

Increasing cucumber resistance to spider mites by biotic plant resistance inducers

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Abstract: Cucumber acceptance by spider mites was studied after treatment with 2 biotic plant resistance inducers. The plants were treated with *Pseudomonas fluorescens* (plant growth-promoting rhizobacteria) and with an extract of the giant knotweed *Reynoutria sachalinensis* Schmidt (1% solution of Milsana Bioprotectant Concentrate). Experiments testing plant acceptance by 2-spotted spider mites (*Tetranychus urticae* Koch, 1836), density of their population, and fecundity of females were conducted in the glasshouse and in laboratory conditions. A lower preference of spider mites for the plants treated with all combinations of plant inducers was observed. Six weeks after infestation of plants with spider mites, the density of the mite population was 2-fold lower on bacteria-treated and on Milsana-treated plants, and 24% lower after simultaneous application of both resistance inducers, as compared to untreated plants. Fecundity of mite females also decreased on leaves of the plants treated with resistance inducers. The lowest oviposition (35% lower than in the control) was observed for the plants treated with Milsana.

Key words: resistance inducers, plant growth-promoting rhizobacteria, *Reynoutria sachalinensis* extract, *Tetranychus urticae*

INTRODUCTION

In recent years attention has been given to the possibility of using natural inducers of plant resistance to diseases and arthropod pests in plant protection. Usually some rhizosphere bacteria and non-pathogenic fungi are used for increasing the level of plant resistance to harmful organisms (WEI et al. 1991, ZEHNDER et al. 1997, VAN LOON et al. 1998). The phenomenon of induced resistance is connected with activation of biochemical and physiological reactions leading to changes in the chemical composition of plant tissues in a direction that is disadvantageous for phytophagous organisms (BOROWICZ et al. 1992, BI et al. 1997, TOMCZYK & KIEŁKIEWICZ 2001). This is often related to the level of allelochemicals and their influence on pathogens and pests (ZEHNDER et al. 1997, TOMCZYK 2002). The induced resistance can have a systemic or local character. Systemic resistance to diseases (systemic acquired resistance = SAR) is connected with activation of the salicylic acid path-

way, while resistance to insects is usually connected with jasmonic acid and ethylene (induced systemic resistance = ISR); but in some cases opposite relations are observed and cross-talk between both pathways can occur (VAN LOON et al. 1998, PIETERSE et al 2001). Plant growth-promoting rhizobacteria (PGPR) can usually induce an ISR system in the plant, similar to the reaction caused by insects. Spider mites can activate both SAR and ISR systems. Earlier studies showed that PGPR developed on cucumber roots can inhibit the growth of spider mite populations on bacterized plants (TOMCZYK & KIELKIEWICZ 2000). Plant resistance to harmful organisms can also be induced by other biotic inducers. It was found that an extract of the giant knotweed *Reynoutria sachalinensis* L. (Milsana Bioprotectant Concentrate) can induce resistance to some diseases (SCHMITT 2002). Changes in the level of some secondary metabolites in the tissues of plants treated with Milsana can have a negative influence on both pathogenic organisms and arthropod pests. The aim of this study was to check the level of glasshouse cucumber preference of two-spotted spider mite after an application of 2 biotic resistance inducers.

MATERIAL AND METHODS

Experiments were conducted on cucumber cv. Aramis. Seeds were sown into pots filled with a peat substrate for seedlings, supplemented with 14 kg of chalk, 2.5 kg of MIS-3, and 135 g of microelements per 1 m³ (all elements produced by INTERMAG, Poland). Half of the seeds were previously inoculated for 20 minutes with *Pseudomonas fluorescens* P-112 isolated from cucumber roots. The inoculum contained 3×10⁹ bacterial cells per 1 cm³. The other half of the seeds were treated with water. The total number of experimental plants was 48. Plants were used in 3 tests after application of a second resistance elicitor, the extract of *Reynoutria sachalinensis* (Milsana Bioprotectant Concentrate).

