

Potential of the mycoinsecticide Naturalis® for use in strategies of crop protection against *Diabrotica virgifera virgifera* LeConte, 1858



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Abstract: Entomopathogenic fungi are important and promising bio-control agents for controlling insect pests. *Beauveria bassiana* strain ATCC 74040 (Naturalis®) was selected for testing because it is commercially available to farmers in EU for use against pests of different crops in greenhouses and in the open field. In laboratory conditions, the effect of different conidial concentrations ($2.3 \times 10^3 - 2.3 \times 10^7$ conidia/ml) of *B. bassiana* strain was evaluated on the western corn rootworm, *Diabrotica virgifera virgifera*, (WCR) mortality in Petri dishes. Ten days post treatment the mean corrected mortality of WCR adults in response to the conidia concentrations reached 19% to 92% for the lowest to the highest conidia concentration, respectively. In 2022, a monitoring of the seasonal flight activity of WCR adults was conducted using Csalomon® KLP traps baited with dual attractant lures in maize fields near Knezha and Dolni Dabnik (northern Bulgaria). At both localities, two baited traps were set up in maize fields with biological seed treatment containing Naturalis® (200 ml/100 kg seeds) and one baited trap was set up in maize fields, where the seeds were treated with Gaucho FS 600 (700 ml/100 kg seeds). In Knezha, catches of the target species were recorded from the end of June to the beginning of September while the period of the flight in Dolni Dabnik was recorded from the beginning of July to the beginning of August. Catches of the adults in traps in crops with biological seed treatment were somewhat lower than those in fields treated with the chemical insecticide. The two-factorial ANOVA (treatment and locality as factors) revealed a significant interaction between the type of seed treatment and locality/maize hybrid on the grain yield of maize.

