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Short communication

Pyrrolizidine alkaloid plant species in the area of large-scale chamomile cultivation in Croatia

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Introduction

Agriculture in Croatia still holds a high economic significance despite a declining trend. In Virovitica-Podravina county (Continental Croatia) 58% of the total land area is used for arable agriculture. Although cereals account for 41.3% of production in the county, the production of chamomile has increased rapidly in recent years and currently accounts for 6.6% of total production in the county (CROSTAT, 2019).

Pyrrolizidine alkaloids (PAs) are produced by numerous plant species (mainly within plant families Boraginaceae, Asteraceae, and Fabaceae) and are widespread. A great number of PAs are not only hepatotoxic but also carcinogenic, mutagenic and teratogenic. They can contaminate crops and therefore cause health problems both in humans and livestock (Moreira et al., 2018). In Croatia, species from the genera *Myosotis* L., *Senecio* L., and *Symphytum* L. are common weeds in agriculture and can cause challenges for farmers when exporting agricultural products due to EU regulations (EFSA, 2011).

The aim of this study was to determine the presence of PA plants in the area of organic chamomile cultivation (both within the crops and in their immediate proximity) and employ spatial analyses in order to evaluate the level of contamination.

Materials and methods

The survey of the PA plant species was conducted from March to August 2018 on the area of 30 hectares in the vicinity of Lozan (northern part of Virovitica-Podravina county, Croatia). The research area was divided into 5x5m plots (135 plots in total). PA species were mapped using the Garmin Etrex Vista GPS device. Plant species were identified using floras and standard identification keys (Domac, 2002; Rothmaler, 2009).

For recorded PA plant species (where it was possible) spatial centrographic analysis was conducted in order to describe the spatial distribution of the species. The global intensity of the species was calculated as a ratio of recorded plants to the research area studied. Local density was calculated as well for each of the 135 plots applying the formula for global intensity. Finally, the index of dispersion was calculated as a ratio of variance to the mean of the recorded number of plants. All analyses were performed using the 'spatstat' package for R and QGIS 2.18 software.

Results and discussion

Three species containing PAs were found: *Myosotis arvensis* (L.) Hill, *Symphytum officinale* L., and *Symphytum tuberosum* L. The most widely distributed of the three species in the researched area was *M. arvensis*, with 1,259 individuals recorded predominantly on meadows (949 individuals) and areas under chamomile cultivation (312 individuals). The other two species were recorded in far lesser numbers, 10 individuals of *S. officinale* (seven on the edges of cultivation areas and three in the canal) and a single plant of *S. tuberosum* in the forest habitat.

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The global intensity for *M. arvensis* in the researched area was 3,730 individuals/km^2, 30 individuals/km2 for *S. officinale* and three individuals/km^2 for *S. tuberosum*. The local intensity of *M. arvensis* varied from 0 individuals/km^2 (58 plots) to 62,400 individuals/km^2. The hotspots of *M. arvensis* distribution were found on the meadows surrounding the cultivation areas. An increased number of individuals were also recorded in the western part of the researched area, along the farm track. Based on the results of the spatial analyses we can infer that the source of the *M. arvensis* contamination is the meadows surrounding the chamomile cultivation areas. The species dispersal to the agricultural surfaces is mitigated by both wildlife and human activity in the form of agricultural workers (Strykstra et al., 1997). The intensity drops towards the eastern part of the research area (forest habitat). The index of dispersion for *M. arvensis* was 50.49 which additionally confirms the aggregation of the species on before mentioned areas. *S. officinale* was distributed mainly along the edges of the research area so it remains unclear whether these individuals are a part of a larger population beyond the research area or remnants of former populations prior to conversion of meadows into farmlands.

Preventing and reducing PA contamination of food and feed is a challenge for agriculture and an objective of various research projects and studies (BfR, 2015). One of the important factors is weed management following a combination of agricultural, mechanical, and chemical methods (THIE, 2018). However, the total eradication of PA-containing plants is not technically possible nor ecologically desirable.

Conclusion

Three Pyrrolizidine alkaloid (PA) species were recorded in the survey conducted near Lozan (northern part of Virovitica-Podravina county, Croatia): *M. arvensis*, *S. officinale*, and *S. tuberosum*. *M. arvensis* was most widely distributed (1,592 individuals recorded), followed by *S. officinale* (10 individuals recorded) and *S. tuberosum* (single record of the species in the forest habitat). Spatial analyses revealed local hotspots of *M. arvensis* on meadows and along the farm track used to access the cultivation areas from where it gradually spreads to surrounding chamomile fields indicating anthropogenic factors in this species dispersal.

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