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Mass Production and Utilization of the Predatory Midge, *Aphidoletes aphidimyza* Rondani for Controlling Aphids

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INTRODUCTION

Three species within the family Ceccidomyiidae are known as predators of aphids *i.e*, *Aphidoletes aphidimyza* Rondani, *A. urticariae* Kieffer and *Monobremia subterrana* Kieffer [1]. Only *A. aphidimyza* has been investigated with respect to its suitability for the control of aphids in greenhouses [2]. Previous investigators [1,3,4] stated that the predatory midge, *A. aphidimyza* is a general aphid predator, attacking many different species of aphids on different host plants. Havelka and Zemek [5] showed that the various geographic populations of *A. aphidimyza* differ in most biological parameters. Since the beginning of the 70's at twentieth century, the predatory midge, *A. aphidimyza* has been used for biological aphid control in greenhouses [6], mainly in Finland, USSR, Denmark, Germany and Netherlands on commercial vegetables and roses growing in greenhouses and outdoors [7]. During the past twenty years, research on *A. aphidimyza* has increased in its mass production and its effectiveness to control aphids on various plants, especially in greenhouses [8-10]. Harris [7] and Markkula and Tiittanen [2] referred to the following reasons for the success of the predatory midge, *A. aphidimyza* in biological control of aphids: a) Mass production is easy and hence economical, b) Cocoons readily withstand transport and distribution, c) The aphid midge forms a permanent population in the glasshouse; surviving the winter if the growth substrate of the plants is not changed and no harmful disinfectants used, d) Adults are able to fly to aphid-infested plants even in large glasshouses, e) The midge larvae kill and eat all the pest aphids in a greenhouse, f) The larger the aphid population, the more aphids the midge larvae destroy and g) The midge larvae are motile and thus are able to find new prey. Aphids do not readily escape.

Mass production methods: Bondarenko and Asyakin [11] used *M. persicae* (as prey) and sweet pepper plants (as host for aphids) in mass rearing of *A. aphidimyza*. When the larvae reached the final instar, the leaves that contain the larvae were detached and placed in containers containing sand. After pupation, cocoons were sieved out from the sand, their number estimated and they were then transferred to the greenhouse without sand.

Markkula and Tiittanen [12] concluded that the peach aphid is the most suitable prey for the midge larvae and green pepper and eggplant are the most suitable host plants for the aphid because these two plants foster a high rate of reproduction of the peach aphid while on the hosts and because they tolerate damage caused by the aphids. They finalized a method for mass production in the beginning of the growing season 1975. It is based on the following five stages at the Agricultural Research Center in Finland:

- Green pepper and eggplant are sown at 2-week intervals and cultivated in a greenhouse as food for *M. persicae*.
- When the plants are 20-30 cm high, they are placed in cages 3 plants in each cage and about 50 aphids are placed on each plant.
- When the number of aphids has increased to about 2000 per plant, 70 female and 30 male midges are placed in each cage. After two days, the midges will have deposited about 3000 eggs. The plants are removed and the adult midges are killed.
- When the larvae have reached the final instar, leaves containing larvae are detached from the plants and placed on sand within small plastic containers (9 cm high and a diameter of 16 cm). The containers are filled with moist sand to a height of 4 cm to maintain even humidity. On top of this is placed a sheet of nylon gauze and a thin layer of peat. 3 or 4 leaves are usually put into each container so as to give about 200 larvae per container. The larvae pupate in the peat layer.

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- The peat layer containing cocoons is sent to greenhouse growers a couple of days before the adult midges emerge.

The method is so simple that it could be easily used for industrial production of the predatory midge. Predatory midge can be reared in 16 hours day length throughout the year, they can be kept also in diapause to start the rearing when required.

Rimpiläinen [13] concluded a plan for a rational mass production of *A. aphidimyza* in glasshouses:

- In one glasshouse with optimal conditions for growing *capsicum* peppers, the plants are grown to a hight of about 30 cm.
- In the next glasshouse, peach aphids are fed on these plants.
- In the third glasshouse, the aphid midges are reared. Aphid infested plants are planted 6 per m^2 and a suitable amount of *A. aphidimyza* pupae are spread out. Maximal larvae production occurs then each three weeks.
- These plants are changed after 3-4 weeks at a time when most of the midges are in the soil as pupae. The aim is that there are always fresh, new plants for egg laying.
- Other natural enemies of aphids and possible hyperparasites of the aphid midge are kept in control.
- The glasshouse for mass rearing of the midge should be shaded and the relative humidity held high through watering/dust. The temperature of the air should be about 20°C and that of the soil never under 10°C. The soil must also be kept moist enough. He mentioned that in this way on area of 5-10 m^2 gives thousands of pupae per day.

