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Cytotaxonomic studies on *Galium verum* L.

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Abstract

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A cytological investigation of *Galium verum* L. occurring in Europe revealed diploids ($2n=22$) and tetraploids ($2n=44$). The tetraploids are distributed throughout Europe, the diploids have a more southerly distribution.

A comparative morphological investigation showed three groups within the diploids. One of these is identical to the subspecies *wirtgenii* (F. Sch.) Ob., the other two fall entirely within the subspecies *verum* as far as their morphology is concerned. Within the tetraploids no well-defined correlation in morphology and geographical distribution could be demonstrated, except in the case of a taxon occurring along the Atlantic coast of Europe. A clear-cut separation, however, proved to be impossible due to the large degree of overlap in the distinguishing characters.

Introduction

The extremely polymorphic Eurasian *Galium verum* group comprises several taxa whose delineation and distribution are still unclear. The group displays its highest degree of polymorphy in the Asiatic part of its distributional area. Many taxa have been described from the Caucasus and adjacent areas as well as from central and east Siberia. Their systematic position, however, is often doubtful. In the Flora of the U.S.S.R., Pobedimova (1958) mentioned within the section *Xanthogalium*, described originally by De Candolle three subsections containing altogether 15 species. In Europe, with the possible exception of the Balkans the situation does not seem to be so complicated. Ehrendorfer and Krendl (1976) in Flora Europaea, distinguished two species within the *verum* group e.g.: *G. tunetanum* Lam – a more or less isolated taxon from the south of Spain, Sicily and North West Africa –, *G. verum* L. with two subspecies *verum* and *wirtgenii* (Sch.) Ob. and *Galium* × *pomeranicum* Retz. a hybrid of the subspecies *verum* with the tetraploid *G. album* Mill. ssp. *album*, which is a representative of the *G. mollugo* group and which is widely distributed over Europe.

Within *G. verum* L. two cytotypes are known: a diploid and a tetraploid, with $2n=22$ and $2n=44$ chromosomes respectively. In Europe these cytotypes are not equally distributed. This already has been known since the studies of Fagerlind (1937). Among a majority of tetraploids, distributed all over Europe, this author found a minority of diploids in the south-eastern parts of Europe. This picture was confirmed by a

number of authors. Most of them report only tetraploids (Löve and Löve 1956, Piotrowicz 1959, Zhukova 1966, Levêque and Gorenflot 1969, Murin and Váchová 1970, Schotsman 1973, Sokolovskaja 1972, Surova 1973, Murin 1974, Murin and Zabor-sky 1976, van den Brand, van Meel and Wieffering 1979). Tetraploids and diploids were reported by Kliphuis (1962) and Løvkvist (1962). A locality with diploid plants has been recorded for Bulgaria by Ančev (1974).

Material of *G. verum* was collected from different parts of Europe. Plants obtained directly from the wild and plants grown from seed collected in the field were cultivated under uniform conditions in an experimental plot. During the years of cultivation this material showed differences in its morphology and a number of characters proved to be variable. Some differences were subtle and hard to describe, whereas others were striking. A number of differences were influenced by the environment. It seems worthwhile subjecting this collection to a cytological and comparative morphological investigation. The results of this investigation are given and discussed in this paper.

Material and methods

Plants collected in the field and plants grown from seed were cultivated in the experimental garden of the State University of Utrecht during several years. From each seed sample 3–5 plants were used for cytological purposes.

The material was collected partly by the author, partly by colleagues and partly with the aid of other botanical institutes. In this study we used only material collected in the wild and of which the place of origin was registered.

The chromosome counts were made from the root tips of potted plants. The tips were fixed in Karpechenko's fixative, embedded in paraffin wax, sectioned at 15 micron and stained according to Heidenhain's method.

A comparative morphological investigation was carried out on living material as well as on voucher specimens collected in the experimental plot.

Results

1. Cytology

The chromosome number of 322 plants from 161 localities in 19 European countries was determined. The results of the counts are given in an appendix at the end of this paper. The material is arranged according to the alphabetical order of countries of provenance, and the place of origin and plant number are given. Diploids ($2n = 22$) and tetraploids ($2n = 44$) were observed. The tetraploids were found throughout Europe. The diploids were in the minority and were encountered in the central and southern parts of Europe only. Figure 1 shows the distribution of localities. The diploids are represented by an open dot, the tetraploids by a black dot. – The chromosome portrait of both cytotypes is regular and no B-chromosomes or satellites were observed.

