

## Article

# Evaluation of the *Spiranthes spiralis* Population's Phenotypic Density in the Plant Community of *Medicagini minima*–*Festucetum valesiaca* Meadows in the Natura 2000 Jiului Corridor Site, Romania (ROSCI0045)

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**Abstract:** There are few reports related to the presence of *Spiranthes spiralis* in the Oltenia region in southern Romania. This study develops the information concerning the presence of this type of orchid in Gorj County. The studies on the populations of this orchid species have been conducted in the Natura 2000 site of the Community ROSCI0045 Jiului Corridor. In the researched territory, during the investigations, twelve populations of *Spiranthes spiralis* were sampled for the first time. In 2022–2023, we studied the distribution of this species and assessed its population densities. Our observations indicate that it is a relatively widespread species in the studied area and its populations are more abundant in meadows, in the *Medicagini minima*–*Festucetum valesiaca* plant community.

**Keywords:** population; density; phenotypic characters; *Spiranthes spiralis*; plant community; coenology; undesirable species



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## 1. Introduction

The *Orchidaceae* family is one of the richest families in the plant kingdom and it includes approximately 28,000 species and 880 genera [1]. The species and subspecies of this family are present on all continents, the most important centers of their diversity being Indochina, Southwest Australia, Europe, Northern Asia, and North America [2]. About 250 species and subspecies of 35 orchid genera have been identified in Europe [3], but they are not uniformly distributed throughout the area. The plant is widely distributed in Southern Europe, in the Mediterranean region, where it can be found in suitable open habitats. In Romania, 58 species of wild orchid exist, all terrestrial, widespread in meadows, swamps, forest edges, and clearings [4].

*Spiranthes spiralis* (L.) Chevall. is a small, beautiful, pleasant-smelling perennial orchid, included in the National Red List as a rare plant since 1994 [5]. It is a perennial geophytic species, with a height of 10–20 cm, rare in the sessile oak and beech forests' grassy layer, in meadows, and the edge of the woods [4]. In the NATURA 2000 site Sighișoara–Târnava Mare (ROSCI0027), it was identified for the first time in Mălâncrav (Laslea commune, Sibiu County, at the end of June 2011), in two types of dry meadows [6,7]. *Spiranthes spiralis* occasionally occurs in the meadows in Gheboieni, Gorgota, Bucani, and Mija [8]. In floristic investigations conducted in the Grădișteța Muncelului Cioclovina Natural Park, we identified 30 species of orchids belonging to 11 genera, including *Spiranthes spiralis* [9]. The species is rare in the Moldova province, being recorded in the Neamț and Bacău counties (Cașin Monastery) [10]. In the Oltenia, *Spiranthes spiralis* has a limited spread: in the Olteț river basin, it was only collected from Gârnicet Șuiștei village [11] and near Cărbunari





Table 1. Cont.

