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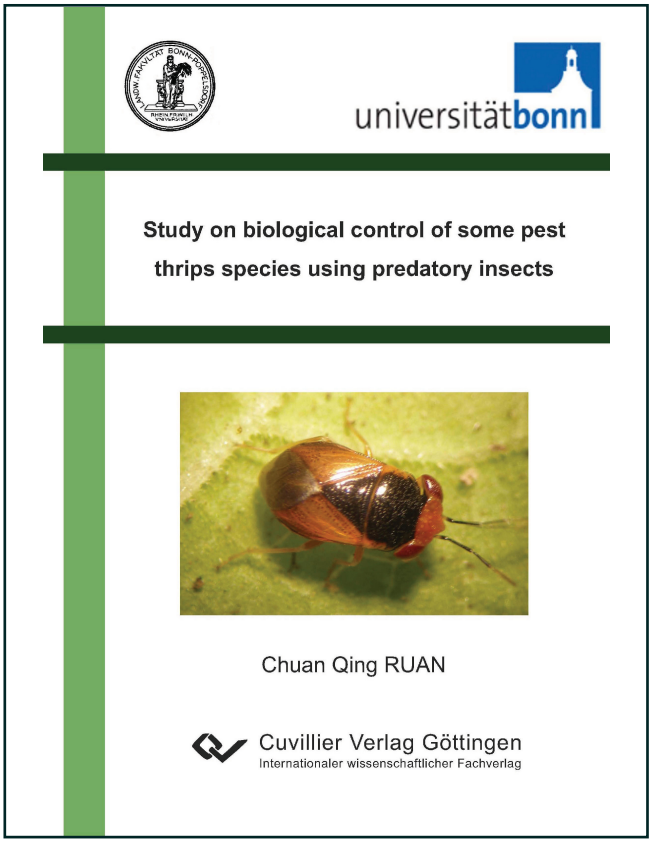
Study on biological control of some pest thrips species using predatory insects



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2 MATERIALS AND METHODS

2.1 Laboratory experiments

2.1.1 Rearing of the insects

2.1.1.1 Rearing of the pest insects

2.1.1.1.1 *Frankliniella occidentalis*

The stock culture of *F. occidentalis* was established on bean plants (*Phaseolus vulgaris* L., cv Marona) with individuals obtained from the original stock culture at Institute of Crop Science and Resource Conservation, University of Bonn. The rearing was carried out in a climatically controlled room at 25±1°C temperature, 60±10% RH and 16:8 h (L:D) photoperiod with an artificial light intensity of about 4000 Lux. Bean plants were usually planted in trays (60×40 cm) under greenhouse conditions before transferring to the climate room. The fresh bean plants were used to replace the heavily infested ones weekly.

To obtain desired life stages of *F. occidentalis* for different experiments, a kind of cage, "Rearing Cage", for breeding thrips or other insects in incubators had been developed from round Plexiglas cages with 11 cm in diameter and 3 cm in height (Fig. 1).

Fig. 1: Rearing Cages using for the obtaining of the appropriate stages of insects for experiments

The cages were filled with 0.5 cm thick agar gel layer to keep the leaves fresh, and had three meshed holes in the lid for aeration. To prepare appropriate age of *F. occidentalis*, freshly bean leaves were placed upside down onto the agar gel layer in the cages, and 20-30 adult females of *F. occidentalis* for each cage were picked up from stock culture and transferred into the cages for egg laying. After 24 h, the adult females were removed to the newly prepared cages to obtain eggs again. The eggs obtained in the cages were kept in an incubator at the above-mentioned climatic conditions for development until the thrips reached the life stage desired for the experiments.

2.1.1.1.2 *Thrips tabaci*

The stock culture of *T. tabaci* was established on leek plants (*Allium porrum* L.) in a climatically controlled room at the above-mentioned temperature, humidity and photoperiod with the same artificial light intensity. The stock culture started with female adults of *T. tabaci* obtained from the original stock culture at Institute of Crop Science and Resource Conservation, University of Bonn. Leek plants were usually planted in pots (11 cm in diameter and 11 cm in height) under greenhouse conditions before transferring to climate room. The fresh leek plants were used to replace the heavily infested ones weekly.

To obtain the desired life stages of *T. tabaci*, the same procedure as for *F. occidentalis* was used, except that the thrips species was replaced with *T. tabaci* and the bean leaves were replaced with leek leaves.

