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# 2. MATERIAL AND METHODS

## 2.1. The species investigated

The species group of *Scabiosa columbaria* is of southeuropean- westasiatic origin and consists of about 30 species. In the area of the Alps about a dozen species occur. Three widely distributed species have been choosen for the investigations:

- a. Scabiosa gramuntia L. (S. triandra L.). The species has a southwesteuropean distribution and grows in regions with a submediterranean climate. In the Alps it is restricted to the warmest central and southern alpine valleys. The distribution in Switzerland is shown in Fig. 1.
- b. Scabiosa columbaria L. The species has a central european distribution north



Fig. 1. Distribution map of species of *Scabiosa columbaria* s.l. in Switzerland and surroundings (according to LANDOLT 1971).

Verbreitungskarte von Scabiosa columbaria s.l. in der Schweiz und Umgebung (nach LAN-DOLT 1971).





....



S. portae



S. columbaria

S. lucida S. g

S.gramuntia

1

S. dubia

of the Alps from central France to western Russia and north to southern Scotland and southern Scandinavia. In Switzerland it is widely distributed at lower altitudes north of the Alps (Fig. 1). However, it is becoming more and more rare as a result of intensive fertilization of the meadows.

**Table 1.** Origin and original habitat of the species studied.

 Herkunft und Standort der untersuchten Arten.

- 1 from SCHUEPP (1960) for the stations Davos-Schatzalp, Zürich and Martigny-Bourg
- 2 from UTTINGER (1965) for the stations Davos-Schatzalp, Boppelsen and Martigny-Ville
- 3 from MÖRIKOFER (1932) for the stations Davos-Platz, Zürich and Sion. Sunny days are days with less than 2/10 cloudiness
- 4 from SCHUEPP (1962) for the stations Davos-Platz, Zürich and Sion
- 5 from KUHN (1973) for the stations Rigi, Zürich and Sion. The length of the dry period is defined as the number of days with less than 5 mm precipitation.

Remarks on the climate stations : The stations Davos-Platz and Davos-Schatzalp are situated in a more continental climate than the collection locality of *S. lucida* (Davos-Parsenn). The precipitations are about 25% higher. On the other hand, the station Rigi is more oceanic having a somewhat lower value of length of dry periods than Davos-Parsenn. However, all three stations show the tendency towards the habitat conditions of *S. lucida*. The other stations (Martigny and Sion for *S. gramuntia* and Zürich and Boppelsen for *S. columbaria*) show only minor differences in the actual climate at the habitat station.

S. lucida	S. columbaria	S. gramuntia
Grisons	Zürich	Valais
Davos	Boppelsen	Martigny
SE	SSE	ESE
30	25	40
1950	560	500
6.4	7.2	7.5-8.0
	0	
6.7	12.7	14.9
76	74	45
39	39	64
1162	1336	1484
a a - ar	215 B	
12.3	17.1	32.6
121	303	626
	<i>S. lucida</i> Grisons Davos SE 30 1950 6.4 6.7 76 39 1162 12.3 121	S. lucida         S. columbaria           Grisons         Zürich           Davos         Boppelsen           SE         30           25         1950           6.4         7.2           6.7         12.7°           76         74           39         39           1162         1336           12.3         17.1           121         303

c. *Scabiosa lucida* Vill. *S. lucida* is distributed in the mountains of central Europe: viz. Alps, Carpathians, northern Apennins and Jura. In Switzerland it occurs at higher altitudes (above 1500 m a.s.l.) (Fig. 1).

The origin and habitat of the investigated populations is shown in Table 1. For more details see LANDOLT et al. (1975, p. 88). CaCO<sub>3</sub> is present in all three types of soil, the nitrogen content being rather low (1.7-3.2 mg/100 g soil after incubation) of six weeks). The most characteristic differences in climate are the amount of precipitation and the temperature. *S. gramuntia* grows in a dry climate with warm summers, *S. lucida* in a humid one with cool summers. *S. columbaria* has intermediate climatic demands. The climatic differences of the habitats of the three species are also shown in the ecological indicator values of the different species (Table 2). The values for temperature and continentality are highest in *S. gramuntia* and lowest in *S. lucida*. The humidity value is lowest in *S. gramuntia*.

**Table 2.** Indicator values of the species investigated (from Landolt 1977).Zeigerwerte der untersuchten Arten.

Species	F	R	N	H	D	L	Т	K
S. gramuntia	1	3	2	3	3	4	5	4
S. columbaria	2	4	2	3	4	4	4	3
S. lucida	2	4	2	3	4	4	2	3

Table 3. Some characteristics of the species investi	gated.
Einige typische Eigenschaften der untersuchten Ar	ten.