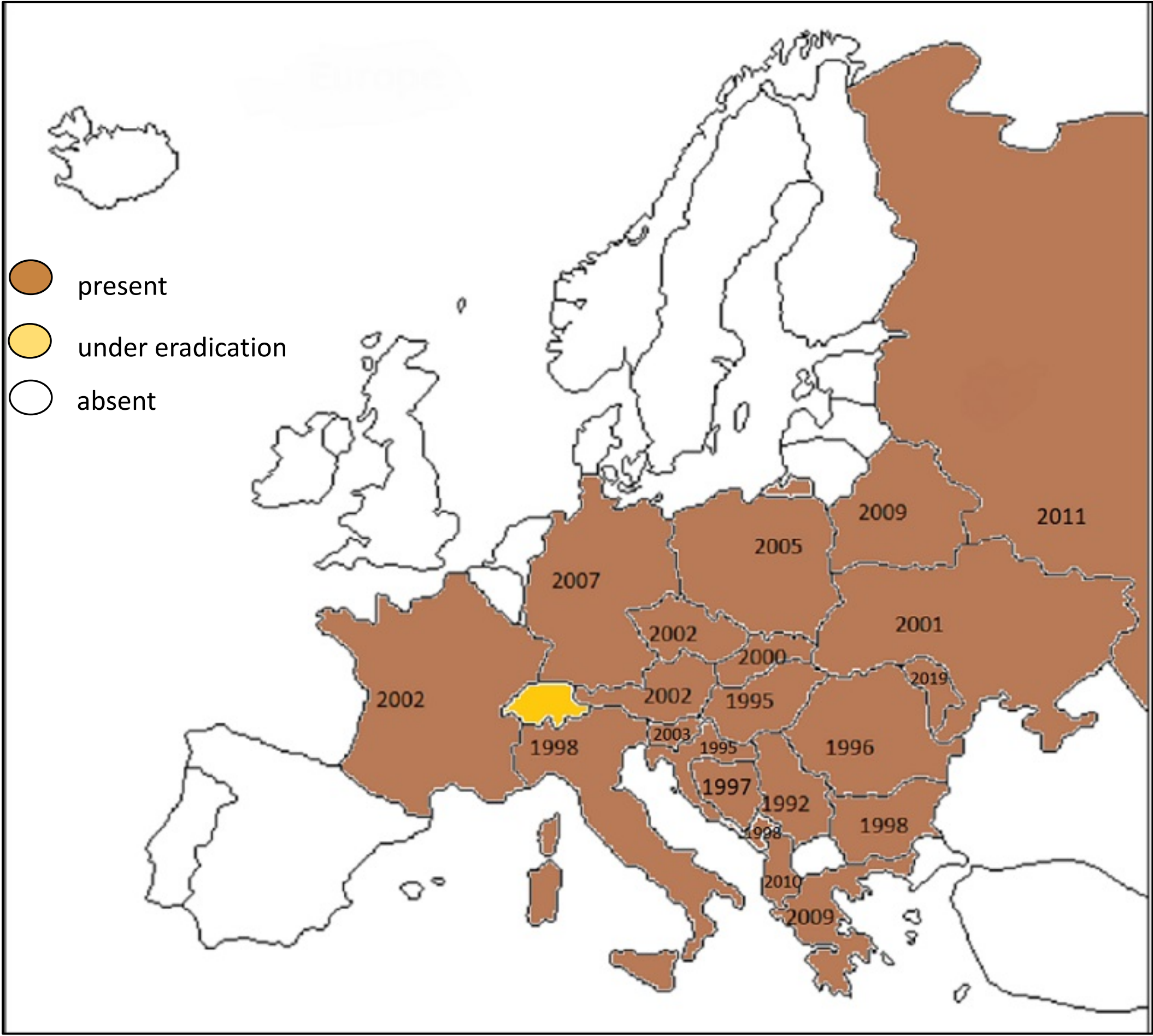


Diabrotica v. virgifera adults (A, B) and damages caused by the larvae - "goose neck" (C, D)

The Western corn rootworm (WCR), *Diabrotica virgifera virgifera* LeConte, 1858 is native to North America and it was first discovered in Europe in 1992 near Belgrade airport, Serbia (former Yugoslavia). The pest is currently distributed across 21 European countries. *D. v. virgifera* is univoltine, overwintering as an egg in the soil. Maize is the primary host of WCR larvae, which are the harmful stage damaging maize roots but alternative host plants may contribute to the invasion potential of the pest.

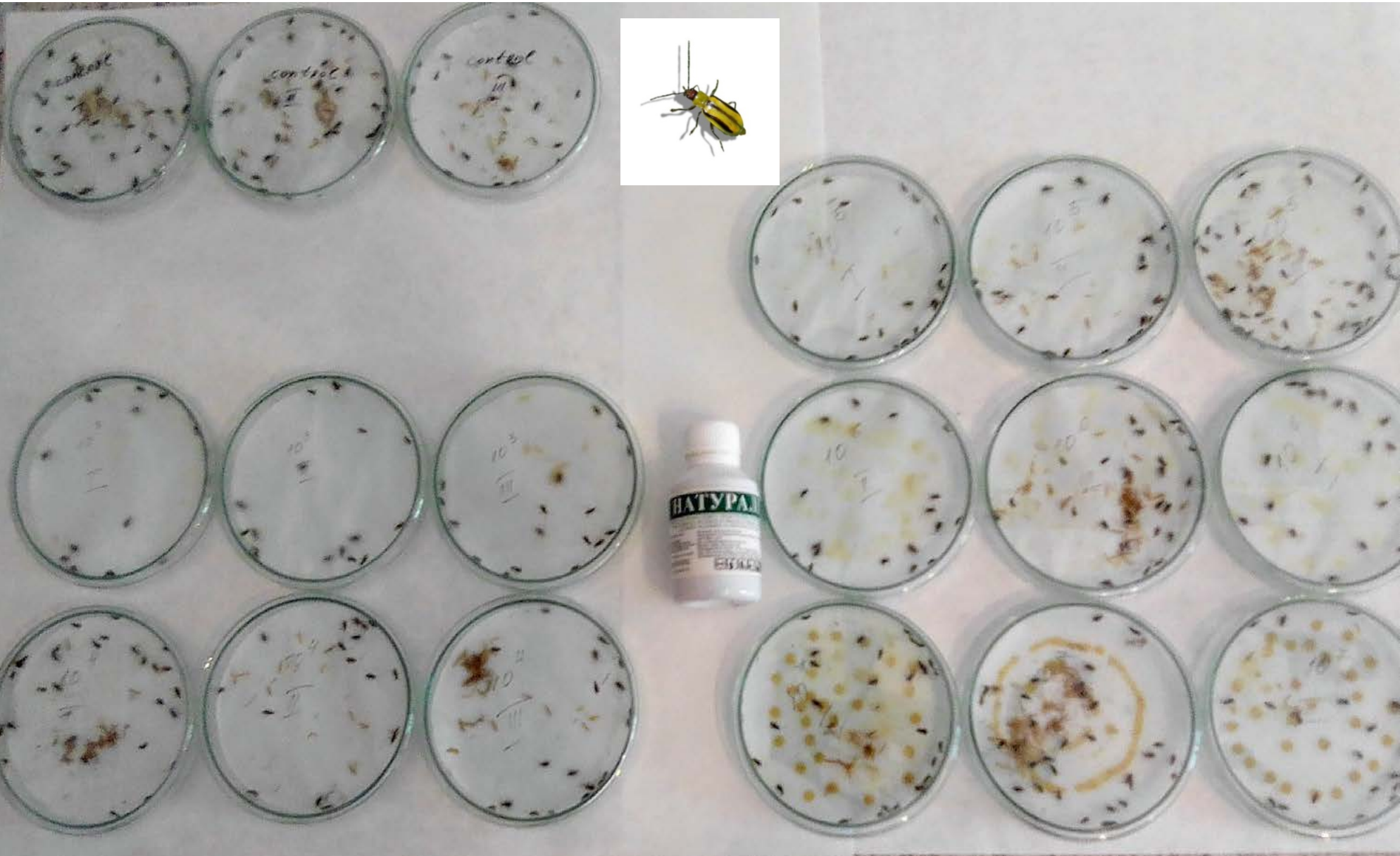
Nowadays adopting cropping system approaches and fostering the adoption of nonchemical tactics is a challenge toward a reduced reliance on chemical insecticides against insect pests.

