eating pesticide-tainted produce, and has contaminated soil and water.

To avoid these consequences, but achieve adequate, economical, and sustainable control of the pest, it is logical that the biological forces that keep this pest under control in Europe be employed elsewhere. This requires the importation and introduction of European parasites into areas where they do not exist. Indeed, introduction of one such European parasite, *Diadegma semiclausum* (Hellén), has reduced to a tolerable level the diamondback moth damage in Australia and New Zealand,

and in the highlands of Indonesia, Malaysia, Taiwan, and the Philippines. Insecticide use for combating diamondback moth and other crucifer pests in these areas is far less than elsewhere.

At the Asian Vegetable Research and Development Center we emphasize the importance of biological control as part of integrated pest management (IPM) strategies to combat the diamondback moth. For the past dozen years we have been importing, rearing, and introducing these parasites in interested countries where they do not exist. We have encouraged national program scientists and other researchers to do the same. To this end, we have trained developing-country scientists in the rearing and multiplication of these beneficial parasites.

We are constantly upgrading our rearing procedure to enable us to produce large numbers of healthy parasites with minimum material and labor cost. Hence this latest slide set and guide booklet. The purpose of this publication is to explain, including as many essential details as feasible, our protocols for mass rearing of five parasites; *Diadegma semiclausum*, *Cotesia plutellae* (Kurdjumov), *Microplitis plutellae* (Muesebeck), *Oomyzus sokolowskii* (Kurdjumov), and *Diadromus collaris* (Gravenhorst), so that interested individuals can rear and use these beneficial insects. All of the parasites are available at AVRDC free of cost to interested scientists willing to satisfy the quarantine requirements of the importing countries.

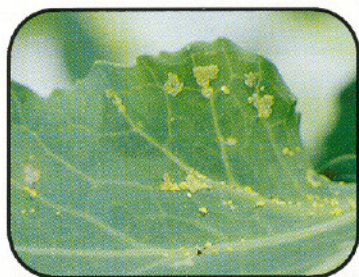
We thank the Swiss Agency for Development and Cooperation (SDC) for their generous support through SDC's Mekong Regional Project grant to the Asian Regional Center - AVRDC, which made production and printing of this publication and slide set possible.

PROTOCOLS FOR MASS REARING OF DIAMONDBACK MOTH PARASITES

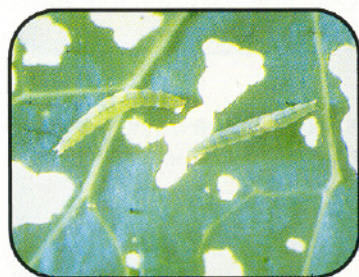
An illustrated slide set and guide



- 1 Diamondback moth (DBM), *Plutella xylostella* (L.), is the most widespread and destructive pest of all economically important crucifers, such as cabbage, cauliflower, radish, mustard, kale, and others, throughout the world, but especially in the tropics.



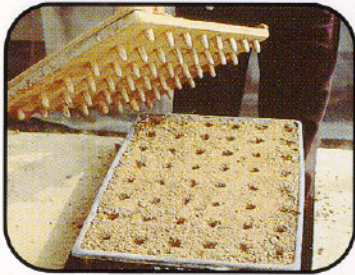
- 2 DBM adults lay tiny eggs, mostly on the lower leaf surface. The eggs do not damage the plant.



- 3 The larva that hatches from the egg feeds on foliage and other above-ground parts of the plant. This reduces the yield and quality of the produce.



- 8 In order to rear the parasites, you must first rear DBM, the host insect. DBM can be reared easily on cabbage plants.



- 9 Prepare soil or a suitable soil and compost mixture normally used for growing plants inside a greenhouse. If possible, use well dried autoclaved soil.



- 10 Sow cabbage seeds in seedling flats, placing 2-3 seeds per spot to assure adequate germination. After plant establishment, thin out and maintain 1 plant per spot



- 11 Water plants regularly.



- 12 You should see germination within 1 week.



- 13 Allow the plants to grow for 4-5 weeks (they should have 6-8 leaves). Start applying dilute ammonium nitrate solution (3 g/liter) once a week soon after germination.



- 14 Transplant 4- to 5-week-old seedlings, 1 seedling per 15 cm diameter clay or plastic pot. Water plants daily and apply 1-3 g of ammonium nitrate, depending on the amount of foliage, once a week after transplanting.