2.1.1.1.3 *Gynaikothrips ficorum*

Population of *G. ficorum* was maintained on young banyan trees (*Ficus microcarpa* L.) in a climatically controlled room at the above-mentioned temperature, humidity and photoperiod with the same artificial light intensity (Fig. 2). The individual for the stock culture of *G. ficorum* were collected from the banyan trees in Fuzhou City of southern China. Stems with diameter of 0.3-0.5 cm were also detached from the banyan trees in Fuzhou City, and cut into 20 cm long. The prepared stems were cultured by the method of cuttage in pots (12 cm in diameter and 12 cm in high), in order to obtain little young banyan trees. Fresh banyan trees in pots were transferred to the room in order to replace the heavily infested ones.

For obtaining the desired life stages of *G. ficorum*, young banyan trees were exposed to *G. ficorum* infestation in the stock culture. After 24 h, the adults were removed from the trees. The laid eggs were used directly or reared further in meshed cages (80×50×60 cm) at above-mentioned climatic condition, and checked daily till they reached the required ages.

Fig. 2: Rearing *Gynaikothrips ficorum* on young banyan trees (*Ficus microcarpa*) in pots

2.1.1.1.4 Other prey insects

The rearing of other pest insects as prey, like *Aphis fabae* SCOPOLI (Hom., Aphididae) on broad bean plants, *Aphis gossypii* GLOVER (Hom., Aphididae) on cotton plants, *Bemisia tabaci* (GENN.) (Hom., Aleyrodidae) on cucumber plants and *Tetranychus urticae* KOCH (Acari, Tetranychidae) on bean plants, were maintained exclusively in meshed cages (80×50×60 cm). The rearing of these four pest species were started with the individual obtained from original stock cultures at Institute of Crop Science and Resource Conservation, University of Bonn. All the plants were usually planted in pots under greenhouse conditions. All the rearing of these pest species were took place at 25±1°C temperature, 60±10% RH and 16:8 (L:D) photoperiod with an artificial light intensity of about 4000 Lux.

A. fabae was used as prey to establish or obtain the stock culture and uniformly aged individuals of *S. subula*. Mixed population of *A. fabae* with the infested host plant leaves were picked up from its stock culture, and offered as prey for *S. subula*.

One or two-day-old nymphs of *A. gossypii* for experiments were prepared by transferring the adult aphids onto cucumber leaves in Rearing Cages mentioned in Fig. 1. After 24 h, the adult aphids were removed, and the nymph aphids were used directly or reared further for one day.

Pupae of *B. tabaci* for experiments were prepared by exposing the cucumber plants to *B. tabaci* infestation in the stock culture for 24 h, and then *B. tabaci* adults were removed. The plants with the eggs were fostered under the same climatic conditions as used for the stock culture. The development of the eggs was checked daily until the individuals reached the pupa stage. The cucumber leaves containing the pupae were excised into leaf discs which would be used in experiment. *B. tabaci* on the leaf discs were removed until 20 pupae per leaf disc were remained.

To obtain the adult females of *T. urticae* for experiments, its mixed population as well as the infested bean leaves were picked up from its stock culture, and were identified under a binocular microscope. Adult females were collected gently by using a camel-hair brush.

2.1.1.2 Rearing of the predatory insects

2.1.1.2.1 *Geocoris ochropterus*

G. ochropterus was collected from the soybean plantation in Fuzhou City of southern China (Fig. 3). The stock culture of *G. ochropterus* was maintained on cotton or pepper plants with *F. occidentalis* or *T. tabaci* as prey in the meshed cages (80×50×60 cm). The climatic condition for the rearing was 25±1^{o}C temperature, 60±10% RH and 16:8 h (L:D) photoperiod with an artificial light intensity of about 4000 Lux.

Fig. 3: Eggs, N_1 nymph and adult of predatory bug *Geocoris ochropterus*

Desired stages of *G. ochropterus* for different experiments were obtained from the Rearing Cage as described in Fig. 1. Every 5 adult females and 2 adult males of the predator were transferred onto pepper or cotton leaves in each Rearing Cage. Individuals of different life stages from *F. occidentalis*, *T. tabaci* or *G. ficorum* were offered into the cages as prey. After 24 h, the adults of *G. ochropterus* were moved to new cages to lay eggs again. The eggs laid in 24 h were reared with mixed population of the desired thrips species as prey on the host plant leaves in the Rearing Cages. The Rearing Cages with the eggs were placed in an incubator at the same climatic condition as used for the stock culture. The development of the eggs was checked daily until they reached the life stages required in experiments.