No. of Relevée	1	2	3	4	5	6	7	8	9	10	11	12
<i>Reseda lutea</i>	+	-	+	+	-	-	+	+	-	-	+	+
<i>Falcaria vulgaris</i>	-	-	-	-	-	+	+	-	-	-	-	-
<i>Lotus corniculatus</i>	-	+	-	-	-	+	+	+	-	+	+	-
<i>Thymus pannonicus</i>	+	+	+	+	-	-	+	+	+	+	+	+
<i>Astragalus onobrychis</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>Senecio jacobaea</i>	-	-	-	-	-	+	-	-	-	-	-	-
<i>Achillea setacea</i>	-	+	-	-	-	+	+	+	-	+	+	-
<i>Onobrychis viciifolia</i>	-	-	-	-	-	+	-	-	-	-	-	-
<i>Muscari tenuiflorum</i>	-	-	-	-	-	+	+	-	-	-	-	-
<i>Linum perenne</i>	+	+	+	+	-	-	+	+	+	+	+	+
<i>Fragaria viridis</i>	+	+	+	+	-	-	-	+	+	+	+	+
<i>Anthemis tinctoria</i>	+	+	+	+	-	-	+	+	+	+	+	+
<i>Chamaecytisus austriacus</i>	+	+	+	+	-	-	+	+	+	+	+	+
<i>Potentilla arenaria</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>Medicago minima</i>	+	+	+	+	-	-	-	+	+	+	+	+
<b>Festuco–Brometea</b>												
<i>Galium verum</i>	+	+	-	+	-	-	-	+	-	+	+	+
<i>Echium vulgare</i>	+	+	+	-	+	+	+	-	-	-	-	+
<i>Stachys germanica</i>	+	+	+	+	-	+	+	+	+	+	+	+
<i>Coronilla varia</i>	+	+	+	+	-	-	-	+	+	+	+	+
<i>Berteroa incana</i>	-	+	-	-	-	+	+	+	-	+	+	-
<i>Potentilla argentea</i>	+	+	-	+	-	+	-	+	-	+	+	+
<i>Eryngium campestre</i>	+	-	-	+	-	+	+	-	-	-	-	+
<i>Agrimonia eupatoria</i>	+	-	-	+	-	+	+	-	-	-	-	+
<i>Scabiosa ochroleuca</i>	+	-	-	+	-	+	-	-	-	-	-	+
<i>Asperula cynanchica</i>	+	-	-	+	+	-	-	+	+	+	+	+
<i>Hypericum perforatum</i>	+	+	+	+	-	+	+	+	+	+	+	+
<i>Euphorbia cyparissias</i>	+	+	+	+	-	-	-	+	+	+	+	+
<i>Hieracium pilosella</i>	+	-	+	-	+	-	+	+	-	-	+	+
<i>Trifolium campestre</i>	+	-	+	+	-	+	+	-	-	-	-	-
<i>Salvia verticillata</i>	+	+	-	+	-	-	+	-	-	-	-	+
<i>Achillea collina</i>	-	-	+	+	+	-	+	-	-	-	-	-
<i>Potentilla recta</i>	-	-	+	+	-	+	-	-	+	-	-	-
<i>Agrostis capillaris</i>	-	+	-	-	+	-	-	+	-	+	+	-
<b>Molinio–Arrhenatheretea</b>												
<i>Spiranthes spiralis</i>	+	+	+–1	+–1	1	1	1–2	1–2	2	2–3	2–3	2–3
<i>Vicia carcca</i>	-	-	-	-	-	+	+	-	-	-	-	-
<i>Trifolium repens</i>	+	-	-	+	-	-	+	-	-	-	-	+
<i>Taraxacum officinale</i>	-	-	-	-	+	+	+	-	+	+	-	-
<i>Lolium perenne</i>	-	-	+	-	+	+	-	-	+	-	-	-
<i>Poa pratensis</i>	-	+	-	+	+	-	-	+	-	+	+	+

Table 1. Cont.