In this study, we evaluated for first time the insecticide properties of the mycoinsecticide Naturalis® (*Beauveria bassiana* strain ATCC 74040) against field-collected adults of WCR under laboratory conditions and the influence of seed treatment practice on the yield of maize grain in crops in northern Bulgaria, where *D. v. virgifera* occurs.



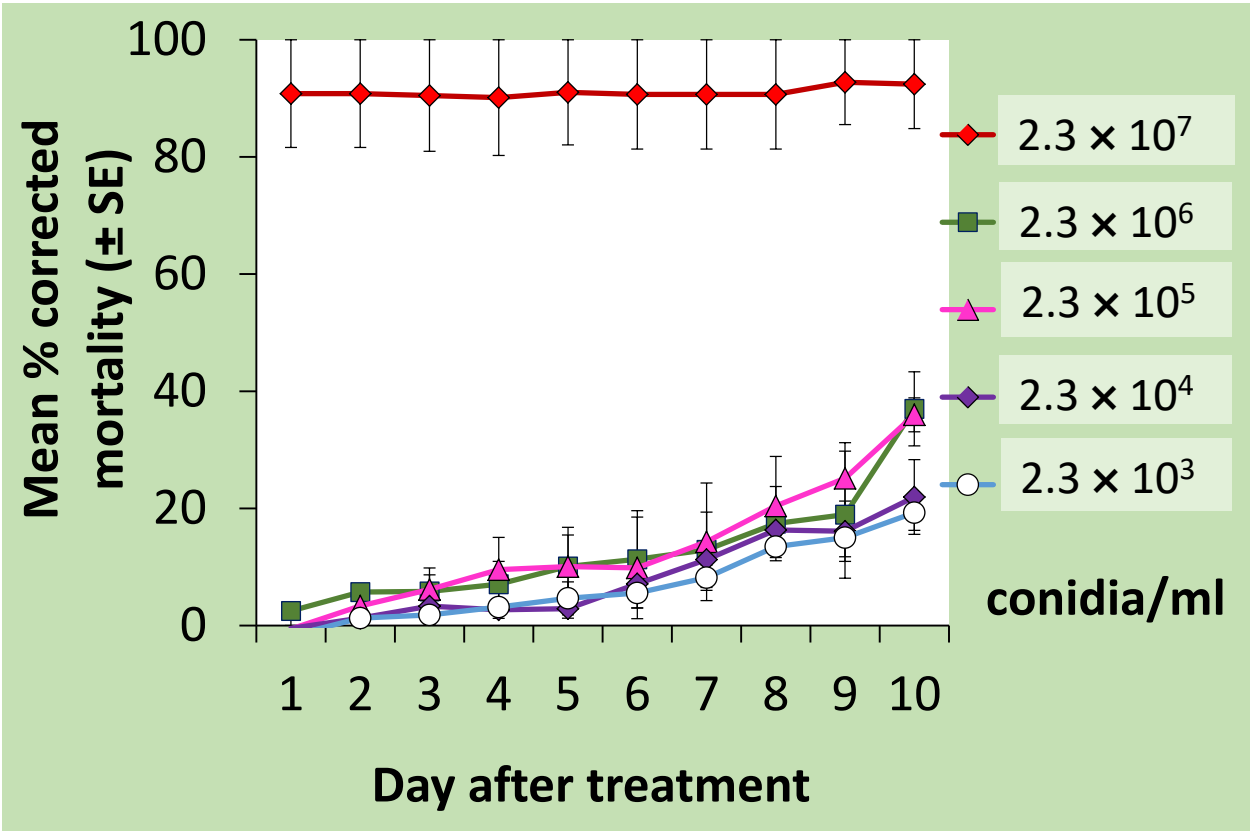
Distribution of *Diabrotica v. virgifera* in Europe and years of its appearance in the countries according to EPPO database [accessed November 2022]

