Application of biological agents to protect cucumber plants against multiple pests in greenhouses in Northwest Russia

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Abstract. Dynamics of the main pests of cucumber over a five-year period were analysed. The impact of meteorological conditions on the pest dynamics was observed when growing photo culture cucumbers in a greenhouse all year round. The possibility of successful and efficient use of cotton aphid, spider mite, tobacco thrips, greenhouse whitefly and noctuid moth entomophagous was demonstrated. Application rates for several entomophagous were proposed for prevention and protection of cucumber plants against the above-mentioned pests.

1 Introduction

There is a long list of natural enemies that could be used for biocontrol of pests on cucumber indoors. But only a few of them were selected by practice as the most effective and handy in usage biocontrol agents (BCA). There are the predatory mites Neoseiulus (Amblyseius) cucumeris (Oudemans), Amblyseius swirskii Athias-Henriot, Phytoseiulus persimilis Athias-Henriot, the predatory bug Macrolophus pygmaeus Rambur and the parasitoid Aphidius colemani Viereck [1, 2]. The application rate of these entomophagous depends mostly on the climate and weather conditions in the region, where they are used. Also, other factors have the considered impact such as cultivated plants, structure and volume of the greenhouses floor area, usage of new technologies of plant growth, and the unique phytosanitary history, which every greenhouse has.

Currently, in Russia, the indoor floor area is growing, mostly because of the modern industrial greenhouses in which new plant growth technologies are widely used. In the North-West region, there is one of the leaders between greenhouse facilities - “Year around” on which base our institute has been conducted the assessment of BCA efficacy during the last five years. The goal of these researches is to optimize the system for pest biocontrol on cucumber indoors in the North-West of Russia.

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2 Materials and methods

In view of the above, the analysis of cucumber crop protection system performed at “Year around” greenhouse facility (Boksitogorskiy District, Leningrad Region) can be useful for similar facilities located in the regions with similar climatic conditions. The facility designed by a Dutch company Dalsem Horticultural Projects B.V. has been operating since September 2014. The facility with an area of 6 ha is used for growing tomatoes, cucumbers and lettuce under conditions of photo culture and intense photo culture. Cucumbers are grown in three units with a total area of 29,900 m². Intense photo culture – that is, supplementary lighting at the level of tops of the plants and inside the coenosis, is used in two units. In the third unit (11,600 m²) where observations were performed for 5 years from 2015 till 2019, there is no supplementary lighting inside the coenosis, which results in less intensive growth and a higher possibility of accumulation of pests and development of diseases. This requires increased attention during planning and implementation of protective measures. Mean cycle duration for cucumber plants is 10–14 weeks.

The main pests identified in the greenhouses include Tetranychus urticae Koch., Trialeurodes vaporariorum Westw., Thrips tabaci Lind., Aphis gossypii Glov., Agrotis segetum Schiff, Mamestra brassicae L., Autographa gamma L. and Agrotis exclamationis L., which are very common for indoors in North-West Russia. Greenhouse whitefly, tobacco thrips and spider mite have found in greenhouses all year round, so preventive releases of natural enemies were performed as early as at the plant propagation stage.

The predatory mite N. cucumeris (Oudemans) and predatory bug Orius laevigatus Fieb. have been used for thrips control. Preventive releases of N. cucumeris (5 specimens/2 plants every 2 weeks) continued after transplanting of seedlings, for control of thrips population. In the cases where thrips hot spots were found, the application rate was increased up to 10 specimens/plant. Application rate of the predatory bug O. laevigatus was 2 specimens/m².

As natural enemies of whitefly A. swirskii and M. pygmaeus have been used. A. swirskii was used on seedlings at the application rate of 10 specimens/plant every 2 weeks. Two weeks after transplanting of seedlings, sachets with A. swirskii were used (1 sachet contained 250 specimens of the mite). Release rate of M. pygmaeus was 1 adult per 1 m².

Against spider mites the predator Ph. persimilis has been used at the application rate of 2 specimens/m² once in 3 weeks. Also predatory gall midge Feltiella luboviae Fedotova et Kozlova was released in rate 0,1-0,2 specimens/m² weekly [3, 4, 5]. To suppress A. gossypii the parasitoid A. colemani was released. The recommended application rate for aphid prevention was 1-2 specimens per 1 m² once in 2 weeks [6]. The predatory bug Podisus maculiventris (Say) has been used as natural enemy of noctuid caterpillars. Its application rate was 0,1-0,2 specimens/m² monthly.