Forsberg [14] studied the possibility of using diapause in mass rearing of *A. aphidimyza*. He showed that the simple method of making the predatory gall midges enter diapause is to keep both eggs and larvae in short photoperiod at a low temperature [L8 (25°C) and D16 (10°C)]. The highest percentage of diapausing larvae in cocoons was obtained by this method; ranging from 92.2 to 100%.

Hansen [15] used "open rearing units" into glasshouses in Denmark. This meant that the midge was provided with an aphid species not infesting the glasshouse crop involved. He used boxes (30 x 70 x 30 cm) in which 100 broad beans which had been sown were placed in the glasshouse and 100-200 vetch aphids (*Megoura viciae*) which fed on Leguminous plants only, were transferred to each box. After two weeks gall midge pupae, kept in moist earth, were distributed in the boxes. In this way a successful control of *M. persicae* on sweet pepper could be obtained. Survival of the *M. viciae* population was crucial for a good control and it had to be kept in balance with the bean plants. Supplementary introductions of *Aphidoletes* pupae proved to be necessary when the crop was infested with aphids before the development from pupae to adult was completed in the units.

Lieburg and Ramakers [16] described a method for mass rearing of *A. aphidimyza*. Sweet pepper were grown, infested with *M. persicae* and finally placed in an emergence cage containing adults of the predator for oviposition. The predatory larvae later descending spontaneously from the plants fell on to slanting plates and were washed by running water into a collector, where they were able to survive for up to a week at a water temperature of 20°C. About 2000 larvae could be gathered daily by this method and placed in plastic lidded petri dishes filled with cotton wool for pupation. Adults emerged from 85% of the pupae thus formed. According to the authors, this method is considered more convenient than picking infested and predatized leaves and placing them on sand, which necessitates separating the larvae from the substrate by sieving.

Gilkeson and Hill [17] showed that in greenhouses, radiation from a 60-W incandescent bulb would prevent diapause in more than 50% of *A. aphidimyza* larvae within a 10 meter radius.

Gilkeson and Hill [18] selected genetically a line of *A. aphidimyza* that did not diapause under winter greenhouse conditions from 4 lines from Finland and North America under 21°C and L (8): D (16). In three selected lines, diapause incidence dropped rapidly in the first four or five generations, with means of 3-11% thereafter. There was no clear response to selection in the fourth line. Neither morphology nor sex ratio was affected by nondiapause selection, nor the fecundity affected in the longest reared line. Selection for nondiapause under L (8): D (16) with fluctuating thermoperiods was unsuccessful.

Popov and Belousov [19] reared (in USSR) *A. aphidimyza* with larval feeding on cereal aphids (*Schizaphis graminum* and *Rhopalosiphum padi*). They gave details of a procedure for their collection.

Popov and Belousov [20] mentioned that in the USSR, laboratory colonies of cereal aphids fed on wheat or barley were used for rearing large numbers of *A. aphidimyza*.

Tiittanen [21] investigated the possibility in the laboratory of using long-term storage during diapause of *A. aphidimyza*. Larvae failed to survive storage in moist peat at 2°C. But at 10°C, 66% of diapausing larvae emerged after storage for 7 months, as compared with 71% during continuous rearing without diapause. Emergence was poor following storage for less than 3 or more than 8 months. The shorter the storage period, the longer it took for development to begin and to be completed.

Kuo-Sell [22] used an open rearing unit of *A. aphidimyza* on cereal aphids (*Sitobion avenae, Metopolophium dirhodum* and *R. padi*) to control the peach aphid on sweet peppers. In the midges treated greenhouses, the aphid population densities remained at very low levels throughout the entire vegetation period. In the midge free greenhouses, Population growth of the aphid increased very rapidly reaching peak population densities 4-5 weeks after aphid infestation. Cereal aphids are suggested as prey species for mass rearing of *A. aphidimyza*. However, he discussed the effectiveness of *A. aphidimyza* in suppressing aphid populations and the advantages of using open rearing in form of a "cereal aphids-*A. aphidimyza*"-system for control of greenhouse aphids.

