# Finding a needle in a haystack: case-control studies can identify measures to prevent weeds in grassland

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#### Abstract

Prevention is essential to effectively manage weeds in grasslands. Yet, the identification of preventive measures can be tedious because numerous interacting management and environmental factors can influence grassland infestation by a weed. Case-control studies carried out on-farm allow a risk value to be ascribed to potential factors. We conducted a paired case-control study to assess the risk of the occurrence of *Rumex obtusifolius* in intensively managed permanent grasslands. Following a common protocol, in Switzerland, Slovenia and United Kingdom, parcels of land with high density of *R. obtusifolius* were compared with nearby parcels that had very few or no *R. obtusifolius* plants. Measured parameters included data about management practices, vegetation, and soil nutrients and texture. Analysed with multiple logistic regression, we showed that increased vegetation cover reduced the relative risk of *R. obtusifolius* occurrence while increased soil phosphorus and high soil bulk density raised the risk. Each of these effects was of comparable size across countries, as no interactions between country and any of the factors were observed. We conclude that case-control studies are a suitable tool to identify factors driving the infestation risk of grasslands regarding weeds such as *R. obtusifolius*. Results were achieved under the conditions of applied management, sometimes lasting for more than 10–20 years and allowed for direct recommendations to integrated weed management.

Keywords: integrated weed management, vegetation cover, soil P content, soil K content, soil bulk density, indicator species

## Introduction

Weeds are a major problem for forage production and quality in intensively managed, temperate grasslands. The management and the environment can influence weed infestation of grasslands; however, given the wide variety of farm practices, identifying preventive measures through manipulative experiments can be very laborious. On-farm case-control studies are an effective tool for identifying risk factors associated with management practices that favour a particular weed, by comparing parcels with high weed density (cases) with nearby parcels with very low weed density (controls). This type of study was used in medicine as early as the 1950s to investigate diseases (Doll and Hill, 1950) and has later been adopted to grassland systems to study weeds (Suter et al., 2007; Suter and Lüscher, 2008). In the current study, a case-control design was chosen to assess the risk factors that make productive grasslands prone to infestation with broad-leaved dock (*Rumex obtusifolius* L.). Known as one of the most problematic weeds in European temperate grasslands, R. obtusifolius is able to tolerate frequent defoliation once it is fully established (Niggli et al., 1993). R. obtusifolius produces large amounts of long-lived seeds that can contribute to a seed bank in the topsoil (Suter et al., 2023) from which the species can potentially recruit for many years. Based on previous studies, we identified factors that could potentially influence the occurrence of R. obtusifolius and defined a set of variables to be measured. The objective was to identify management practices and environmental factors that affect the risk of *R. obtusifolius* occurrence with the aim of improving strategies for the integrated weed management of the species.

# Materials and methods

Following a common protocol, a case-control study was conducted in Switzerland (CH), Slovenia (SI) and the United Kingdom (UK) during the 2019-2020 growing seasons. The study was carried out onfarm by comparing parcels of land with high density of *R. obtusifolius* (case) with nearby parcels free of or with very few *R. obtusifolius* plants (control). Forty (CH), 20 (SI), and 18 (UK) pairs of parcels were sampled per country. Parameters measured included data about the environment, management practices, soil nutrients and texture, and vegetation. The influence of the recorded variables on the occurrence of *R. obtusifolius* was analysed using multiple logistic regression and forward selection, the response variable being the presence or absence of *R. obtusifolius* in high density, equivalent to case-control parcels (Agresti, 2002). Further details on design and analysis can be found in Klötzli *et al.* (2023).

#### **Results and discussion**

The risk of the occurrence of *R. obtusifolius* in high densities was explained by three factors: percentage vegetation cover (measured by the line-point intercept method), soil P content ( $P_{Olsen}$ ), and soil bulk density (Table 1; factors were not correlated to each other). Increase in vegetation cover by 10%, for example from 80 to 90%, reduced the relative risk of *R. obtusifolius* occurrence to about half (Table 1). The two other variables raised the relative risk: an increase in  $P_{Olsen}$  of 13 mg kg<sup>-1</sup> (the mean difference between case and control parcels) resulted in a relative risk of 1.24, and an increase in soil bulk density of 0.1 g cm<sup>-3</sup> in a relative risk of 1.32 (Table 1). Despite differing soil conditions and a gradient of climate from Atlantic to continental, these effects were consistent across countries, indicated by non-significant interaction terms (P>0.2 each) between country and the three selected variables. Based on the selection procedure, no other recorded variable, such as soil pH, land-use intensity, or soil clay content, explained the occurrence of *R. obtusifolius*.

In this on-farm study, the factors influencing *R. obtusifolius* (vegetation cover, soil phosphorus content and soil bulk density) were all related to non-adapted management (soil bulk density most likely so), either directly or indirectly, such as unbalanced fertilisation or increased soil compaction. Importantly, these factors can be seen as the result of medium- and long-term processes of more than 10-20 years, acting much longer than a typical grassland experiment of less than five years.

Variable	df	χ2	P value	<b>Relative risk</b>
Country <sup>a</sup>	2	2.02	0.364	-
Vegetation cover	1	9.07	0.003	0.56 <sup>b</sup>
P <sub>Olsen</sub>	1	7.63	0.006	1.24 <sup>c</sup>
Soil bulk density	1	4.20	0.040	1.32 <sup>d</sup>

Table 1. Variables with significant effects on the relative risk of the occurrence of *R. obtusifolius* in high density in grasslands of Switzerland, Slovenia, and United Kingdom.

Variables were tested with forward selected using a generalised linear model with a logit link function. Terms added sequentially (first to last) given country; only variables that lowered

the AICc (Akaike Information Criterion) upon inclusion in the model are given.

<sup>a</sup>Inference of 'Country' based on single term deletion from the final model.

<sup>b</sup>Relative risk for an increase of vegetation cover by 10%.

<sup>c</sup>Relative risk for an increase of soil P<sub>Olsen</sub> by 13 mg kg<sup>-1</sup> (mean difference between case and control parcels).

<sup>d</sup>Relative risk for an increase of soil bulk density by 0.1 g cm<sup>-3</sup> (mean difference between case and control parcels)

The case-control approach proved very effective in identifying risk factors among a multitude of management and environmental factors likely to influence the infestation of intensively managed, permanent grasslands by *R. obtusifolius* (under conditions practiced over several years). Moreover, this type of design has the advantage of allowing a combination of management and environmental conditions to be tested that would be difficult to establish in a manipulative experiment. However, given the potential correlations between explanatory variables under practical conditions, causal interpretations in case-control studies must be made with caution.

#### Conclusion

For successful control of *R. obtusifolius* in intensively managed grasslands, preventive measures combined with direct control measures are decisive (Schaffner *et al.*, 2022). The case-control study provided evidence for the role of medium- to long-term factors driving the infestation of grasslands by *R. obtusifolius*, and all factors were indicative of poor management practices. Preventive strategies can be implemented through appropriate management, for example by adapting fertilisation to the needs of the forage plants, minimising soil compaction, and promoting dense and competitive swards.

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