No. of Relevée	1	2	3	4	5	6	7	8	9	10	11	12
<i>Dactylis glomerata</i>	+	-	+	+	-	+	-	-	+	-	-	+
<i>Cichorium intybus</i>	-	-	+	+	-	+	+	-	-	-	-	-
<i>Plantago lanceolata</i>	+	-	-	+	-	-	+	-	+	-	-	+
<i>Ononis arvensis</i>	-	-	+	+	+	-	+	-	-	+	+	-
<i>Rhinanthus minor</i>	+	+	+	-	+	+	+−1	-	+	+−1	+	+
<i>Achillea millefolium</i>	-	+	-	-	-	-	-	+	-	+	+	-
<i>Ranunculus repens</i>	+	+	-	-	-	+	-	-	-	-	-	-
<i>Trifolium fragiferum</i>	-	-	-	-	-	+	-	-	-	-	-	-
<i>Prunella vulgaris</i>	+	+	+	+	-	+	+	-	+	+	-	-
<i>Veronica chamaedrys</i>	+	-	-	+	-	-	+	+	+	-	-	+
<i>Centaurea indurata</i>	+	+	+	+	-	-	+	-	-	+	+	-
<i>Verbena officinalis</i>	-	+	+	-	-	+	-	-	-	-	-	-
<i>Leucanthemum vulgare</i>	+	+	+−1	+	+−1	-	+	+	+	+	+	+
<i>Potentilla reptans</i>	-	+	+	+	-	+	-	+	+	+	-	+
<i>Stachys officinalis</i>	+	+	+	+	-	-	-	+	+	+	+	+
<i>Inula britannica</i>	+	+	+	+	-	-	+	+	+	+	+	+
<i>Eryngium planum</i>	-	+	+	+	-	+	-	-	-	-	-	-
<i>Polygonum aviculare</i>	+	+	+	+	-	-	-	+	+	+	+	+
<b>Stellarietea mediae</b>												
<i>Lathyrus tuberosus</i>	+	+	+	+	-	-	+	+	-	-	-	-
<i>Anagallis arvensis</i>	+	+	-	-	+	-	-	-	-	-	-	-
<i>Carduus acanthoides</i>	-	-	-	-	-	+	+	-	-	-	-	-
<i>Matricaria perforata</i>	+	-	-	+	-	-	+	-	-	-	-	+
<i>Linaria vulgaris</i>	-	+	+	+	-	-	+	-	-	-	-	-
<i>Bromus arvensis</i>	-	-	+	-	-	+	-	-	+	-	-	-
<i>Xanthium strumarium</i>	-	+	-	-	+	-	-	+	-	+	+	-
<i>Cardaria draba</i>	+	-	+	+	-	+	-	-	+	-	-	+
<i>Consolida ragalis</i>	-	-	-	-	-	+	+	-	-	-	-	-
<i>Picris hieracioides</i>	+	-	-	+	-	-	+	-	+	+	-	+
<i>Lepidium campestre</i>	-	-	+	+	+	-	+	-	-	-	-	-
<i>Erodium cicutarium</i>	+	+	+	+	-	-	-	+	+	+	+	+
<i>Lappula squarrosa</i>	+	+	+	+	-	-	+	+	+	+	+	+
<i>Lactuca serriola</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>Malva sylvestris</i>	-	-	-	-	-	+	-	+	+	+	-	-
<i>Capsella bursa-pastoris</i>	+	+	+	+	-	-	-	+	+	+	+	+
<i>Senecio vernalis</i>	+	+	+	+	-	-	+	+	+	+	+	+
<i>Stellaria media</i>	-	-	-	+	+	+	-	-	-	+	+	+
<b>Variae Syntaxa</b>												
<i>Dianthus armeria</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>Cirsium canum</i>	-	-	+	-	-	+	+	+	+	-	-	-
<i>Rubus canescens</i>	3	3	2–3	2–3	2	1–2	1–2	1–2	1	1	1	1

Table 1. Cont.

No. of Relevée	1	2	3	4	5	6	7	8	9	10	11	12
<i>Rosa canina</i>	-	+	-	-	+	+	+	+	-	+	+	-
<i>Crataegus monogyna</i>	-	+	+	+	-	-	-	+	-	+	+	-
<i>Xanthium orientale</i>	-	+	-	-	+	+	+	+	-	+	+	-
<i>Althaea officinalis</i>	-	+	-	-	-	-	-	+	+	+	+	-
<i>Ambrosia artemisiifolia</i>	1	1	+−1	-	-	-	+	+−1	+	+	+	+
<i>Erigeron annuus</i>	-	+	-	1	+	+	+	+	1	+	+	-
<i>Conyza canadensis</i>	+	+	+	+	+	+	3	3	3	-	-	-
<i>Pyrus pyraister</i>	+	+	+	-	1	1	+	+	+	+	+	+
<i>Prunus spinosa</i>	+	+	+	-	-	-	1	1	1	+	+	+

**Place and data of the relevées:** Hill of Bran, Urdari, 23.V.2021; 12.VI.2022; 18.VII.2023. Source: performed by the authors based on their own research.



**Figure 2.** *Spiranthes spiralis* in meadows, from Urdari © Mariana Niculescu.

In addition to the characteristic species of the plant communities *Festuca valesiaca*, *Medicago lupulina*, *M. falcata*, and *M. minima*, most species belong to the classes of Festuco–Brometea, Molinio–Arrhenatheretea, and Stellarietea mediae. This plant community belongs to an important Natura 2000 grassland habitat: the 6240\* Sub-Pannonic steppe grasslands [24].