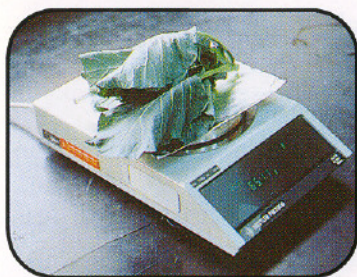


- 15 Six weeks after transplanting, plants should have 10-12 leaves. These are ideal for rearing DBM larvae.

DBM Rearing



- 16 Healthy eggs, larvae or pupae are essential for trouble-free rearing of all parasites.



- 17 Take about 65 g of healthy, pesticide-free cabbage leaves



- 18 Place them in a blender and add 500 ml water.



- 19 Blend leaves in water and prepare slurry/juice.



- 20 Autoclave the juice (120°C , 1.05 kg/cm^2 for 20 minutes).



- 21 Filter the autoclaved juice through an ordinary sieve and discard plant debris.



- 22 Wrinkle, then flatten, sheets of aluminum foil. Dip the sheets into the autoclaved juice until the foil is uniformly wet.



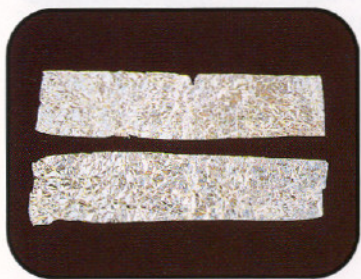
- 23 Air dry the foil, cut it into $2\text{ cm} \times 10\text{ cm}$ pieces (henceforth called "oviposition foils") and store them in a refrigerator until use.



- 24** Cut a slit in the lid of a 3-4 liter plastic container and hang 2-3 oviposition foils through the slit into the container. Place a 150-ml flask filled with 10% honey solution, mixed with yellow food color (20 mg/100 ml solution), inside the container. One end of a 10-cm long cotton wick is dipped in the honey solution and the other end is exposed out of the mouth of the flask, inside the container.



- 25** Release 200 DBM adults inside the container and allow them to lay eggs for 24 hours. The yellow honey solution absorbed in the exposed portion of cotton wick serves as food for the DBM adults. Place the container (the oviposition chamber) inside a cabinet away from direct light or cover it with a black cloth.



- 26** Adults readily lay eggs on the oviposition foils.

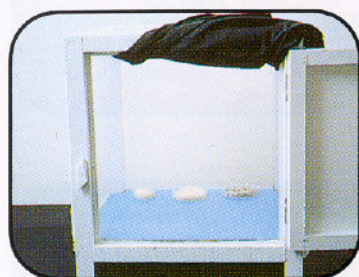


- 27** Dip oviposited foil (foil on which eggs have been laid) in 10% formalin for 30 minutes and then rinse the foil in running tap water for 15 minutes to completely wash off the formalin disinfectant.

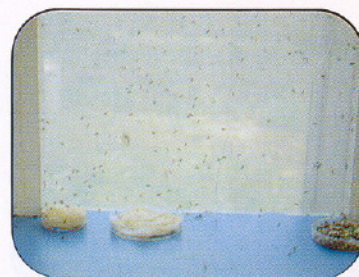


- 28 Rearing *Diadegma semiclausum*, *Cotesia plutellae* or *Microplitis plutellae*

Place a six-week-old potted cabbage plant inside a nylon net cage (50 cm x 50 cm x 40 cm) with a wooden board floor. Place the disinfected oviposition foil (bearing about 800 eggs) on the cabbage plant and allow larvae to emerge and feed on the cabbage leaves until they are in the 2nd or 3rd instar. Second instar larvae are about 3 mm long and 3rd instars about 5.5 mm long. These larvae are ready to be used to rear any one of the three larval parasites.



- 29 Place about 200 pupae of *Diadegma* or *Cotesia* or *Microplitis* in a cage and allow the parasite adults to emerge. Hang a plastic sheet smeared with 10% honey solution in the cage. Spray the honey solution on the plastic sheet daily. Better yet, in addition to the spray, pour about 40-50 ml of honey solution into a petri dish containing absorbent cotton. Change this food source once every 2 days. Hang a black cloth curtain inside the cage to cover the door opening. This will minimize escape of parasite adults during opening and closing of the cage.