2. Morphology

Comparative morphological investigations were carried out and attention was paid to the height of the plant, the length of the stem, the length of the internodes, the shape of the panicle and the length of their flowering branches, the length and width of the leaves, the length of the pedicels, the diameter of the flowers, the length of the stamens and the size of the fruits.

a) Diploids

Within the diploids three groups could be recognized. One group of well-developed plants, 50–80 cm high, with erect stems. The lower parts glabrous, the upper parts becoming increasingly puberulent with short curved (not uncinata) hairs. Flowers and fruits glabrous. Flowering branches short, shorter than or exceptionally as long as their corresponding internodes, forming a narrowly cylindrical poorly flowering and interrupted panicle. The leaves are linear, up to 2.5 cm long and up to 2 mm wide. Compared to the other two groups the plants of this group have relatively long pedicels (up to 3 mm) and relatively large flowers (3–4 mm). – The plants characterized in this way were collected in the French Alps (Hautes Alpes, Alpes Maritimes and Savoie) and in

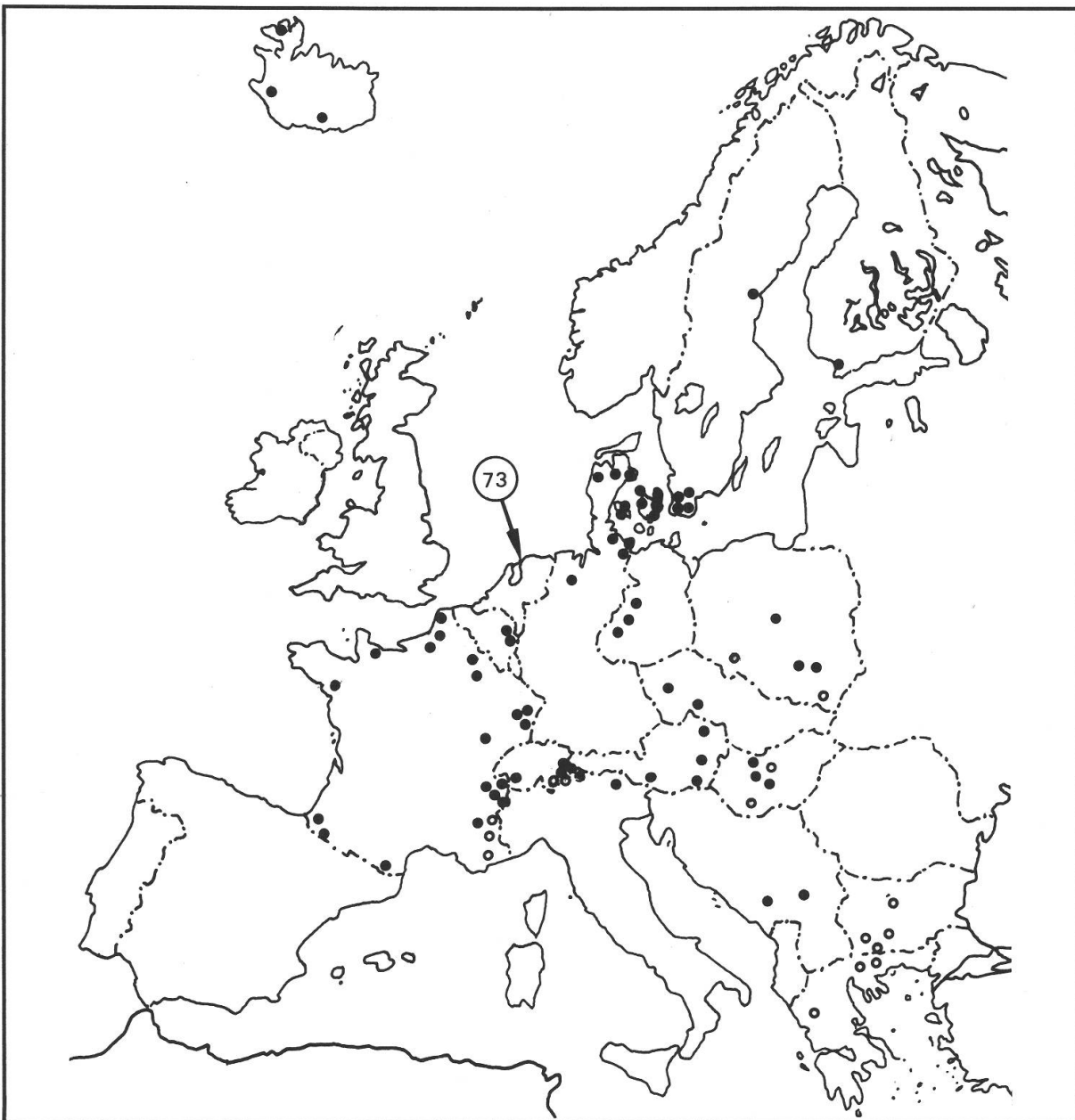


Fig. 1. Localities of the cytologically investigated plants of *Galium verum* L. The diploid is represented by an open dot, the tetraploid by a black dot. The no. 73 represents the number of localities investigated for the Netherlands.

the south of Switzerland (Ticino). (Collection numbers: K 111, K 224, K 559, K 618, and K 3162). In these regions this type of *G. verum* is commonly distributed in meadows that are fairly moist. – The flowering time in nature as well as in the experimental plot extends from the second half of June into the first week of July.

The second group comprises plants collected in Bulgaria and Greece. (Collection numbers: K 1497, K 1502, K 3392, K 3409, K 3418, and K 3427). These plants are robust, up to 125 cm high with stout erect stems. The flowering branches, which are always longer than their corresponding internodes, form a loose elliptic or elliptic to narrow-ovoid, rich-flowering panicle, which is sometimes faintly interrupted. The leaves are narrow-linear, up to 4 cm long and 2 mm wide; outside the inflorescences the leaves are often much longer than within. The pedicels are short, up to 1 mm in length and the flowers whose diameter is generally less than 3 mm, are relatively small. With exception of the flower parts the whole plants may be densely hairy. – The flowering time last from the first half of July into the first half of August.