In the structure of the phytocoenoses analyzed, many plants were ruderal, segetal, and invasive, providing them with an anthropophilic character. Among the invasive species identified in the phytocoenoses floristic composition, the most frequently encountered were *Xanthium orientale*, *Erigeron annuus*, *Ambrosia artemisiifolia*, and *Conyza canadensis*.

A number of species are considered undesirable and affect the conservation status of these meadows, such as *Rosa canina* (Figure 3), *Pyrus pyraister* (Figure 3), *Rubus canescens*, and *Crataegus monogyna*. In particular, *Rubus canescens* (Figure 4) presented exacerbated development within the analyzed phytocoenoses; it had a high abundance–dominance values and it affected both the conservation status of the meadow and populations of *Spiranthes spiralis*. Thus, in the phytocoenoses where *Rubus canescens* was established and developed excessively, the populations of *Spiranthes spiralis* were very low, with a maximum of six individuals. *Rubus canescens* developed in some phytocoenoses to such a great extent that it formed a tangle of branches that could not be penetrated by the other species. Also, in the structure of phytocoenoses, other shrubby species, such as *Rosa canina*, *Pyrus pyraister*, and *Crataegus monogyna*, influenced the development and conservation state of the plant community indirectly of the habitat. This can be explained by the lack of mowing and maintenance work required for these meadows and their abandonment by the locals. Regarding the phytocoenoses of the meadows under study, they are located on soils that lack moisture during summer and they develop in conditions of xerophytism with temperatures that can frequently reach up to 40 °C during summer, favoring high

evaporation rates of surface water. The lack of mowing and the abandonment of these meadows has led to the establishment of bushes and a syndynamic evolution into forest vegetation. These results, corroborated with the lack of necessary studies on these meadows, overgrazing, the presence of invasive species, and the frequent use of all-terrain vehicles by the locals, have led to the irreversible degradation of the habitat and the populations of *Spiranthes spiralis* (Figure 5). Although the populations of this species are still high, the gradual degradation of the habitat, especially through the installation of *Rubus canescens* thickets, can destroy them.



**Figure 3.** *Rosa canina* and *Pyrus pyranthes* in the habitat of *Spiranthes spiralis*, Urdari © Mariana Niculescu.



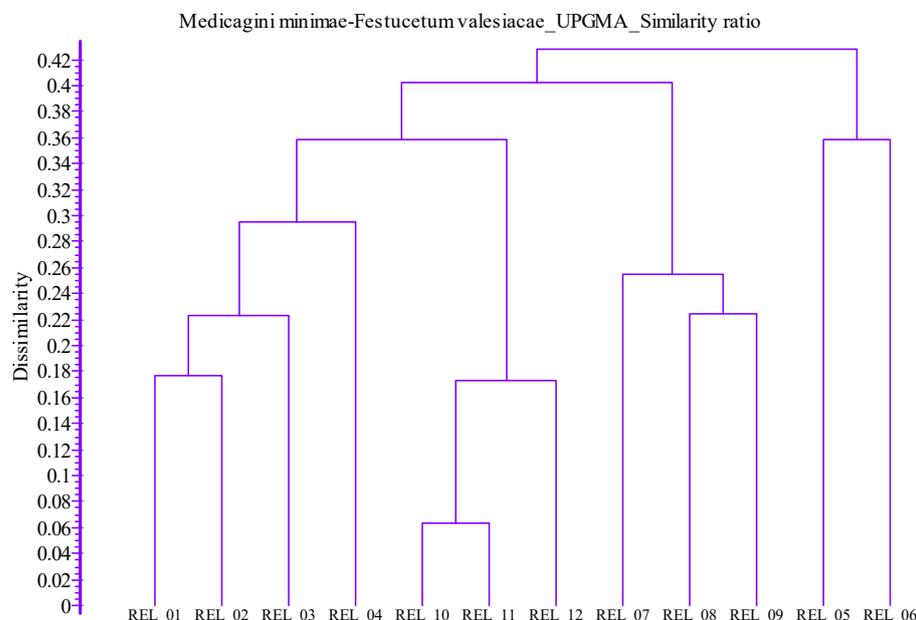
**Figure 4.** *Rubus canescens* in the habitat of *Spiranthes spiralis*, Urdari © Mariana Niculescu.