The errors of average were calculated using Statistica v.9 software package.

3 Results and discussion

Seedlings are treated with mites A. swirskii and N. cucumeris against thrips and greenhouse whitefly. The application rate is 5–10 specimens/plant per 1 m² every 2 weeks. Then, after transplanting of seedlings, application of the above-mentioned mites continues for prophylactic purposes. However, after transplanting of seedlings, the positive dynamics of pests are observed in some cases despite the preventive application of entomophages. An increase in pest population differs for different species, depending on the season and meteorological conditions during the year.

The minimum number of N. cucumeris was applied in 2015 when thrips population in harbourage areas was not more than 3 specimens/plant. The maximum number (three times as large as the minimum number) was applied in 2018 when harbourage areas with average
thrips population of 8–14 specimens/leaf were observed throughout spring/summer season (Table 1). Predatory bug *O. laevigatus*, are additionally applied in harbourage areas where average thrips population amounts to 6–8 specimens [6-7].

**Table 1. Change rates of application of thrips predators during observations (2015-2019)**

<table>
<thead>
<tr>
<th>Rate of application (average annual)</th>
<th>Neoseiulus cucumeris</th>
<th>Oritus</th>
</tr>
</thead>
<tbody>
<tr>
<td>min</td>
<td>151.4</td>
<td>0</td>
</tr>
<tr>
<td>max</td>
<td>469.8</td>
<td>3.58</td>
</tr>
<tr>
<td>for 5 years</td>
<td>366.5±50.93</td>
<td>2.65±0.601</td>
</tr>
</tbody>
</table>

Predatory bug *O. laevigatus* was used in all years, except for 2015 when minimum thrips population was observed, and there were no harbourage areas with critical numbers of the pest. Throughout the remaining observation period, the application rate of the predatory bug varied depending on the pest population (Table 1).

It should be noted that the application rates of predatory mite *N. cucumeris* throughout the observation period were generally below the recommended rate. For example, for thrips prevention, it is recommended to apply up to 500 specimens (in a sachet) per 10 plants once a month, while in the case of the pest outbreak in a greenhouse (more than 35 % of the area) a release of 100–200 specimens of the predatory mite per plant every 2 weeks is recommended [2].

For control of greenhouse whitefly population during transplanting of seedlings, yellow glue bands were used for detection of first specimens of the pest. The glue bands were placed at the level of tops of the plants, 1 band per 1 mat. As the plants grew, the glue bands were placed on a trellis above them, 1 band per 3 plants. Overall, up to 6,500 glue bands per unit were used. The application rate was increased where necessary. The minimum number of *A. swirskii* was applied in 2015 when greenhouse whitefly population was very small. In spring, only occasional specimens were observed on glue bands, so the use of the predatory mite was limited to preventive releases (Table 2).

**Table 2. Change rates of application of whitefly predators during observations (2015-2019)**

<table>
<thead>
<tr>
<th>Rate of application (average annual)</th>
<th>Amblyseius swirskii</th>
<th>Macrolophus pygmaeus</th>
</tr>
</thead>
<tbody>
<tr>
<td>min</td>
<td>514.5</td>
<td>0</td>
</tr>
<tr>
<td>max</td>
<td>710.4</td>
<td>0.26</td>
</tr>
<tr>
<td>for 5 years</td>
<td>634.1±31.57</td>
<td>0.052±0.046</td>
</tr>
</tbody>
</table>