The third group is formed by plants from the central parts of Europe (Poland and Hungary). These plants are up to 100 cm high with a loose faintly interrupted relatively poor-flowering panicle; the flowering branches in the lower parts are shorter than their corresponding internodes and in the upper parts they are as long as or longer to much longer than the corresponding internodes. During the years of cultivation this character turned out to be variable. In different years of cultivation the same plant would have all flowering branches longer than their corresponding internodes. This phenomenon is reversible and it is unclear by which factor or factors it is induced. – The leaves are narrow-linear, up to 4 cm long and up to 2 mm wide. The flowers have an average diameter of 3 mm and the pedicels are up to 1.5 mm in length. The indument does not differ much from that found in the plants from the first group. The plants from this group are the collection numbers: K 1611, K 1680, K 1683, K 1686, and K 1687. – The flowering time is from the last week of June into the second half of July.

b) Tetraploids

The material investigated was not uniform in its morphology. The characters varied. Taken together they occur in almost every possible combination. Taken individually they may show a difference, but there is almost always a degree of overlap present.

Flower characters do not much differ between plants and over the years of cultivation the size of the flowers (2.5–4 mm, with an average of 3.3 mm) and the fruits (1–1.5 mm) and the length of the stamen (ca. 1 mm) remain remarkably constant. The bright, striking yellow colour of the flowers is also a constant factor and does not show much interindividual variety. During the years of cultivation the length and width of the leaves may show some variation. They may become longer and wider (up to 3.5 cm long and 2 mm wide), but in spite of this the leaves cannot be used as a discriminating character. This is partly due to a degree of overlap, partly because the length/width ratio remains rather constant. The leaves are always linear, mucronate, one veined, dark green and rough above, pale green and pubescent beneath and their margins are always strongly recurved. The pedicels with an average length of 1.5 mm are relatively short and they do not show much variation between plants and within plants from various years. The indument is rather uniform. The stems are finely puberulent. Corolla, ovaries and fruits are glabrous. This seems to be the normal picture for *G. verum* in Europe. Plants with hairy fruits and closely related to *G. verum* were found only in material of Asiatic origin. The shape of the panicle is usually oblong ovoid to ovoid with all possible intermediates. In a few cases a broad ovoid, almost pyramidal inflorescence

was found with well-developed flowering branches from about the lowest half of the stem. In the experimental plot this character proved to be very variable. From year to year the panicle of the same plant may show considerable differences.