**Figure 5.** *Spiranthes spiralis* overwhelmed by *Rubus canescens*, Urdari © Mariana Niculescu.

From a coenotic point of view, from the analyzed phytocoenoses, it can be seen that this species shows abundance–dominance results that vary according to the ecological conditions, in close correlation with the anthropogenic factors. In some phytocoenoses, the undesirable species *Rubus canescens* influences the abundance–dominance of *Spiranthes spiralis* (Figure 6). We can also observe the grouping of two clusters: in the first cluster, the relevées 1, 2, 3, and 4 (REL\_01, REL\_02, REL\_03, and REL\_04, respectively) are highlighted, based on the greater abundance–dominance of the species *Rubus canescens* in

correlation with the low abundance–dominance of the studied species *Spiranthes spiralis*. The relevées 10, 11, and 12 are differentiated by the greater abundance–dominance of the species *Spiranthes spiralis*. Also, the relevées 7, 8, and 9 are differentiated by the great abundance–dominant of the invasive species *Conyza canadensis* and the undesirable shrub species *Prunus spinosa* that invade the meadow and suffocate the valuable species in the floristic structure, including *Spiranthes spiralis*.



**Figure 6.** Dendrogram of the *Medicagini minimae–Festucetum valesiacae* plant community; similarity ratio coefficients.

The second cluster was formed by separating two relevées, 5 and 6 (REL\_05, REL\_06, respectively), based on the abundance of the larger dominant shrub species *Pyrus pyraeaster*.

The analysis conducted highlighted the negative impact of shrub and shrub species as well as invasive species on *Spiranthes spiralis* populations and also on the meadow community where it was found.

### 3.2. Statistical Analysis of the Morphological Traits of *Spiranthes spiralis*

The studies were conducted taking into account the correlation with the population density.

In relation to the height of the plant, the highest mean value was recorded in the surfaces smaller than 15 pl./m<sup>2</sup>, which was significantly distinguished from all other mean values. The second-highest mean value was recorded at densities of 25–35 pl./m<sup>2</sup>, which was still significantly higher than those found at densities ranging from 40 to 100 pl./m<sup>2</sup> (Table 2).

**Table 2.** Analysis of the influence of the density of pl./m<sup>2</sup> on the variability of *Spiranthes spiralis*'s morphometric characteristics. LSD (0.05) Least significant difference at 5% probability level, values with same letters are statistically insignificant for the same variable.

Density (pl./m <sup>2</sup> )	Characteristic	Vegetation Height (cm)	Vegetation Height (cm)	Mean Inflorescence Length (cm)	Mean Plant and Inflorescence Length/Height Ratio
>100 pl./m <sup>2</sup>		16.019 <sup>bc</sup> ± 2.51	10.701 <sup>b</sup> ± 1.25	5.150 <sup>d</sup> ± 0.22	0.72 <sup>a</sup> ± 0.05
45–55 pl./m <sup>2</sup>		15.071 <sup>c</sup> ± 2.75	12.321 <sup>b</sup> ± 1.29	5.782 <sup>c</sup> ± 0.21	0.661 <sup>b</sup> ± 0.04
25–35 pl./m <sup>2</sup>		21.047 <sup>b</sup> ± 3.27	15.66 <sup>a</sup> ± 1.14	6.573 <sup>b</sup> ± 0.25	0.619 <sup>b</sup> ± 0.04
<15 pl./m <sup>2</sup>		47.502 <sup>a</sup> ± 5.11	16.267 <sup>a</sup> ± 1.34	7.521 <sup>a</sup> ± 0.33	0.476 <sup>c</sup> ± 0.04
LSD 5%		5.48	2.31	0.59	0.058

Regarding the characteristic of the mean length of inflorescence, the highest value was recorded on surfaces smaller than 15 pl./m<sup>2</sup>, this being 7.52 cm, a value that was significantly higher than all the others. The second average value was recorded on the surfaces with of 25–35 pl./m<sup>2</sup>, a value that was also significantly higher than the others. A significant difference was recorded for the last two value averages calculated for surfaces of 45–55 pl./m<sup>2</sup> and those larger than 100 pl./m<sup>2</sup>.