Acceptance test

The experiment started when plants were at the stage of 2 leaves. Thirty-two plants (16 treated with bacteria and 16 untreated) were selected and divided into 4 groups of 8 plants each. One group of bacteria-treated and one group of untreated plants were sprayed with 1% (by volume) water solution of Milsana, by using a small, 2-dm³ sprayer. Other plants were sprayed with water. In this way, 4 experimental combinations were obtained: (1) control – non-bacterized and non-sprayed with Milsana; (2) bacteria – bacterized but non-sprayed with Milsana; (3) Milsana – sprayed with Milsana but non-bacterized; and (4) Milsana+bacteria – sprayed with Milsana and bacterized.

Two days after Milsana treatment, the plants were transferred together with the substrate into open-ended rings of polyvinylchloride filled with a peat substrate, supplemented with 14 kg of chalk, 2.5 kg of MIS-4, 135 g of microelements per 1 m³ (all elements produced by INTERMAG, Poland). They were planted in 2 rows. One row was for plants treated with Milsana, and the other for untreated plants. In each row, bacterized and non-bacterized plants were located alternately. Six days after the spraying with Milsana (i.e. 4 days after transferring the plants into the rings), the plants from all combinations were used in a test estimating their acceptance by spider mites. For this purpose, between every 4 plants belonging to different combina-

tions (2 in one row and 2 in another), cardboard squares were placed, as special arenas. Eight arenas were used in the experiment. Every angle of each square touched the stem of one plant, as shown in Fig. 1. Thirty females of *Tetranychus urticae* were introduced to the central part of the arena, giving them free choice in plant acceptance. Four days later, the arenas were removed and the number of females was checked on every plant.

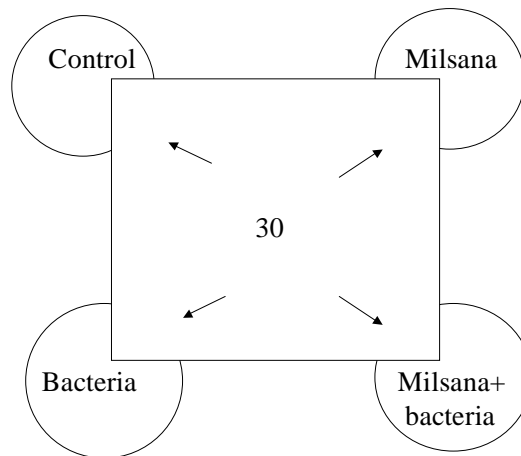


Fig. 1. Scheme of the acceptance test

Development of the spider mite population

In this test, plants infested in the acceptance test were used 6 weeks after arena removal. During this period, Milsana was applied 4 times in one row of experimental plants (Milsana and Milsana+bacteria). First time, Milsana was used 5 days after arena removal and then plants were sprayed in 10-day intervals. At the same time, other plants were sprayed with water. For an estimation of the density of the spider mite population, 12 leaves from the middle of all 8 plants of each combination were collected. From every plant, 2 leaves were taken, in total 16 leaves, but 4 of them were used for another purpose. The number of spider mites was assessed by using a magnifying glass and calculated per leaf.

Fecundity of spider mite females

For the tests, 16 cucumber plants uninfested by spider mites were used. The same experimental combinations (4 plants per combination) were used as for other tests.

Ten leaves were taken from the plants of every combination and used for the fecundity tests in the laboratory. Disks with a diameter of 2 cm were cut from the leaves and placed on wet cotton wool in Petri-dishes. Two females of *Tetranychus urticae* reared on cucumber were released onto each plant disk and left in a growth chamber. Thirty 1–2-day-old inseminated females were used in each plant combination. The number of eggs laid by females was checked after 3 days. The fecundity of females was calculated per female per 3 days.

Statistical methods

One-way ANOVA was used and significance of differences was determined by Fischer's test, with significance threshold of 0.05.