It proved impossible to find groups that were well defined by a distinctive combination of the above-mentioned characters. The only characters by which it appeared possible to distinguish two groups, each of which correlated with a certain eco-geographical distribution, proved to be the height of the plants and connected with this, the lengths of the stem, the internodes and panicle. Two groups could be distinguished on this basis. One group of plants which may grow in to large plants with a height up to 100 cm and one group of plants which, with a maximum height of 60 cm never reached this length. There is a degree of overlap on both sides which prevented a clear-cut separation of the two groups, but nevertheless a difference possibly may be present. Plants from this last group (with a maximum length of 60 cm) turned out to be plants from the coastal regions of Western-Europe. Plants of the first group (with a height up to 100 cm) were collected all over Europe, but outside this area.

Discussion

The cytological investigation confirmed the occurrence within *G. verum* of two cytotypes: diploids and tetraploids. In Europe the tetraploids are widely and commonly distributed. The diploids are less frequent and have a more southerly distribution. This picture is in agreement with data found in the literature. A number of authors reported mainly tetraploids for *G. verum* in Europe. Diploids were reported by Fagerlind (1937) for nine localities in the Balkans, by Løvkvist (1962) for Italy (north of Udine), Austria (Carinthia) and Poland (vic. of Lodz) and by Ančev (1974) for a plant found in the Ljulin Mountains in Bulgaria.

The position of the plants belonging to group I of the three groups that could be distinguished within in the diploids is quite clear. The characters investigated are well defined and remained stable during the years of cultivation. Slight changes in the length of the flowering branches were observed, but it could never be demonstrated that these branches became longer than their corresponding internodes and the panicle remained interrupted and narrowly cylindrical. All characters are without doubt identical to those as described by F. Schulz in 1855 for *G. wirtgenii* and given subspecies rank by A. Oborny in 1885.

The diploids of group II fall entirely within the limits of the tetraploid *G. verum* as far as their morphological characters are concerned. They tend to have smaller flowers. This, however, is not a decisive character. Relatively small flowers were also observed within the tetraploid cytotype. In the experimental garden the diploids of this group became, when fully developed, the largest plants. This character proved very variable and is induced largely by the environment. Therefore, it cannot be used as a distinguishing character either. Compared with other plants (diploids as well as tetraploids) the plants from this group are relatively hairy. This phenomenon may possibly be in agreement with that found in *G. mollugo* L. In this species the number of plants with indument increases proportionally southwards (Krendl, 1967).

The position of the plants of group III is unclear. Habit characters such as the development of the plants, their height and the size of their panicle seem to be intermediate between those found in plants from group I and group II. The same applies to leaf and flower characters. The important characters of the inflorescences (flowering branches

shorter or longer than the corresponding internodes, poor- or rich-flowering, clearly interrupted or not) by which plants of group I and II can easily be distinguished tend to be intermediate. The picture is highly complicated and obscured by the instability of the structure of the panicle in plants of this third group; the instability became clear when plants were compared over the years of cultivation. It is tempting to suggest that all these characters are due to hybridization between plants of group I and group II. The distribution pattern of the plants concerned seems to support this view. However, there is a gap in flowering time, but in the experimental garden there are still flowers of plants of group I present when the plants of group II are in full flower. It is unlikely that the position is different in nature.

The plants of group III seem to be intermediates, but obviously they stand close to *Galium verum* L. ssp. *verum*. For that reason it appears better to include these plants without further taxonomic recognition within this sub-species than to give them an independent status.

In figure 2, plants from the three groups found within the diploid cytotype are illustrated: I – ssp. *wirtgenii*, collected in Switzerland (no. K 224), II – ssp. *verum* from Bulgaria (no. K 3392) and III – ssp. *verum* from Poland (no. K 1682) in two years of cultivation demonstrating the variability of the panicle.



Fig. 2. Voucher specimens of *Galium verum* L. of the three groups found within the diploid cytotype: I – ssp. *wirtgenii* (F. Sch.) Ob., collected in Switzerland; II – ssp. *verum* from Bulgaria; and III – ssp. *verum* from Poland, showing the variability of the panicle in two years of cultivation.