For the analysis of the inflorescence length and total height ratio index, the highest mean was recorded at densities greater than 100 pl./m<sup>2</sup>, which was significantly greater than the means at other densities.

Certain correlations were seen for the height of the plant and the height of *Spiranthes* (Figures 7–10). Thus, the highest value was calculated for a density of 15 pl./m<sup>2</sup>. In other words, increasing the height of the plant resulted in an increase in the height of the *Spirantes* plants; basically, tall vegetation caused the *Spirantes* plants to etiolate or elongate in their search for light. In other instances, the values of the correlation coefficients were lower, the lowest value being calculated for a density of 105 pl./m<sup>2</sup>.

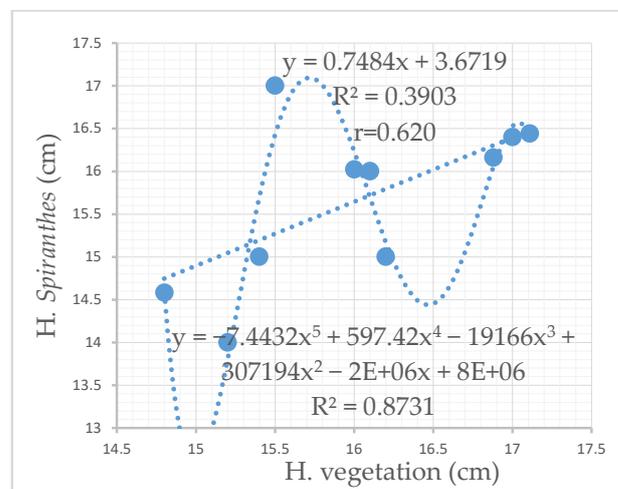


Figure 7. The correlation between plant height and *Spiranthes* height (density higher than 100 pl./m<sup>2</sup>).

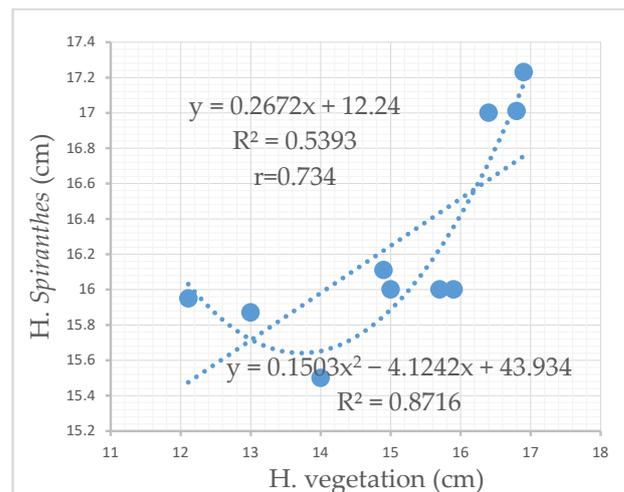
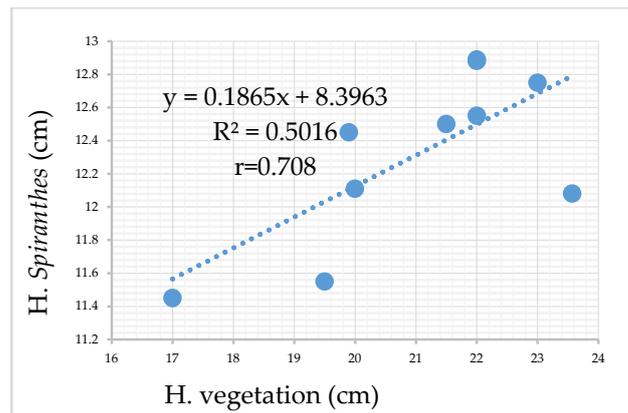
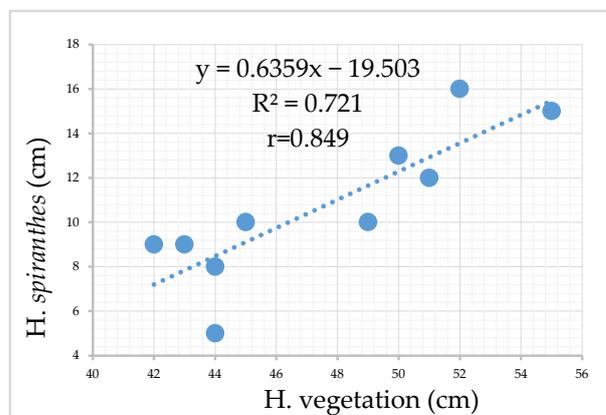