Laboratory bioassay with Naturalis® against the adults of WCR



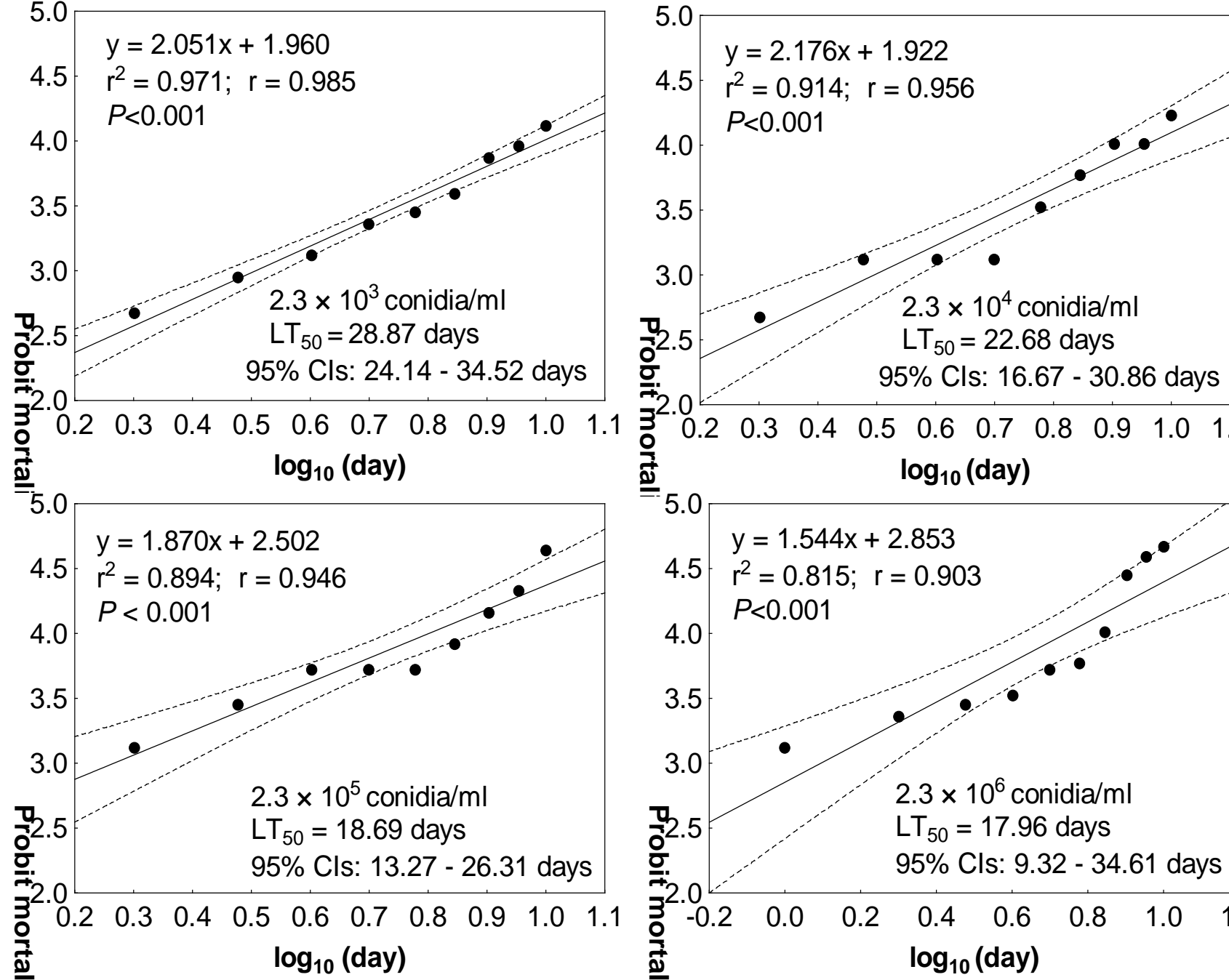
Bioassay of susceptibility of WCR adults treated with different concentrations of Naturalis® (*B. bassiana*) bioproduct