- 30 Parasite adults emerging from pupae feed on the honey solution in the petri dish or on the plastic sheet. From this stage onwards, all operations should be done at the following temperatures for each species: *Diadegma*, 20-22°C; *Cotesia*, 26-28°C; *Microplitis*, 24-26°C.



- 31** Place a potted cabbage plant containing about 800 2nd instar DBM larvae inside the parasite adult cage. Cover the exposed soil in the pot with aluminum foil. On the cage floor surrounding the clay pot, place a single layer of fresh cabbage leaves to trap DBM larvae descending from the plant when parasites try to oviposit. Let the parasites oviposit in the DBM larvae for 24 hours.



- 32** Remove the cabbage plant with parasitized larvae from the cage and carefully strip all leaves, making sure larvae do not fall off. Place 2 -3 of these leaves containing about 150 larvae on a fresh 6-week-old potted cabbage plant placed in a similar but parasite-free cage. At this time transfer all DBM larvae fallen on the leaves placed on the floor of the previous cage on to the fresh cabbage plant. When food supply from the old excised leaves is exhausted, larvae will migrate readily to the fresh leaves of the new plant. The biomass of the 6-week-old plant provides enough food for larvae until pupation in 15 days.



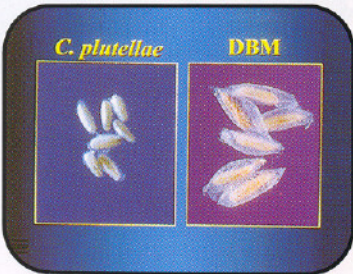
- 33** Collect pupae carefully and store them at 8-10°C until use. Pupae can be stored at this temperature for 15-30 days without significant loss of viability.



- 34 Initially, pupae of DBM and *Diadegma* look alike. However, as the pupal period progresses, *Diadegma* pupae darken, whereas DBM pupae remain light brown. In addition, *Diadegma* pupae are short with a blunt abdominal end, compared to the pointed abdominal end of the slightly longer DBM pupae.



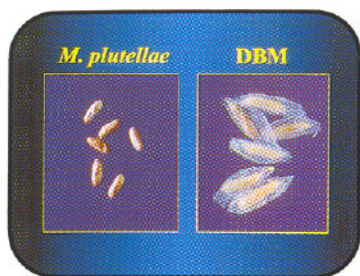
- 35 *Diadegma* pupae are covered by an opaque silken web. After about a week at room temperature, parasite adults will start emerging from the pupae. These insects are 0.45 cm long.



- 36 *Cotesia* pupae are smaller and yellowish white, whereas DBM pupae are larger, light brown, and covered loosely with a silken cocoon.



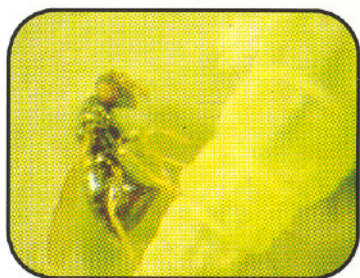
- 37 After 4-5 days, parasite adults will begin to emerge from the pupae. *Cotesia* adults are smaller than *Diadegma* adults.



- 38 *Microplitis* pupae are smaller and dark brown, whereas DBM pupae are larger, light brown, and covered loosely with a silken cocoon.

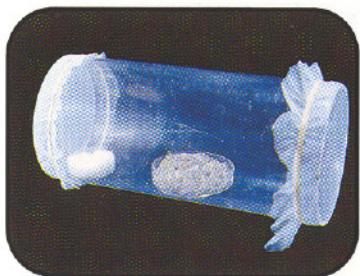


- 39 After about 2 weeks, parasite adults will begin to emerge from pupae. *Microplitis* adults are slightly smaller than those of *Cotesia*.

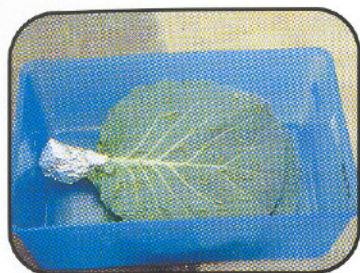


- 40 Rearing of *Oomyzus sokolowskii*

Oomyzus is a very tiny insect which is also a larval parasite of DBM.