Characteristics	S. gramuntia	S. columbaria	S. lucida
Length of calyx setae (mm) Width of calyx setae (mm) Colour of setae Number of hairs per mm <sup>2</sup> on the lower surface of upper rosette leaves Width of lobes of cauline leaves (mm) Height of stems (cm) Length of stalks of flower heads (cm) Length/width ratio of terminal lobe of upper cauline leaves Number of flower heads per stem	1-3 0.08-0.15 light brown 4-8 0.5-1.8 20-100 20-50 12-25 5-70	3-5 0.10-0.20 dark brown 0-2 1-3 20-60 20-50 8-16 5-30	5-8 0.20-0.40 black 0-2 2-8 10-35 10-25 4-8 1-10

Typical morphological characteristics of the three species are put together in Table 3 and *S. columbaria* has morphologically and ecologically an intermediate position between *S. lucida* and *S. gramuntia*.

Best characteristics to distinguish the species are the length and width of calyx setae and the number of hairs on the lower leaf surface.

# 2.2. Experimental conditions

The experimental conditions are described in detail in LANDOLT et al. (1975). During winter season the plants were cultivated in the Institute's garden, all under the same conditions. The soil consisted of one part Löss loam, poor in nutrients, and three parts quartz sand. After the first part of the experiment (1970) half of the plants were harvested for measurements. The remainder stayed in the plots for the second part of the experiment. Since 1968 the plants were cross-pollinated and set numerous seeds. Weeds were removed periodically but the seedlings of *Scabiosa* were left in the experimental plots.

Two groups of experiments were carried out :

## a. Greenhouse experiments

From the beginning of April to the end of October the plants stayed under two different temperature conditions in the greenhouse (Table 4).

The mean cool temperature of 11°C is two degrees lower than the mean temperature of April to October of the habitat of *S. columbaria* and only slightly higher than the warmest month of Davos/Parsenn (origin of *S. lucida*). The mean warm temperature of 24°C is by far higher than the summer temperature at the habitat of *S. gramuntia*. It is five degrees warmer than the warmest month in Martigny (origin of *S. gramuntia*).

Plants of the three species S. gramuntia, S. columbaria and S. lucida were grown in culture containers (45x45x45 cm). At the beginning of the experiment six

**Table 4.** Temperature conditions in the greenhouse experiment.Temperaturbedingungen im Gewächshausexperiment.

Series	day temperature (10 hours) (°C)	night temperature (14 hours) (°C)
warm conditions cool conditions	30 17	20 7

plants were planted either in pure cultures of each of the three species or in mixtures of two or all three species. Four replicates of each combination were made in the warm chamber but only two in the cool chamber (on account of limited available space).

Pollination: Since no insects were allowed in the greenhouse, the pollination had to be done with a small brush. Twice a week every flower was pollinated at random.

## b. Experiments in the garden

During the vegetation period the following conditions were established in twelve directly adjacent basins with controlled water table level in the garden (Table 5). Only two species (*S.gramuntia* and *S. columbaria* were used in this experiment. The basins (4.25x1.20 m) were divided into three plots and planted with *S. gramuntia*, *S. columbaria* and a mixture of both species, respectively.

The fertilizer used, contained constant amounts of nitrogen, phosphorus and potassium as well as some trace elements. The yearly fertilization corresponded to a supply of 30, 3 and 0 g nitrogen per squaremeter, respectively. The fertilization of

Conditions	Water table level in cm below surface	Yearly supply of each N, P, K in g/m <sup>2</sup>	Dry periods in days between watering
$G_{45}D_0$	45	0	natural
G <sub>45</sub> D <sub>3</sub>	45	3	natural
G <sub>45</sub> D <sub>30</sub>	45	30	natural
$G_{145}D_0$	145	0	natural
$G_{145}D_0$	145	3	natural
G <sub>145</sub> D <sub>30</sub>	145	30	natural
B <sub>7</sub> G <sub>45</sub>	45	3	- 7
B <sub>7</sub> G <sub>95</sub>	95	3	7
B <sub>7</sub> G <sub>145</sub>	145	3	7
$B_{28}G_{45}$	45	3	28
B <sub>28</sub> G <sub>95</sub>	95	3	28
B <sub>28</sub> G <sub>145</sub>	145	3	28

**Table 5.** Conditions in the plots of different ground water tables.Bedingungen in den Grundwasserbecken.

Dry periods of 28 days are very uncommon in the distribution area of S. columbaria (in Switzerland) but not too rare in the central alpine valleys (origin of S. gramuntia)(see also Table 1).

The pollination was achieved by the insects in the garden. Cross-pollination between plants of different conditions was possible.

The climatic conditions during the study period are summarized in Table 6. Relatively cool years were: 1972, 1980 and 1974.Warm years: 1983, 1971 and 1982. 1971 was also a very sunny year whereas 1980 and 1981 received little sunshine. Wet years were 1975 and 1970, dry years 1983 ( dry July) and 1985 (dry Fall). Longer dry periods occurred in 1976 from March to June, and in 1969 and 1972 during the Fall.

**Table 6.** Mean temperatures, precipitation, and sunshine hours during the study period (from Ann.Schweiz.Meteorol.Anst. 1968-1985).

Mitteltemperaturen,	Niederschläge und Sonnenscheindauer	<sup>.</sup> während der	Untersuchungs-
periode.			