Within the tetraploids studied the differences in morphology are mainly of a quantitative character. Most of them do not justify a separation into groups, neither taken individually nor when taken in combination. Except in the case of the height of the plants, the length of the stems, the length of the internodes and the size of the panicle it was impossible to demonstrate a correlation with a certain geographical distribution.

In the coastal regions of Western-Europe, particularly in the dunes, there occurs a type of *G. verum* that is strongly adapted to the extreme environment. The plants are small, 10–20 cm high, procumbent or decumbent to ascending or ascending, with a tremendously branched root system. The internodes are shorter than or as long as the leaves. The inflorescences are narrowly ovoid, compact, with the flowering branches always longer than their corresponding internodes. This type is often considered as a separate taxon. It was described as a variety by De Candolle in 1805 (var. *maritimum*) and later on as var. *littorale* by De Brébisson (1859). Recently it was given subspecies rank (ssp. *maritimum*) by Adema (1981). Transplanted to the experimental plot these plants are the ones that may show remarkable phenoplasticity in habit characters such as the height of the plants, the length of the stem and internodes and the size of the panicle. As a matter of fact, these are the characters used to distinguish the variety.

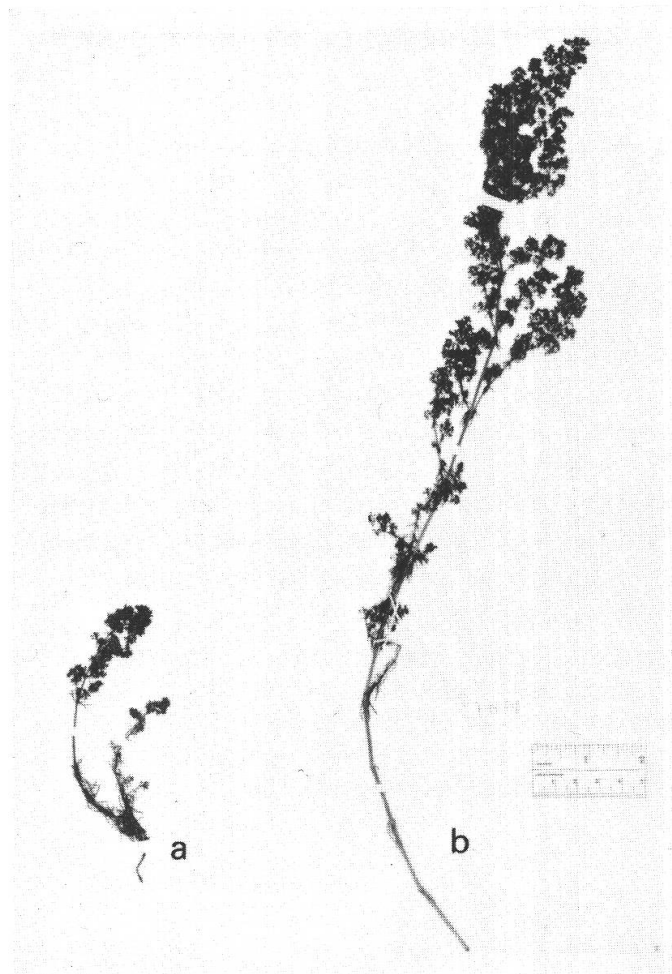


Fig. 3. Phenoplasticity of *Galium verum* L. ($2n=44$). Voucher specimens: **a** – plant collected in the coastal region of the Netherlands (dunes) and **b** – the same plant in the fourth year of cultivation in the experimental plot.

Retransplantation from the experimental plot into the original habitat was unsuccessful. The plants did not strike root and all died. In the experimental garden the root system of the plants did not develop as extremely as it does in the dunes. In this respect these plants also show a considerable phenoplasticity. The time for adaptation to the extreme conditions in the dunes was evidently for the retransplanted plants too short to survive.

Plants outside the coastal regions are as a rule large plants, 30–85 cm high, with ascending to erect or erect stems, with the leaves always shorter than the internodes, with a well-developed panicle and the flowering branches always longer than the corresponding internodes. In the experimental plot these plants may often become larger plants, which can be distinguished by their height from the plants transplanted from the coastal regions. The characters, however, are variable and a large degree of overlap may be present.