- Five conidial concentrations in distilled water (from 2.3×10^3 to 2.3×10^7 conidia/ml and control - distilled water (1 ml of a conidial concentration was applied on filter paper disc on the bottom of a Petri dish; 1 ml water in control))
- Groups of 24 ± 1 adults in Petri dish; 3 replications of each concentration and control
- Mortality was recorded at 24-h intervals for 10 days.



Cumulative mortality (%) of *D. v. virgifera* adults caused by different concentrations of *B. bassiana* ($2.3 \times 10^3 - 2.3 \times 10^7$ conidia/ml)

The mean corrected mortality of WCR adults in response to the conidia concentrations of Naturalis® after 10 days post treatment was in the range from 19% to 92% for the lowest to the highest conidia concentration, respectively. The conidial concentration, which cause LC_{50} of WCR adults after ten days was 4.3×10^5 conidia/ml (95% CIs: $9.0 \times 10^3 - 3.0 \times 10^7$ conidia/ml), whereas LC_{90} was 3.3×10^7 conidia/ml (95% CIs: $4.7 \times 10^5 - 2.2 \times 10^9$ conidia/ml).



Probit analysis on median lethal time LT_{50} of *D. v. virgifera* adults treated with *B. bassiana* at different concentrations: ($2.3 \times 10^3 - 2.3 \times 10^6$ conidia/ml)