Belousov and Popov [23] described a method for mass rearing of *A. aphidimyza*. Sprouts of cereals are used as food plants for *Schizaphis graminum* and *R. padi*, on which the Cecidomyiid larvae feed. This makes it possible to rear them at a higher density than previously. The aphids are used more economically than before and the predator cocoons can be collected using a simple apparatus. Any room is suitable for rearing the prey and predators. Many-tier shelves allow a 70-80% reduction in the area required. The output of biomaterial from an effective area of 1 m^2 is 140 g of aphids or about 20000 cocoons of the predator. One person can serve a line with a capacity of 15000 cocoons per day.

Gilkeson [8] investigated *A. aphidimyza* as a way to reduce rearing costs and to preserve genetically important lines. Prolonged cold storage of last instars in cocoons at temperatures of 1-11°C in total darkness was sufficient, as an environmental cue to induce diapause, even though larvae had been reared under conditions that would not induce diapause. Mortality and emergence patterns of midges' diapausing in response to cold storage did not differ from those of larvae reared under diapause inducing conditions (8-h day at 18°C, 16-h night at 15 or 18°C) before cold storage. For commercial application, cold storage regimes with lowest mortality (<10%) and highest percentage of emergence in the first 4 days of the adult emergence period were: 2 weeks at 10-11°C, up to 4 weeks at 5°C and up to 2 months at 1°C after acclimation for 10 days at 5°C. Larvae stored at 5°C survived 8 months with a mortality rate that did not reach 9% and fecundity of females did not significantly differ than in unstored controls.

Applications

A) Without other natural enemy: Markkula [24] used three *A. aphidimyza* pupae per each ten peach aphids on roses and green peppers in experimental greenhouses. He stated that if less predatory midges were distributed, the aphids had time to increase and started to cause damage before the effect of midges started to be prominent.

Markkula *et al.* [25] mentioned that the commercial use of *Aphidoletes* by vegetable growers has been introduced in Finland in 1977, followed by commencement of mass production and marketing in 1978. They used one pupa per 3 aphids or, depending on the number of aphids present, 2-5 pupae per m^2 should be applied. Treatment should be repeated after 2-4 weeks to ensure good results. The method was successful; the reduction of the aphid population prevented further damage throughout the growing season.

Markkula and Tiittanen [26] mentioned that during 1978, over 100 000 pupae, half of which was spread on the soil in the greenhouses for 70 growers while the other half of the pupae were used on small greenhouse cultures of agricultural schools and hospitals in the Soviet Union. The control was generally successful and growers have been satisfied with the new control method (*A. aphidimyza*).

Markkula and Tiittanen [27] studied the effect of chemical and biological control of *M. persicae* on sweet peppers. In one greenhouse, the pesticide Mevinphos was used when the aphids began to damage the plants. In the other, *A. aphidimyza* cocoons were applied at a rate of 1 cocoon for 3 aphids. The aphid midges overwintered in the greenhouse although it was not heated during mid-winter; it re-appeared on the plants the following spring, when heating began. The distribution of a single batch of aphid midge pupae into the soil gave better control than 6 treatments of Mevinphos.

Meadow *et al.* [28] used in USA the predatory gall midge to control the peach aphid *M. persicae* in greenhouse and field experiments. In a greenhouse with low aphid density 2 releases of *A. aphidimyza,* 7 days apart effectively suppressed aphid populations on tomatoes (5 pupae/m2). When midges were released on a 14-day interval into high densities of aphids on tomatoes

and peppers in the greenhouse, they significantly reduced the number of aphids and aphid-induced injury, compared to an untreated control. In a field grown peppers, two releases (2-3 pupae/plant) of *A. aphidimyza*, 21 days apart, effectively controlled green peach aphid by maintaining aphid populations at low levels throughout the season compared to the untreated control plots which were heavily colonized by the aphids.

Bondarenko [29] mentioned that a ratio 1: 5 larva of predator to prey is required for effective application of the gall midge to cucumbers. Sometimes a ratio of 1: 9 can be successful when aphid colonies are small while relative air humidity in the greenhouses is more stable and exceeds 70%.