2.1.1.2.2 *Montandoniola moraguesi*

M. moraguesi was collected from the banyan trees (*F. microcarpa*) in Fuzhou City of southern China (Fig. 4). Its stock culture and uniformly aged individuals for experiments were conducted in the same procedure as for *G. ochropterus* in capital 2.1.1.2.1. The climatic condition for the rearing was the same as mentioned above.

Fig. 4: Eggs, N_1 nymph and adult of predatory bug *Montandoniola moraguesi*

2.1.1.2.3 *Orius similis*

O. similis was obtained from the cucumber plantation in Fuzhou City of southern China (Fig. 5). The stock culture and desired stages of *O. similis* were established or obtained by the same procedure used for *G. ochropterus* in capital 2.1.1.2.1. The climatic condition for the rearing was the same as described above.

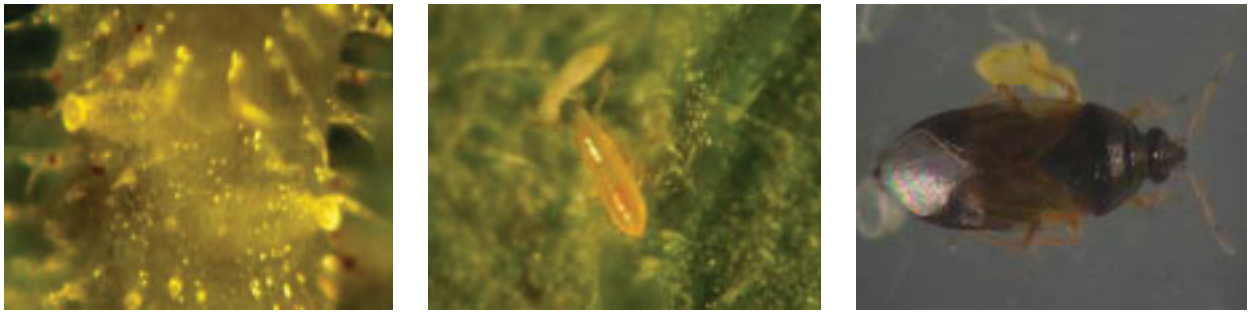

Fig. 5: Eggs, N_1 mymph and adult of predatory bug *Orius similis*

2.1.1.2.4 *Scipinia subula*

S. subula was collected from the plantation of Garden Balsam (*Impatiens balsamina* L.) in Fuzhou City of southern China (Fig. 6). The stock culture of *S. subula* was maintained in meshed cages (80×50×60 cm) with *A. fabae* as prey on broad bean plants, or with *A. gossypii* as prey on pepper plants. The climatic condition for the rearing was the same as mentioned before.

Desired stages of *S. subula* for experiments were obtained from the Rearing Cages as described in Fig. 1. Every 5 adult females and 2 adult males of the predator were picked from the stock culture, and then transferred into each cage, which previously contained broad bean leaves infested with *A. fabae*. After 24 h, the adults were moved to new cages to lay eggs again. The laid eggs were used directly or reared with aphid as prey for a certain period of time in an incubator. The rearing of the eggs were daily checked until they reached the uniformly life stages required in experiments. The incubator was at the same climatic condition used for its stock culture.

Fig. 6: Eggs, N1 nymph and adults of predatory bug *Scipinia subula*

2.1.2 Biology and prey consumption of *Geocoris ochropterus*, *Montandoniola moraguesi* , *Orius similis* and *Scipinia subula* at temperature 25°C

2.1.2.1 With *Frankliniella occidentalis* as prey

The biology and prey consumption of *G. ochropterus*, *M. moraguesi*, *O. similis* and *S. subula* were studied in the laboratory at 25±1°C temperature, 60±10% RH and 16:8 h (L:D) photoperiod. Experiments on embryonic and nymphal development, mortality, longevity, fecundity as well as the prey consumption were carried out in a kind of arenas (Fig. 7), which were developed from the round Plexiglas cages with 3.6 cm in diameter and 1.5 cm in height. The cages were filled with 0.3 cm thick agar gel layer and had a meshed hole in the lid to allow aeration. Sex ratio, on the other hand, was carried out in the Rearing Cages mentioned in Fig. 1.