Crossing experiments between the coastal plants and tetraploid plants from other parts of Europe failed. *G. verum* is self-incompatible. Strange pollen may induce pseudo self-compatibility (Fagerlind 1937, Kliphuis 1970). For that reason all flowers were emasculated.

From a biosystematic point of view the coastal plants should be considered as an independent taxon. It is the variability of the distinguishing characters which renders the taxonomic treatment difficult and consequently recognition as a subspecies or even as a variety. This taxon probably has a much wider distribution than is often assumed. It is certainly not restricted to the dry dunes. Probably all *G. verum* plants in a broad strip along the Atlantic coast of Europe belong to this taxon. Due to the wide variation in the distinguishing characters of *G. verum* inside and outside these areas confusion is bound to occur between the two groups.

Figure 3 shows a plant from the dunes in the Netherlands (no. 15), and the same plant in the fourth year of cultivation in the experimental plot.

One of the three plants collected in Iceland died in the first year of cultivation. One is similar to the *G. verum* found outside the coastal regions. One plant (no. 1053) at first sight seemed to be identical to *G. verum* of the Atlantic area. However, during the years of cultivation this plant remained small, 15–25 cm high, with erect stems and a densely flowering narrowly ovoid to ovoid panicle. The flowering branches were longer than their corresponding internodes and the internodes were always longer than the leaves. A separate taxon, therefore, seems justified. The systematic position of this taxon, however, is unclear for the moment due to the fact that only one plant was available.

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Appendix

Cytologically investigated material of *Galium verum* L.

Diploids: 2n = 22

Bulgaria: K 3392 – Troyan pass, alt ca. 1000 m; K 3408 – Backovski monastir, alt ca. 500 m; K 3409 – vic. of Rila, alt ca. 700 m; K 3418 – vic. of Pestera, alt ca. 400 m.

France: K 111 – Termignon (Sav.) alt ca. 1350 m; K 559 – Thorenc (Alpes Mar.); K 3162 – vic. of Plampinet, valley of the Clarée, near Briancon (H. tes Alpes) alt ca. 1500 m.

Greece: K 1497 – vic. of Purgoi, Macedonia, alt ca. 600 m; K 1502 – vic. of Pentapolis, Macedonia, alt ca. 100 m; K 3427 – Mt. Lakmos, West of Ampelakai, prov. of Ioannina, alt ca. 1200 m.

Hungaria: K 1686 – vic. of Pésc, Baranya; K 1687 – S.E. of Budapest.

Poland: K 1611 – vic. of Kujbyscew; K 1680 – vic. of Jelcz, Wroclow; K 1683 – vic. of Rzeszów Strzyżów, 49.52 N/21.47 E.

Switzerland: K 224 – South of the San Salvatore, near Lugano, Ticino, alt ca 600 m; K 618 – Monte Verita, near Ascona, Ticino, alt ca. 300 m.

Tetraploids: 2n = 44

Austria: K 504 – Hohe Tauern; K 515 – vic. of Krems; K 687 – vic. of Gramsbach. östliches Alpen-Vorland; K 1545 – vic. of Neudorf, Nieder-Österreich.

Belgium – K 159, K 160 – vic. of Kanne, prov. of Limburg.

Czechoslovakia: K 723 – Jihlaské Vrehy, E. Bohemia; K 727 – vic. of Praha.

Denmark: K 207 – Tommerup, Sjaelland; K 360 vic. of Copenhagen; K 1202 – vic. of Ebeltoft, Jylland; K 1207 – near Løgstør, Jylland; K 1208 – near Gilleleje, Sjaelland; K 1431 – vic. of Holstebro Jylland; K 1663 – Magleby Skov, Møn; K 1664 – Karlstrup strand; K 1665 – Lammerfjorden, Sjaelland; K 1666 – vic. of Alholm, Sjaelland.

D.D.R.: K 543 – vic. of Zerbst, Magdeburg; K 720 – vic. of Rothenburg, Magdeburg; K 1626 – near Groszvargula, Bezirk Erfurt.

Finland: K 1986 – vic. of Parainen (Pargas), South of Turku.