Year	Temperature ℃	Precipitations mm	Sunshine hours
1985	13.8	571	1306
1984	13.0	683	1091
1983	14.5	540	1123
1982	14.0	810	1143
1981	13.5	848	986
1980	12.4	681	966
1979	13.0	637	1064
1978	12.3	754	1032
1977	12.8	721	1013
1976	13.9	684	1370
1975	13.4	918	1134
1974	12.6	751	1256
1973	13.4	806	1258
1972	12.1	715	1127
1971	14.2	693	1500
1970	13.0	887	1231
1969	13.5	662	1305
1968	13.3	805	1280
1968-1985	13.3	731	1177

### 2.3. The investigated characteristics

The following characteristics have been scored (Fig. 2):

 Hair density (number of hairs per mm<sup>2</sup>) on lower surface of the uppermost rosette leaves

The hair density was described by classes. Class 0: no hairs; class 1: 1-2 hairs/mm<sup>2</sup>; class 2: 2-4 hairs/mm<sup>2</sup>; class 3: 4-8 hairs/mm<sup>2</sup>; class 4: 8-16 hairs/ mm<sup>2</sup>; class 5: 16-32 hairs/mm<sup>2</sup>; class 6: 32-64 hairs/mm<sup>2</sup>.

2) Length of calyx setae The length was measured in mm.



**Fig. 2.** Characteristics of *Scabiosa* used in the present study (see chapter 2.3.). The Figure represents *S. lucida* (from HESS et al. 1972, slightly modified). In der vorliegenden Untersuchung berücksichtigte Eigenschaften von Scabiosa. Die Figur zeigt S. lucida (aus HESS et al. 1972, abgeändert). 3) Width of calyx setae

The width was characterized by classes. Class 1: 0.05-0.10 mm; class 2: 0.10-0.15 mm; class 3: 0.15-0.20 mm; class 4: 0.20-0.25 mm; class 5: 0.25-0.30 mm.

- Length of stalk of flower head The length of stalk was measured in cm.
- 5) Height of stems The height of stems was measured in cm.
- 6) Length of the uppermost rosette leaves The length was measured in mm.
- 7) Length of terminal lobe of the uppermost rosette leaves Distance from the leaf to the point where the margin of the leaf blade reaches first as near as 1.5 mm to the middle nerve.
- 8) Length of the terminal lobe of the uppermost cauline leaves The length was measured in mm.
- 9) Width of the terminal lobe of the uppermost cauline leaves The width was measured in mm.

In addition some ratios of different characteristics were used, e.g. 2/3, 5/4, 7/6, 8/9.

It was supposed that characteristics 1) to 3) were only slightly modified by environmental conditions whereas the characteristics 4) to 8) seemed to be rather strongly influenced.

The characteristics of 20 plants of each plot or container were measured at the beginning. Later the number of full grown individuals was sometimes much smaller (down to 4). Measurements were made in the following years : 1968, 1969, 1970, 1971, 1973, 1975, 1979. The last measurements occurred in 1983 for the ground water plots and 1985 for the greenhouse containers.

# 2.4. Evaluation methods

The mean and single values of the measured characteristics (see Chapter 2.3.) have been plotted versus time. This representation allows a good overview not only of the tendencial development of the characteristics of the different species, but also shows scatter and distribution of the single values within every species under given conditions (see Figs. 5 to 31). Because the hair density on the uppermost rosette leaves is given only as classes, small random values have been added to every single value, thus making visible the cases where more than one indi-

vidual lies within the same hair density class.

Additionally, the differences of the characteristics between the final populations and the original species have been tested by Fisher's *t*-test (Tables 7 and 8).

To show general tendencies of the different species considering all characteristics, a multivariate analysis of the data has been done. Every measured plant is considered as an individual and described with the nine attributes listet in Chapter 2.3. These individuals have been classified into n groups according to species, environmental conditions and year of measurement (e.g. S. columbaria, in ground water table plots with a water level at 145 cm below the ground and well fertilized, in 1968). With this classification, discriminant analysis has been performed. This algorithm creates n - 1 new "attributes" (called discriminant axis) by linear combination of the measured characteristics in a way that the given groups are best separated on the first one or two axes. Thus, the main information of the data set is concentrated on two dimensions and can easily be represented as a scattergram. Because the investigated characteristics are of different kinds, the attribute vectors have been transformed to unit length, i.e. every attribute is considered an m-dimensional vector formed by the values of the given attribute of all m chosen individuals; the measured values are divided by the length of this vector before the analysis:

$$a_j = \frac{1}{\sqrt{\sum_m}a_j^2}$$

 $a_j$  ... transformed value of a given attribute of individual j $a_j$  ... measured value of a given attribute of individual jm ... number of individuals

The contribution of every attribute to the scores ist characterized by the discriminant coefficient, which equals the cosine of the angle  $\alpha$  between the attribute coordinates and the discriminant axis. All multivariate analysis has been done with the program package (now called MULVA-4) of WILDI and ORLOCI (1983 and 1988). Some results are shown as ordination plots in Figs. 41 to 43.