Figure 8. The correlation between plant height and *Spiranthes* height (density between 45 and 55 pl./m<sup>2</sup>).

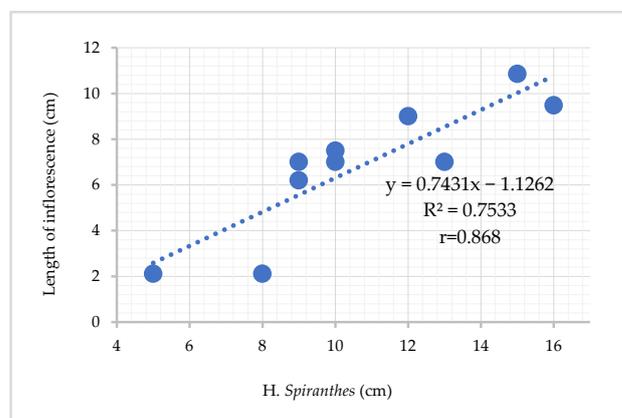


**Figure 9.** The correlation between plant height and *Spiranthès* height (density between 25 and 35 pl./m<sup>2</sup>).



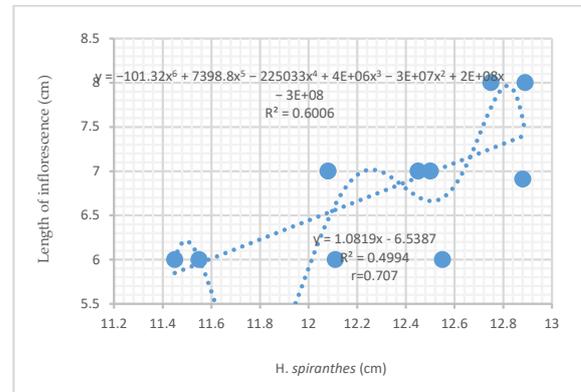
**Figure 10.** Correlation plant height and *Spiranthès* height (density of plants up to 15 pl./m<sup>2</sup>).

At densities higher than 100 pl./m<sup>2</sup>, the correlation between *Spiranthès* height and inflorescence length was 0.868. We assumed that, for this density, there was a very strong connection between the two traits. In other words, at a high density, over 100 pl./m<sup>2</sup>, the increase in the height of the plant also resulted in an increase in inflorescence. The calculated coefficient of determination was as high as 0.753, so the regression model based on the simple linear equation was a valid one. Thus, the regression coefficient was equal to 0.743, which meant that when the plant grew by 1 cm in height, the inflorescence increased by 0.743 cm (Figure 11).



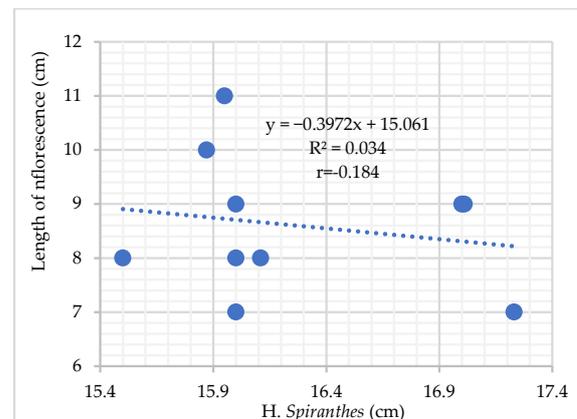
**Figure 11.** The correlation of inflorescence length and plant height (density higher than 100 pl./m<sup>2</sup>).