In the following years greenhouse whitefly population increased, so did the application rates of *A. swirskii*. The maximum application rate of the predatory mite was used in 2018 (Table 2). It was 1.4 times higher than in 2015. Apart from mites, application of predatory bug *M. pygmaeus* is recommended for protection of cucumber plants against greenhouse whitefly. [8] In addition to *M. pygmaeus*, predatory bug *Nesidiocoris tenuis* Reut. has been used at the greenhouse facility since 2015 (one year after commissioning) for protection of tomatoes against greenhouse whitefly. [9] As a result of application in the unit used for growing tomatoes where the vegetation period amounts to 11–11.5 months, accumulation and migration of the pest (as well as migration of its prey, greenhouse whitefly) to other units of the facility were observed. For cucumber plants, the presence of larvae of *N. tenuis* (2–6 specimens/plant) was detected in occasional small harbourage areas of greenhouse whitefly (not more than 2–3 larvae/leaf). Larvae of the pest were eliminated in 10–14 days in such harbourage areas. In general, no significant increase in greenhouse whitefly population on cucumber plants was detected during the observation period. Additional application of predatory bug *M. pygmaeus* (3,000 specimens) was performed only once, on
21 March 2016, in a harbourage area of greenhouse whitefly (7 larvae/leaf) where there were no \textit{N. tenuis} specimens. Subsequent releases of \textit{M. pygmaeus} were not performed, as imagos of \textit{N. tenuis} were found on infected plants 7–10 days after the start of the pest population growth, and larvae of the bug were observed a week later, while larvae of greenhouse whitefly were absent. It should be noted that phytophagy in absence of prey was weak. This phenomenon was more pronounced in \textit{N. tenuis} than in \textit{M. pygmaeus}. [10] \textit{N. tenuis} bug feeding on cucumber plants causes characteristic perforation of leaves due to the damage of tissues in the areas of feeding of the bug on juvenile leaves, which becomes more evident as the leaves grow. However, these damages had no effect on cucumber yield during the observation period. Intensive growth of leaves observed in this intensive technology of cucumber growing, when up to 6 leaves are cut out per week, probably compensates for such damages. Damages of the upper stem, which may result in chipping off of the top when the stem is fixed on a trellis are more dangerous. However, such damages are observed in large \textit{N. tenuis} populations, up to 7–10 imago/plant, which accumulate on some plants (for example, tomatoes) during long-term vegetation. Such accumulation is impossible on cucumber plants as their vegetation period does not exceed 3 months. After a cycle change, the majority of predatory bug population was eliminated, so dangerous damages of cucumber tops at the facility are very rare. Thus, cucumber plants are successfully protected against greenhouse whitefly using \textit{A. swirskii} predatory mite and bugs only. \textit{N. tenuis} is not purposefully applied on cucumber plants. 

According to the research data, the application rate of 75 \textit{A. swirskii} specimens per 1 \textit{m}^2 during transplanting of seedlings, in presence of the pest, is sufficient for protection of cucumber plants against greenhouse whitefly. [11] However, manufacturers of entomophages, such as Koppert, recommend applying 1 sachet (250 mite specimens) per 2.5 \textit{m}^2 once in 4–6 weeks for prophylactic purposes – that is, twice during a cucumber growth cycle [3], which is at least 2 times the application rate we use. Other facilities also use higher application rates of predatory insects for prophylactic purposes. [7] For example, in TK Mayskiy LLC (Kazan), when growing photoculture cucumbers, 200 specimens of \textit{A. swirskii} mite/1 \textit{m}^2 per month and 0.05 specimens of \textit{Macrolophus} bugs are used for prophylactic purposes only [12].

Predatory mite \textit{P. persimilis} was used against spider mite. It was used for prophylactic purposes (2 specimens/\textit{m}^2) as early as at the plant propagation stage However, occasional harbourage areas of the pest were observed on plants, mostly in late winter/spring or in late summer/early autumn, and additional release of \textit{P. persimilis} was performed (\textit{P. persimilis}: \textit{T. urticae} ratio of 1:10). [13] Moreover, in 2015–2016 a new species of predatory midge \textit{F. luboviae} was tested at the facility. [3, 4] \textit{F. luboviae} was brought under cultivation in the biological protection laboratory of the All-Russian Research Institute of Plant Protection. The predatory midge was also used in harbourage areas of the pest together with \textit{P. persimilis}. In 2015 acariphages were used to a lesser extent than in the following years. [5] The maximum number of acariphages was applied in 2018. The application rate of \textit{P. persimilis} was 2.8 times higher, and of midge, 2.3 times higher than the minimum rate in 2015 (Table 3).

\begin{table}
\begin{center}
\begin{tabular}{|c|c|c|}
\hline
Rate of application (average annual) & \textit{Phytoseiulus} & \textit{Feltiella luboviae} \\
\hline
min & 7.76 & 0.95 \\
max & 21.5 & 2.15 \\
for 5 years & 16.01±2.213 & 1.49±0.218 \\
\hline
\end{tabular}
\end{center}
\caption{Change rates of application of spider mite predators during observations (2015-2019)}
\end{table}

For protection against \textit{A. gossypii}, \textit{Macrosiphum euphorbiae} Thomas, \textit{Aulacorthum solani} Kalt., a set of predatory insects and parasitoids (\textit{A. colemani}, \textit{Aphidius ervi} Hal.,)
and predatory midge (*Aphidoletes aphidimyza* Rondani) is used at the facility. [14,15] Occasional small (4–5 plants) harbourage areas of *A. solani* and *M. euphorbiae* (10–15 specimens/plant) can be observed on lettuce plants in summer, and a set of aphidophages ensures their complete elimination. On cucumber plants, only *A. gossypii* is observed, and its mass reproduction takes place in late autumn and winter rather than in summer, which is probably due to migration of the pest from open-ground plants in autumn, and depends on the temperature conditions during the year. The minimum number of the *A. colemani* was applied in 2015 (Table 4).