RESULTS

Acceptance test

The numbers of spider mites found on the plants treated and untreated with resistance inducers in the acceptance test are presented in Fig. 2. The treatment of cucumber plants with resistance inducers caused differences in plant acceptance by spider mites. Mites showed a significant preference for the plants untreated with Milsana and with bacteria.

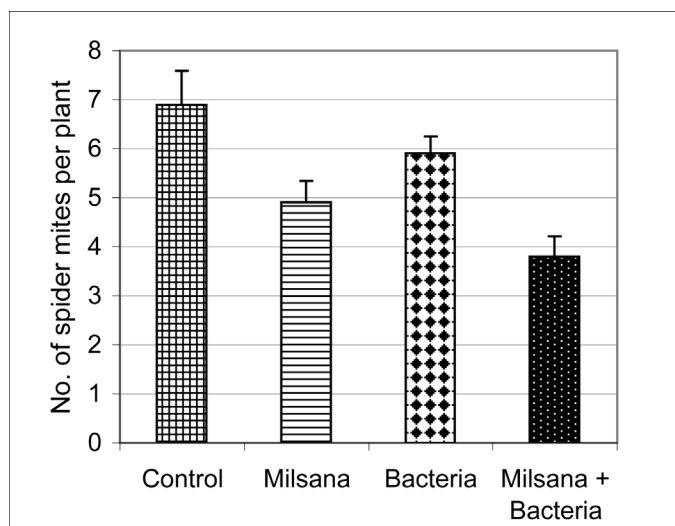


Fig. 2. Numbers of spider mites on cucumber plants in the acceptance test. SE values are presented. Statistical significance was verified with Fisher's test.

The average number of recaptured mites per arena was 21.4. The control plants attracted 32.2% of recaptured mites, compared to 27.5% for bacteria-treated and 22.8% for Milsana-treated plants. The smallest fraction of spider mites, 17.5%, was found on the plants treated with both inducers. The differences in mite preference between plants treated and untreated with resistance inducers were significant in the case of the Milsana application as well as Milsana+bacteria. ($F_{3,28} = 7.43$, $P = 0.008$). No significant differences were found between control plants and plants treated only with bacteria.

Development of the spider mite population

Data on the density of the spider mite population after 6 weeks from the plant infestation are presented in Fig. 3. In every case of plant treatment with resistance inducers, spider mite population density was lower on the treated plants than on

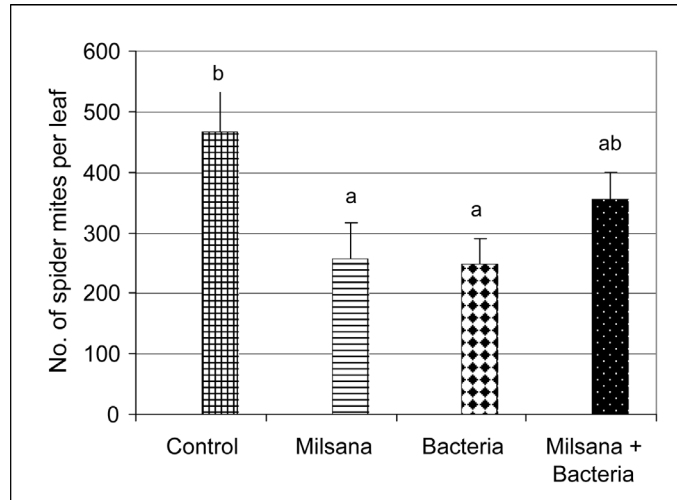


Fig. 3. Densities of the spider mite population on cucumber plants treated and untreated with resistance inducers 6 weeks after plant infestation. SE values are presented. Statistical significance was verified with Fisher's test.

untreated ones. The application of either bacteria or Milsana caused a 2-fold decrease in mite density, in relation to the control population. The difference was statistically significant ($F_{3,40} = 3.38, P = 0.274$). The combination of both inducers resulted in a density decrease of about 24%, as compared to the population developed on control plants. However, this difference was not statistically significant.