France: K 347 – vic. of Herrlisheim, Alsace; K 348, K 601 – vic. of Hipsheim, Alsace; K 469 – vic. of Aix les Bains (Sav.); K 470 – vic. of Nantes (Loire Atl.); K 684 – Arc sur Tille (Côte d'Or); K 689 – Oly les Bains (Sav.); K 690 – Grand-Quevilly (S. Mar.); K 698 – Les Angles (Pyr. Or.); K 706 – near St. Andrien (S. Mar.); K 1568 – vic. of Théhold (M. et M.); alt ca. 420 m; K 1644, K 3014 – Vouziers (Ard.); K 1888 – vic. of Marsous, near Aucun (Pyr. Atl.); K 1896 – vic. of Héas, near Gèdre (Pyr. Atl.); K 3012 – vic. of Ambleteuse (Pas de Calais); K 3013 – vic. of Aure (Calvados); K 3178 – Col d'Izoard (H.tes Alpes) alt ca. 1600 m; K 3195 – vic. of Vallouise, valley of the Onde, Entre les Aigues (H.tes Alpes).

Hungaria: K 1164 – vic. of Vác, near Nagyrillam; K 1580 – near Budapest.

Iceland: K 1053 – Lomatnupur, East slope of Vatnajökull, South Iceland; K 1056 – near Arnarfjörður, N.E. Iceland; K 1185 – vic. of Reykjavik, near Airport.

Italy: K 303 – vic. of Friuli, Vallis Tellinae; K 3003 – Monte Ciaberga, alt ca. 800 m.

The Netherlands: K 15, K 16 – 2 km South of Bakkum, dunes (prov. Nrd. Holl.); K 40–K 43, K 46, K 48, K 50, and K 51 – Kunraderberg, Zuid-Limburg; K 84 – vic. of Westkapelle, Walcheren (Zl.); K 148 – vic. of Callantsoog, dunes (Nrd. Holl.); K 192 – Estate “de Schovenhorst”, Putten (Geldrl.); K 249, K 254 – vic. of Oude Mirdum, Gaasterland (Frl.); K 253 – vic. of Laaxum, Gaasterland (Frl.); K 377, K 378 – Sipkesslag, Oostvoorne (Zd. Holl.); K 409, K 411 – vic. of Mariëberg (Overijs.) K 410 – along the river Vecht, near Mariëberg (Overijs.); K 661 – dunes, 3 km South of de Koog, Texel; K 664 – dunes, 2 km North of de Kooy, Texel; K 665 – de Muy, Texel; K 801, K 807, K 808 – Quakjeswater, Oostvoorne (Zd. Holl.); K 803, K 804 – vic. of Oostvoorne (Zd. Holl.); K 822, K 823 – vic. of Rockanje (Zd. Holl.); K 826–K 839, K 916–K 925, K 956, K 961–K 975 – Weeversduin, Oostvoorne (Zd. Holl.); K 981 – de Bosplaat, Terschelling; K 987 – Paraboolduin, Terchelling; K 989 – Bemelerberg, Zuid-Limburg.

Poland: K 1600 – Deby Szlacheckie (Poznan); K 1622 – Zernike Górne; K 1624 – Góry Swietokrzyskie Bodzentyn.

Sweden: K 277, K 456, K 652 – vic. of Landskrona, Skåne, distr. Malmohüs; K 453 – vic. of Fjellie, Skåne, distr. Malmohüs; K 1542 – Medelpad, Alno, distr. Västernorrland.

U.S.S.R.: K 147, K 639 – Kopet – Dag, Khrebet Mnts. (Turcomaniae); K 317 – vic. of Erevan (Armenia); K 3268 – Jacutia, distr. Nighne Kolynsk, eastern Siberia.

Western Germany: K 259, K 1562 – Heiligenhafen, Sleswig Holstein; K 1557 – vic. of Oldenburg, Niedersachsen; K 1564 – vic. of Dummersdorf, Niedersachsen.

Yugoslavia: K 1341 – vic. of Vičegrad, Bosnia Hercegovina, alt ca. 350 m; K 1345 – vic. of Lovač, Serbia, alt ca. 1100 m.

Switzerland: K 416 – Röntgenweg, Pontresina (Graubünden), alt ca 1800 m; K 417 – vic. of Fex, Fextal (Graubünden), alt ca 1900 m; K 418 – vic. of Pontresina, Punt Muragl (Graubünden); alt ca. 1700 m; K 419 – vic. of Bivio, before Julier-pass (Graubünden), alt ca. 1700 m; K 1510 – vic. of Champex (Valais) alt ca. 1500 m.