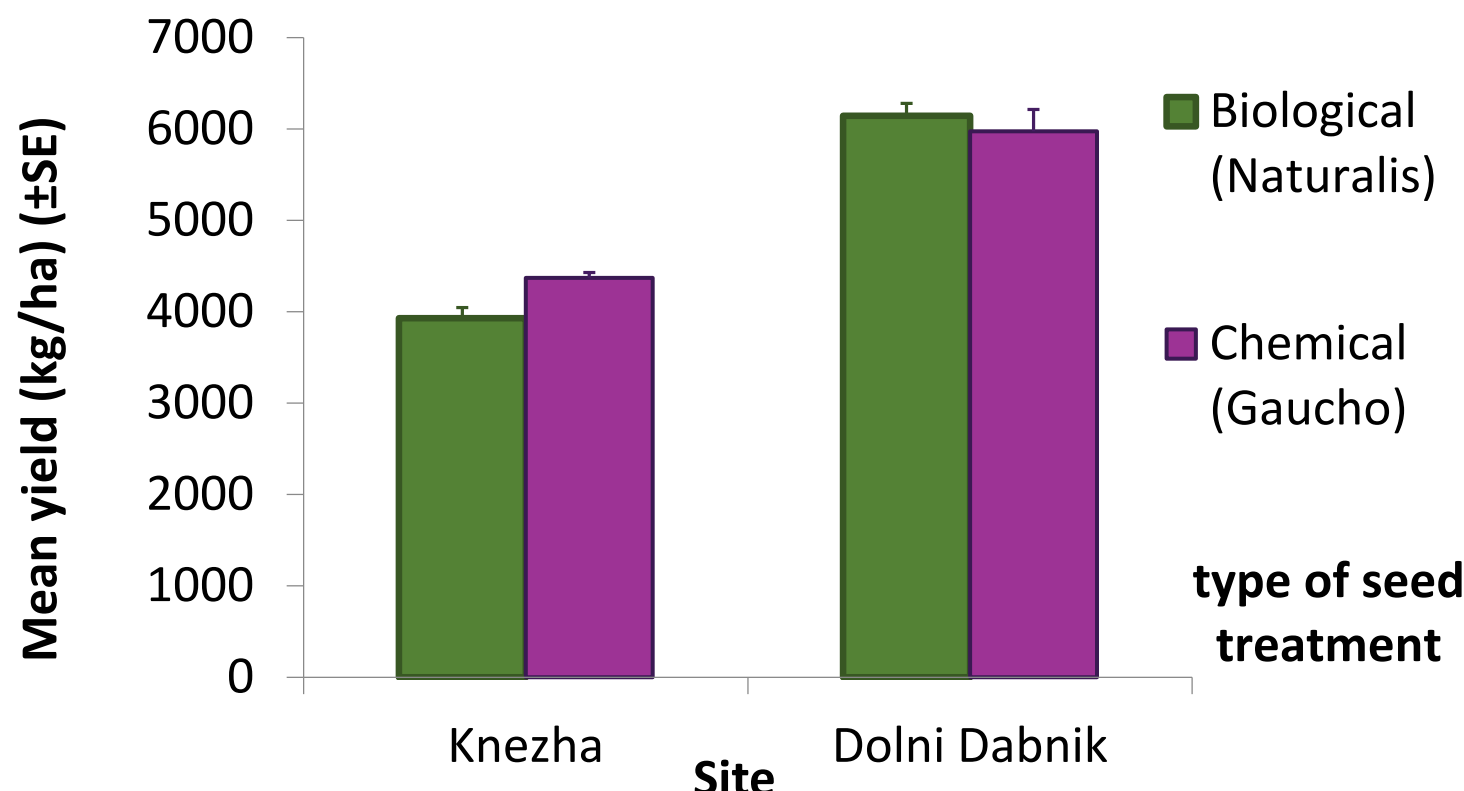
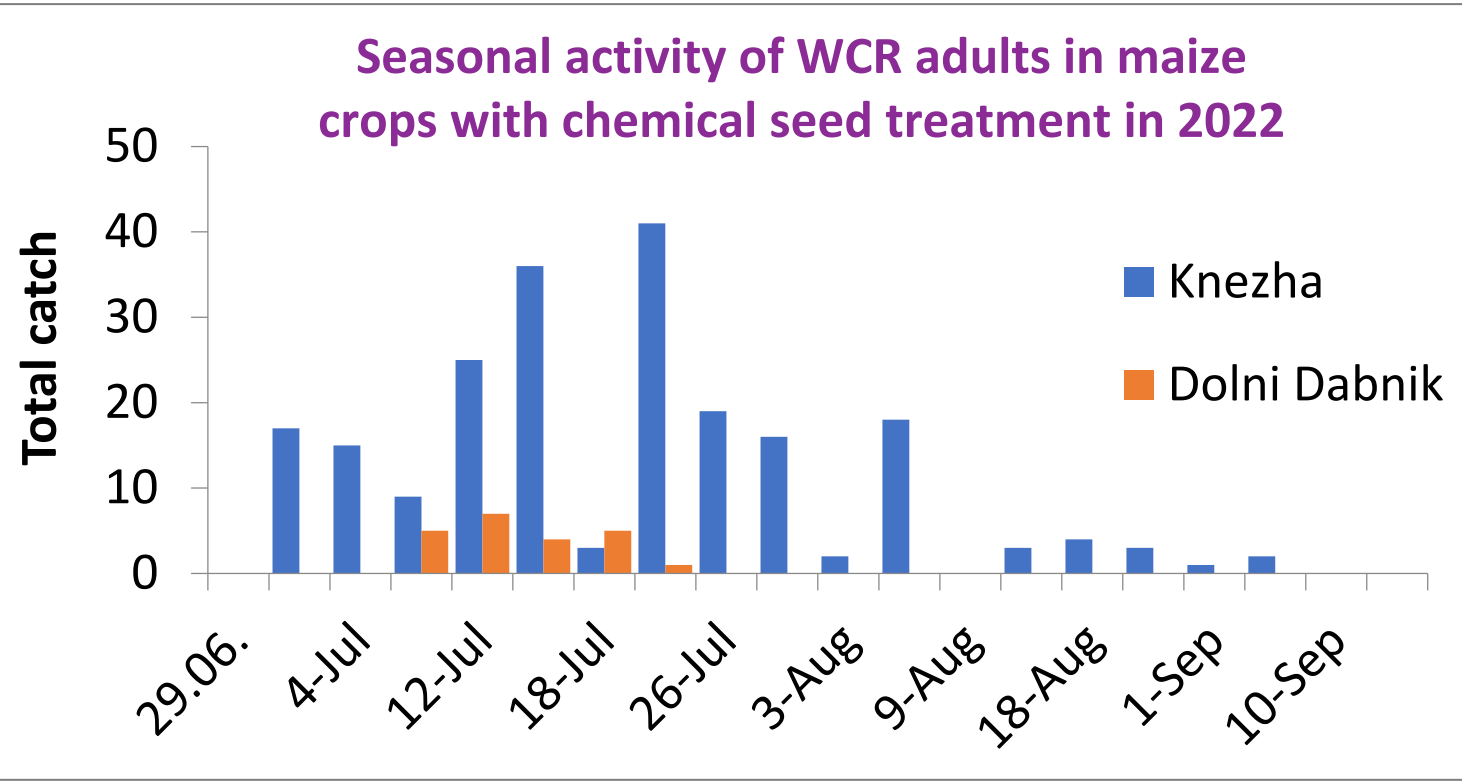
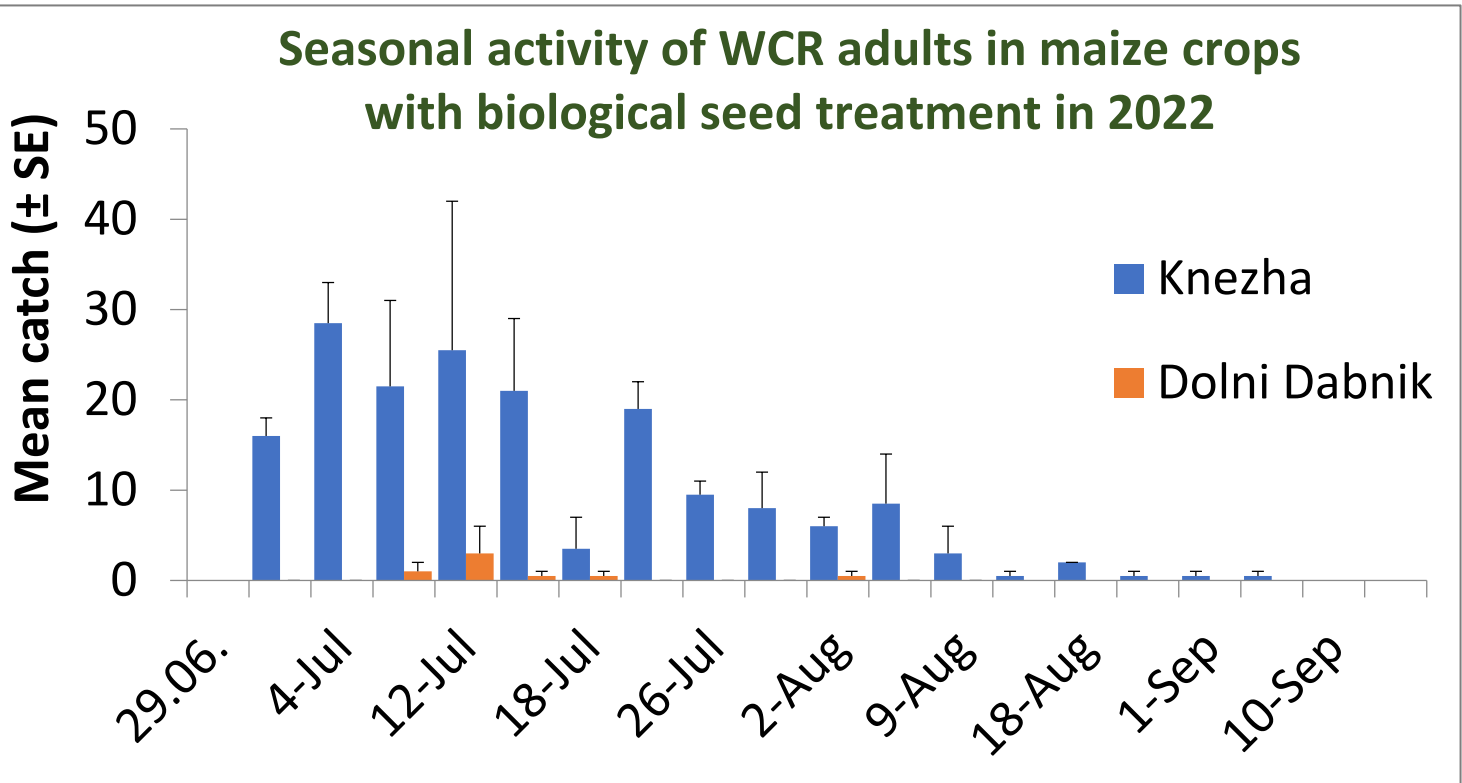
Treatment with the highest conidia concentration (2.3×10^7 conidia/ml) reached LT_{50} for 2.13 min (95% CIs: 0.56 to 8.15 min). The LT_{50} values ranged between 18 and 29 day for lower concentrations.