Lenteren [30] mentioned that *A. aphidimyza* was applied in the world in greenhouses to control the aphids since 1978 for areas of 3 ha. and up to 13 ha. in 1985.

Popov *et al.* [31] indicated that to control the rose aphid and green peach aphid, it is necessary to introduce *A. aphidimyza* at least once a week during a month or a month and a half starting from the moment of the first pest occurrence. Positive results were encountered at the predator/prey ratios 1: 10-1: 15. However, gall midge application at temperatures lower than 20°C is not effective due to great difference in rates of aphid and predator development. Green peach aphid had a lower r_m (0.232) and developed for a long time in the foci. Thus, even one release of the gall midge in the ratio of 1:50 prevented pest reproduction for a month.

Gilkeson and Hill [32] investigated the control of *M. persicae* on green pepper by *A. aphidimyza* under winter greenhouse conditions (21°C daytime maximum, 15°C night minimum). Four release rates (1 predator: 3 aphids, 1:10, 1:50, 1:100) were compared in a randomized complete block design cage experiment from mid-November to mid-January. Best control was achieved with the 1:10 rate. There was significant effect due to location along the temperature gradient on the bench; differences between 1:3 and 1:10 rates appeared to have been due more to location than to initial predator/prey ratio. Aphids were not controlled in a subsequent experiment using the 1:10 rate in late February when daytime temperatures were 23-26°C.

Schmidt *et al.* [33] indicated that the biological control of aphids on cucumber, tomato and *Anthurium* was successfully practised at various culture periods under glasshouse in Germany using *A. aphidimyza*. Satisfactory control on these crops was obtained with 5-6 pupae/m^2. Under very favorable conditions on cucumbers 1-2 pupae/m^2 was sufficient.

Cheng *et al.* [34] controlled *M. persicae* in greenhouses and 2 typical types at plastic tunnels for vegetable production in China by inundative releases of *A. aphidimyza*. Releasing the predator at a ratio of 1 predator: 20 prey when the aphid population reached 200 aphids/plant reduced the aphid populations by 75.1-91.8% and 80.1-90.5% in the greenhouses and small tunnels, respectively. In 1990, a single release was made in each of 5 large tunnels at the same ratio, the aphid population was reduced by 82.2% 10 days after release and was suppressed below 141.2-150.5 aphids/plant for 30 days. In June-July 1991, releases were made on pepper in 16 ha.

Kocourek *et al.* [35] studied the effectiveness of *A. aphidimyza* in 1000 m^2 greenhouse in Czech Republic in 1989-90 in which 2 releases of pupae by an interval of 7 days were made on cucumber plant artificially infested with *A. gossypii*. The subsequent rate of increase of the aphid, expressed in relation to the sum of effective daily temperatures, was evaluated by reference to an intrinsic rate of increase expressed in these terms calculated for this aphid in another work. In 1989, when the rate of release was 1 predator pupa: 2 aphids (12 pupae/m^2), the rate of increase suggested no influence on aphid numbers when viewed against the intrinsic rate. In 1990, when the release rate was 1 pupa to 1 aphid (22 pupae/m^2), there was a slight influence that was, however, insufficient to keep aphid numbers below damage thresholds. They suggested that the poor results might have been connected with high temperatures in the greenhouse.

Cranshaw *et al.* [36] mentioned that cocoons with pupae of *A. aphidimyza* are the stage usually sold. In a base unit of 1000, prices quoted by 10 suppliers showed a threefold range of $ 0.027 to $ 0.075% / insect (mean = $ 0.0443). The recommended use rates ranges 1-5 pupae / 10 ft^2 and usually specified a series of sequential releases.