The application rate did not exceed the rate recommended for prophylactic use. The maximum percentage of the aphidophage was released in November. Aphid population in harbourage areas during this period was 60–350 specimens/leaf. In 2016 and 2018 the application rate of *A. colemani* was 2.3–2.4 times higher as aphid harbourage areas were observed throughout winter. The maximum number of the aphidophage was applied from November till May. In other years the application rate of the aphidophage was 1.7–1.8 times lower than the maximum rate.

Table 4. Change rates of application of *Aphidius colemani* and *Podisus maculiventris* during observations 2015-2019. Rate of application (average annual)

<table>
<thead>
<tr>
<th></th>
<th>Number of individuals per 1 m²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>min</td>
</tr>
<tr>
<td><em>Aphidius colemani</em></td>
<td>28.4</td>
</tr>
<tr>
<td><em>Podisus maculiventris</em></td>
<td>0.049</td>
</tr>
</tbody>
</table>

Lepidopteran pests pose a threat at the facility during periods of migration of butterflies to greenhouses from the environment. Depending on the temperature, it takes place in May–early June and continues till November. Predatory bug *P. maculiventris* Say was used for this purpose at the facility. It should be noted that *P. maculiventris* is extensively used for protection against the Colorado potato beetle and lepidopteran pests. Third instars or eggs of the predatory bug are normally used for releases, both in the open and in greenhouses [16-17]. At the facility, adult *P. maculiventris* specimens were released when noctuid moths were detected in greenhouses. Imago 5–7 days after acquiring wings were used. The minimum number of the bug was released in 2015 when butterflies were detected in a greenhouse on May 23 and were observed till the last third of October (table 4). Noctuid moth larvae on cucumber plants were observed only once (on July 20), on 16 plants, 5 of which had damaged tops. After that, no larvae were detected. *P. maculiventris* was observed in greenhouses throughout the release period and for 37 days after the last release, when there were no butterflies in the greenhouses. It was noted that the bugs fed on adult butterflies.

In the following years the application rate of *P. maculiventris* was increased as first butterflies appeared in greenhouses already in the second third of May and were observed till late October. The maximum number of the predatory bug (1.7 times greater the minimum release) was applied in 2018 which had the warmest summer and autumn in the observation period (table 4). *P. maculiventris* was released from mid-May, when first butterflies were detected in greenhouses, till mid-November as last butterflies were observed in the second third of November. Releases were performed monthly as the lifetime of *P. maculiventris* at 24–26 °C is 55–60 days [18]. Similarly to 2015, noctuid moth larvae were not observed on plants. However, the bugs continued feeding on butterflies. The differences in the time of emergence and presence of noctuid moths in a greenhouse throughout the observation period can be explained by different temperature conditions in different years of observation. In general, application of *P. maculiventris* imago was efficient in terms of protection against lepidopteran pests in greenhouses in Northwest Russia. The bugs stay in greenhouses, travel inside them successfully, have a
prolonged life cycle and eliminate not only larvae of lepidopteran pests, against which their use is recommended, but also butterflies, thus preventing the harmful stage. The application rate at the facility is 0.5–0.9 specimens/m² throughout the hazardous period (5 months on average).

4 Conclusion

The application rates of natural enemies often differ from recommended rates and depend on the climatic conditions during the year. For example, the maximum number of entomophagous during the observation period was applied in 2018, when the period from April through November was the warmest, compared to other years. This apparently promoted the accumulation of pests (aphids and lepidopteran pests) in the environment, with subsequent migration to a lighted greenhouse due to warm weather in autumn. Moreover, the summer of that year was the warmest, which promoted the development of pests (spider mite, noctuid moth and thrips) in greenhouses. The analysis of long-term observations of pest dynamics and application of natural enemies at the facility suggests that application rates of entomophagous shall be adjusted taking into account the geographical location of a greenhouse facility, regional climatic conditions, meteorological conditions of each year, data of signal devices (traps of various types) and the results of greenhouse monitoring for several years in order to prevent both insufficient and excessive application of natural enemies and improve the crop protection system in general.

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