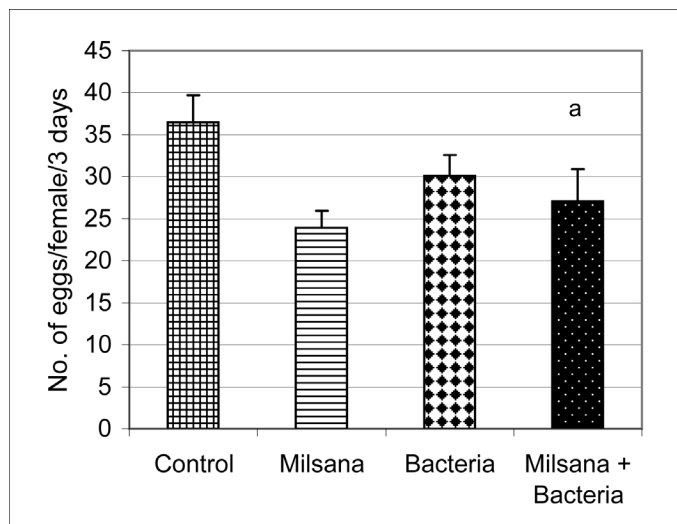


Fig. 4. Fecundity of spider mite females on the leaves of cucumber treated and untreated with resistance inducers. SE values are presented. Statistical significance was verified with Fisher's test.

Fecundity of spider mite females

Data on fecundity of females, feeding on the plants treated and untreated with inducers used in the experiment, are presented in Fig. 4. The application of Milsana, both together with bacteria and separately, caused a significant decrease in the number of eggs oviposited by females as compared to the control ($F_{3,45} = 3.18$, $P = 0.033$). Mite females laid 35% fewer eggs on leaves taken from Milsana-treated plants and 26% fewer eggs on leaves from Milsana+bacteria-treated plants, as compared to control. The differences were statistically significant. A smaller difference was found between oviposition of females on bacteria-treated and control plants, as on the former plants they laid 17.5% fewer eggs than on control plants, so the difference was not statistically significant.

DISCUSSION

Both resistance inducers – Milsana and *Pseudomonas fluorescens* – lowered the preference of spider mites for cucumber plants. This means that both inducers could induce the antixenosis mechanism, which was probably connected with volatile substances emitted from the plants treated with resistance inducers. The experiments conducted on cucumber treated with *Pseudomonas fluorescens* (a strain belonging to PGPR) showed changes in the emission of these substances as a result of bacteria application (PIETERSE et al. 2001). Milsana can also make changes in concentrations of some secondary metabolites in tissues of treated plants (SCHMITT 2002). It is possible that such substances can be detected by spider mites during the process of plant acceptance. The level of spider mite preference for plants treated with Milsana or bacteria was lower than on plants treated with both inducers (inoculated with bacteria and sprayed with Milsana). It is possible that there was cross-talk between the mechanisms induced by Milsana and PGPR. In other experiments, cross-talk between SAR and ISR was observed (VAN LOON et al. 1998).

The results of the presented experiments indicate the possibility for induction of plant resistance to spider mites by use of biotic inducers, such as PGPR and an extract of *Reynoutria sachalinensis*. The reduction in the spider mite population was caused mainly by a decrease in the level of mite preference of the plants treated with bacteria and Milsana inducers. However, the fecundity of *Tetranychus urticae* females was also affected after feeding on the plants with induced resistance. A decrease in mite density and fecundity of females on bacterized plants, as compared to non-bacterized, was found also in earlier experiments (TOMCZYK & KIELKIEWICZ 2000). Changes in the concentration of phenolic compounds and cucurbitacins, as well as in the level of sugars and proteins in bacteria-treated plants, can be responsible for a decrease in the nutritive value of these plants to spider mites (TOMCZYK & KIELKIEWICZ 2001, TOMCZYK 2002).

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