B) With other natural enemies or chemical pesticides: Hofsvang and Hagvar [37] compared two controlling agents on aphids, the parasitoid *Ephedrus cerasicola* Stary and *A. aphidimyza* during two seasons in small greenhouses using paprika plants infested by *M. persicae* one aphid was introduced on every plant in all houses. The parasitoids were introduced as 4 mummies/plant twice at 10-day interval. The first introduction was together with the aphids or in one single experiment 10 days later. In a separate house 18-21 gall midge predator from the last week of May to November. From September the aphid population increased rapidly in the gall midge houses

both years, because the midges then went into diapause. On the contrary, the parasitoids controlled the aphids throughout the season. In 1980, the gall midges and the parasitoids kept the aphid populations at similar and moderate levels until September. The yields of paprika were also similar in all houses that year, 2.1-2.3 kg/plant or 8-9 kg/m^2. In 1981, the aphid populations were considerably larger, being largest in the gall midge house. As a result, the yeild of paprika was less than in the previous year and was most reduced in the gall midge house: 1.6 kg/plant or 5.3 kg/m^2 and 1.1 kg/plant or 3.6 kg/m^2 in the parasitoid and in the gall midge houses, respectively.

Begunov and Storozhkov [38] mentioned that the use of *A. aphidimyza* and *Cycloneda* in Leningrad region USSR for control of aphids has resulted in yield increases of 0.3 kg/m^2 by control of aphids on cucumber and 3 kg/m^2 by control of *M. persicae* on sweet pepper and use of the latter one likewize gave an increase of 0.2 kg/m^2 by control of the melon and cotton aphid, *A. gossypii* on cucumber. They suggested that these two predators successfully and persistently checked aphid populations when used in combination on sweet peppers and ornamental plants.

Elliot *et al.* [39] mentioned that aphids are controlled with *A. aphidimyza* and *Aphidius matricariae* integrated with insecticidal soap or Pirimicarb in interior plantscapes in Western Canada.

Chambers and Helyer [40] mentioned that during 1988, studies were carried out in the UK on the biological control of aphids, *Aphis gossypii* and *Macrosiphoniella sanborni* in greenhouses using *A. aphidimyza*. Larvae of the predator readily became established on chrysanthemum plants following successive introductions of cocoons and attacked and destroyed colonies of both aphids, but aphid number increased to unacceptable levels before further increase was stopped by a combination of *A. aphidimyza* and a natural outbreak of the fungal pathogen, *Verticillium lecanii*.

Matskevich [41] achieved success in controlling *M. persicae* in greenhouses in USSR with combined control measures involving release of the brachonid, *Aphidius matricariae* and *A. aphidimyza* when the pest was already well established.

Bennison and Corless [42] used banker plants (open rearing systems), comprising wheat or barley seedlings infested with *R. padi* to control *A. gossypii* on cucumber in the greenhouses. Banker plants enhanced the early establishment of *Aphidius colemani* and *A. aphidimyza*. Aphid control was excellent even at the lowest rate of banker plant production.

Quentin *et al.* [9] studied the efficiency of *Aphidius matricaria* Haliday (Hymenoptera, Aphidiidae), *A. aphidimyza* and *Chrysoperla carnea* (Stephens) (Neuroptera, Chrysopidae) in controlling aphids *i.e. Aulacorthum solani* (75%), *Macrosiphum euphorbiae* (12%), *Nasonovia ribis-nigri* (2%) and *M. persicae*(11%) on lettuce in greenhouses. *A. aphidimyza* did not control the aphids under the climatic conditions common in greenhouses for growing lettuce, even if pupae had been applied three times. *A. matricariae* controlled *M. persicae* only. The application of *Ch. carnea* (eggs) resulted in reasonable aphid control when eggs were first added to the young lettuce plants before transplanting followed by the three spraying of *Chrysoperla* eggs at weekly intervals (25-30 eggs/m^2).

Granges and Leger [43] showed that the development of biological control measures against arthropod pests as part of integrated control resulted in a reduction of pesticides used by 57% in tomatoes and 68% in cucumbers greenhouses in Switzerland in 1987-1993. Aphids (especially *M. euphorbiae* and *M. persicae*) were controlled by *A. aphidimyza* and *Aphelinus abdominals*.

Sato *et al.* [44] used *A. aphidimyza* with the parasitoid, *Aphidius colemani* in a cucumber-growing vinyl house in Japan to control *A. gossypii*. They showed that *A. aphidimyza* was always present in low numbers, possibly caused by high mortality of mature larvae on mulch.

Mulder *et al.* [10] showed the possibility of controlling *A. gossypii* with banker plants of wheat with *R. padi*. *A. aphidimyza* and *A. coemani* has proved to be effective. They had good result for controlling the aphid with this system (banker plants).

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