- 41 Place 100 *Oomyzus* pupae in a small petri dish inside a 15-cm diameter, 30-cm long acrylic or plastic cylinder. Also place absorbent cotton soaked in honey solution in a small petri dish inside the cylinder. *Oomyzus* adults emerging from pupae will feed on the solution. *Oomyzus* rearing can be done at the same temperature as for *Cotesia*, 26-28°C.



- 42 Place about 200-300 4th instar DBM larvae (normally 8-8.5 mm long) on two fresh cabbage leaves, the petioles of which are wrapped in moist absorbent cotton covered with aluminum foil.



- 43 Place the leaves in the cylinder containing the *Oomyzus* adults. Keep the DBM larvae inside the cylinder for 24-48 hours. *Oomyzus* adults will lay eggs in the DBM larvae during this time.



- 44 After 48 hours, all parasitized larvae will start becoming *Oomyzus* pupae. Initially both *Oomyzus* and DBM pupae are similar. However, after 5 days, *Oomyzus* pupae darken, whereas DBM pupae remain light brown until adult emergence.

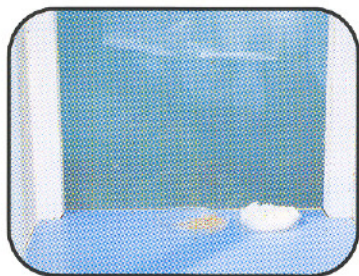


- 45 Tiny, black, *Oomyzus* adults, barely visible to the naked eye, emerge from pupae after 15 to 18 days.

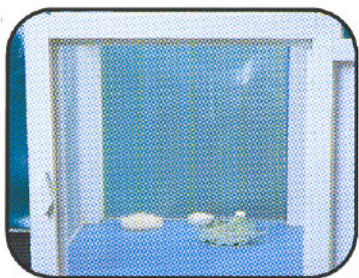


46 Rearing of *Diadromus collaris*

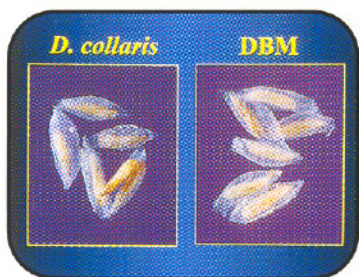
Unlike the 4 parasites so far mentioned, *Diadromus collaris* is a pupal parasite of DBM. This parasite therefore supplements the control achieved by other parasites.



- 47 Place 100 cocoons of *Diadromus collaris* in a 50 cm x 50 cm x 40 cm nylon net cage. Hang a plastic sheet sprayed with honey solution inside the cage and also place a wad of absorbent cotton dipped in 10% honey solution inside the cage to feed *Diadromus* adults emerging from the pupae. *Diadromus* can be reared effectively at the same temperature as that for *Microplitis*.



- 48 After *Diadromus* adults have emerged, place a large number of DBM prepupae or fresh pupae, in a single layer in a petri dish inside the cage. Do not stack the pupae or else the pupae at the bottom will not get parasitized. After 48 hours, remove all DBM pupae and place a fresh lot of DBM pupae for parasitization.



- 49 Since *Diadromus* is a pupal parasite and spends its egg, larval, and pupal stages inside DBM pupae, it is difficult to distinguish between DBM and *Diadromus* pupae. However, towards the end of the pupal period, *Diadromus* pupae turn dark brown, whereas DBM pupae remain light brown.

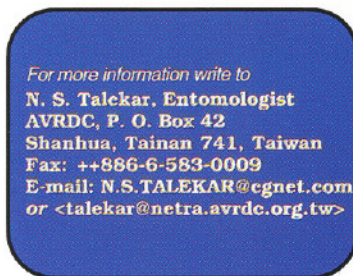


- 50 Maintain pupae in the rearing room for 15 days during which time *Diadromus* larvae will pupate inside the DBM pupae. *Diadromus* adults, which are the same size as *Diadegma* adults, will come out from pupae starting at the end of 15 days.

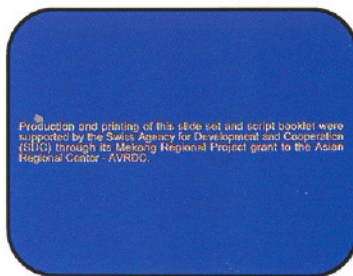


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