At densities between 45 and 55 pl./m<sup>2</sup>, the correlation coefficient was 0.703, a value that expressed a strong link between the two traits, but without being able to establish a trend model based on the simple linear equation, with the value of the coefficient of determination being 0.499 (Figure 12).

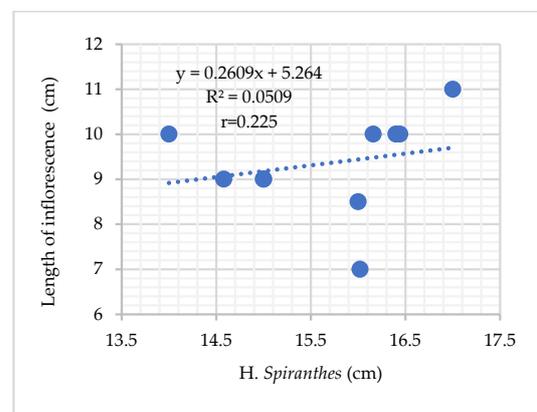


**Figure 12.** The correlation of inflorescence length and plant height.

In the case of surfaces with densities between 25 and 35 pl./m<sup>2</sup> and lower than 15 pl./m<sup>2</sup>, the correlation coefficients of the two traits presented values of 0.034 and 0.255, respectively, values much too low to be able to express a link between the two traits (Figures 13 and 14).



**Figure 13.** The correlation of inflorescence length and *Spiranthes* height (plant density between 25 and 35 pl./m<sup>2</sup>).



**Figure 14.** The correlation of inflorescence length and *Spiranthes* height (plant density up to 15 pl./m<sup>2</sup>).

#### 4. Discussion

The density of *Spiranthes* had a significant influence on the mean height of the vegetation. Thus, on surfaces with high and very high densities of *Spiranthes*, the vegetation had heights that were comparable with *Spiranthes* plants, while on surfaces with low and very low densities of *Spiranthes*, the average height of the vegetation was much higher than the height of the *Spiranthes* plants. Tall (and dense) herbaceous vegetation competes with orchids [25], which can be detrimental for the *Spiranthes* population.

The density of *Spiranthes* (pl./m<sup>2</sup>) also influences the correlation between the mean height of the vegetation and the height of individual *Spiranthes* plants. Thus, at low densities, the height of *Spiranthes* plants correlates very well with the height of the vegetation, in the sense that the increase in the height of the vegetation determines the increase in the height of *Spiranthes* plants. At high densities, the correlation between the two indices decreases, in the sense that the *Spiranthes* compete with each other, but less so with the vegetation.

Related to the average length of the inflorescences, this is strongly influenced by the height of the plant; tall plants usually have longer inflorescences. However, the correlation coefficient between the two traits is high, especially at high densities of *Spiranthes*, where the mean value of the inflorescence is low. The result is supported by the analysis of the ratio between the height of the inflorescence and the total height of the plant. Thus, shorter plants tend to form larger inflorescences compared to their total height, these plants being found at high densities. The density of the orchids on grassland patches correlates with the vegetation height [26]. However, regardless of the density of *Spiranthes*, the length of the inflorescence is more susceptible to modifications than the total height of the plant, this being a characteristic that is influenced by both the density of *Spiranthes* and the height of the vegetation. Thus, in low-density *Spiranthes* areas, tall vegetation determines the height of *Spiranthes* plants, which produces tall inflorescences compared to the inflorescences of shorter plants.

#### 5. Conclusions

The structure and floristic composition of the *Medicagini minima*–*Festucetum valesiacae* plant community as well as its conservation status have a direct influence on the population size of the *Spiranthes spiralis* species, but also on the development of individual plants, especially their height and vitality, and also on the state of conservation of the populations of orchid we studied.

With the abandonment of traditional agro-pastoral practices in recent decades, these meadows have degraded and natural landscapes have lost their value.

The scrubs of *Rubus canescens* and also those of the *Pruno-Crataegetum* type occupy larger areas, together with Turkey oak and sessile oak forests encroaching very rapidly on the deep eutric soils at the edge of grasslands.

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