Field experiments in crops in northern Bulgaria where the WCR occurs – effect of type of seed treatment on yield of maize grain

- Two localities in 2022** – Knezha (maize hybrid Kn-317) and Dolni Dabnik (maize hybrid DEKALB® 4608) (Pleven region, Bulgaria);
- Two crops in each locality with different type of seed treatment practice – biological and chemical;
- Biological seed treatment** - Naturalis® (200 ml/100 kg seeds), biofungicide Mycohelp® (200 ml/100 kg seeds), biofungicide FytoSave 200 ml/100 kg seeds and the fertilizers Ekoprop® (100-200 g/100kg seeds), RhizoVital 42® (100 ml/100 kg seeds), Azotohelp® (50 ml/100 kg seeds), Bulhnova® (250 g/100 kg seeds) and Valtan® (100 ml/100 kg seeds);
- Chemical seed treatment** - Gaucho FS 600 (700 ml/100 kg seeds);
- No insecticide sprayings in the maize fields in 2022;
- Monitoring of the seasonal activity of WCR adults during the period of 29 June – 12 September, 2022 - two baited traps were set up in maize fields with Naturalis® treated seeds, and one baited trap was set up in maize fields, where the seeds were treated with Gaucho FS 600.
- The maize was harvested at full maturity. Maize yield was calculated based on cob weight. The grains obtained from cobs in the fields were corrected according to Shanin (1977), and the grain yield (kg/ha) was calculated.



Csalomon® KLP trap baited with dual (pheromone and floral) lures and catches of WCR adults in maize crop, Knezha



Grain yield (kg/ha) in maize crops with different type of seed treatment in Knezha and Dolni Dabnik, Bulgaria

CONCLUSIONS

- Conidial suspensions of *Beauveria bassiana* strain ATCC 74040 (Naturalis®) had moderate or low effect on mortality of *D. v. virgifera* adults under laboratory conditions. Only undiluted bioproduct caused very high mortality of the tested insects.
- In 2022, the periods of the seasonal activity of WCR adults were from the end of June to the beginning of September and from the beginning of July to the beginning of August in Knezha and Dolni Dabnik, respectively. Longer activity period was observed in Knezha, where the population density of the pest was higher. Adult abundance in maize fields with different type of seed treatment was similar.
- The two-factorial ANOVA (treatment and locality as factors) revealed a significant effect of locality and interaction between the type of seed treatment and locality/maize hybrid on the grain yield of maize. However the type of seed treatment (biological and chemical) had no significant effect of maize yield.
- Further study of the effect of Naturalis® on WCR larvae is required.