Fig. 7: Arenas for the experiments biology and prey consumption

To determine the developmental period of embryonic stage of the four predatory bug species, the adult females and males of *G. ochropterus*, *M. moraguesi* and *O. similis*, which were reared from N_1 instar to adult with mixed population of *F. occidentalis* as prey, were confined to lay eggs in the Rearing Cages as described in Fig. 1, with 5 females and 2 males from the same predatory species in a Rearing Cage. Mixed population of *F. occidentalis* was offered as prey. The adults were moved away after 24 h, and the laid eggs were collected for the experiment. To obtain the eggs of *S. subula*, every 5 adult females and 2 adult males were picked up from its stock culture, and transferred onto broad been leaves in each Rearing Cage as described in Fig. 1. Mixed population of *A. fabae* was offered as prey. After 24 h, the adults were moved away and the eggs

were used in the experiment. The eggs of *G. ochropterus* and *S. subula* were laid on the leaf surface. They were counted directly, and transferred onto the leaf discs in the arenas as described above for embryonic development at the desired temperature. The eggs of *M. moraguesi* and *O. similis* were inserted into the host leaf tissue, and could be counted under a binocular microscope by observing the egg operculum. After counting, the eggs of *M. moraguesi* and *O. similis* were transferred with the host leaves to the arenas and were kept in an incubator with the desired temperature. They were daily checked to record the period of embryonic development for each predatory bug species until the eggs hatched. The experiments were replicated at least 20 times for each predatory species.

To determine the period of nymphal development of the four predatory bug species, the newly hatched N_1 nymphs of each predatory species were singly transferred using a camel hairbrush into arenas, which previously contained cucumber leaf discs for *M. moraguesi*, or bean leaf discs for the other predatory species. The second instar larvae (L_2) of *F. occidentalis* were daily offered as prey in an excess number (20, 30 and 40 per arena for N_1-N_2, N_3 and N_4-N_5 predatory nymphs, respectively). The arenas were kept in an incubator with the desired temperature, and checked daily for the skins of moulted nymphs until adult emergence. During the experiment, the predatory nymphs were daily transferred to the new similar arenas with fresh prey offer. The experiments were replicated 20 times for each predatory bug species.

During the developmental period experiment, the mortality of each predatory species as well as the number of consumed thrips was recorded daily. Thrips, which were consumed by the predator, were empty of fluid and easily distinguished from those of natural mortality.

To establish the sex ratio of each predatory bug species, 200 eggs from each species were developed to adult with mixed population of *F. occidentalis* as prey on cucumber leaves in the Rearing Cages. The emerged adults were sexed under a binocular microscope. Percentage of females and males were calculated for each predatory bug species.

To observe longevity and fecundity, newly emerged adults of each predatory bug species were transferred to the arenas, with one female and male from same species in an arena. Mixed

population of *F. occidentalis* was offered as prey on cucumber leaf discs in the arenas. The adults in the experiment were daily transferred to new arenas with new prey offer. The laid eggs in the old arenas were counted under binocular microscope. The old arenas were also checked to record the longevity of both sexes after the individuals died. Ten replicates were set for the experiment of each predatory bug species.

The prey consumption by the nymphs and adults of the four predatory species were determined throughout the nymphal development and the first 15 days after adult emergence. To obtain the prey consumption by the nymphs, the number of consumed thrips was daily recorded during the above-mentioned experiment on the nymphal development of the four predatory species. The consumed thrips were empty of fluid, and easily distinguished from those of natural mortality. To determine prey consumption by the adults, freshly emerged adults of each predatory bug species were singly kept on cucumber leaf discs in the arenas. L_2 larvae of *F. occidentalis* were daily offered as prey in an excess number (40 per arena). The females accessed to males for the first time on the 2nd day after emergence and then once weekly. During the experiment, the adults were daily transferred to new arenas with fresh prey offer. The number of *F. occidentalis* consumed by each predatory individual was daily recorded. The experiments were conducted from 1st to 15th day after adult emergence. Fifteen replicates were used with each sex of each predatory bug species.

2.1.2.2 With *Thrips tabaci* as prey

Experiments on biology and prey consumption of *G. ochropterus*, *M. moraguesi*, *O. similis* and *S. subula* with *T. tabaci* as prey on cucumber leaves were conducted in the laboratory at 25±1^{o}C temperature, 60±10% RH and 16:8 h (L:D) photoperiod. The experiments were carried out in the same procedure as described in capital 2.1.2.1, except that the host plant leaves were all replaced with cucumber leaves, and the thrips species was replaced with *T. tabaci*. In the experiments on nymphal development and prey consumption of the four predatory species, L_2 larvae of *T. tabaci* were daily offered as prey with numbers of 20, 30 and 40 per arena for N_1-N_2, N_3 and N_4-adult of the predators, respectively. The experiments were replicated in the same way as described in capital 2.1.2.1.