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INVESTIGATION OF A POPULATION OF MENTHA × NILIACA JUSS. EX JACQ. FOUND IN NATURAL CONDITIONS AT OUDEMOLEN (DR.)

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SUMMARY

The results of an investigation (floristic determination, chromosome number and the composition of the essential oil) of a mint-population, growing at Oudemolen (Dr.) have been rendered. According to a morphological determination and the composition of the essential oil it proved to be a hybrid between *Mentha longifolia* (L.) Hudson and *Mentha suaveolens* Ehrh. that is *Mentha* × *niliaca* Juss. ex Jacq. The seedlings of the hybrid did not differ from the original population concerning their habit and the composition of the essential oil, of which piperitone oxide is the main constituent. Chromosome countings gave for the population found 2n = 24; the same number as often found for *Mentha longifolia* and for *Mentha suaveolens*. From these data we voted for the hybrid form of *Mentha* × *niliaca* Juss.ex Jacq. with a gene composition of cc aa PP rr.

1. INTRODUCTION

Some years ago, one of us (van Os) found a mint-population along the side of a ditch at Oudemolen (Dr.), a little hamlet in the neighbourhood of Assen.

After a morphological determination it seemed to be *Mentha* × *niliaca* Juss. ex Jacq., which is a hybrid between *Mentha longifolia* (L.) Hudson and *Mentha suaveolens* Ehrh.

Within the scope of investigations into the genus *Mentha*, during which special attention was paid to the section *Spicatae* (HENDRIKS 1970, 1971, 1974; HENDRIKS & VAN OS 1972) it was interesting to examine this population of *Mentha* × *niliaca* more thoroughly. Special attention was given to the morphological determination, the chromosome number and the composition of the essential oil.

2. EXPERIMENTAL

2.1 Morphological determination

For the morphological determination of the vegetable material the "Flora van Nederland" (HEUKELS & VAN OOSTSTROOM 1973), the "Flora Europaea" (HARLEY 1972) and the determination scheme of OUWENEEL (1967) were consulted.

2.2 Determination of the chromosome number

For the determination of the chromosome number the lacto-orcein method was used on root tips of very young seedlings, which were treated in a mixture of glacial acetic acid, formaline and a solution of chromic acid (1%) (1:4:10). After that the root tips were stained in an orcein solution for 20 min. and transported into a drop of acetic acid (45% v/v) on a slide. The root tips were carefully squashed by pushing a cover-glass on them. The obtained microscopic slide was examined at a 1500 × magnification.

Chromic acid solution: Dissolve 5 g of potassiumdichromate in a mixture of 45 ml of water and 50 ml of concentrated sulfuric acid. Fill up with water to 500 ml.

Orcein solution: Reflux for 1 hour 1 g of orcein with 9.4 ml of lactic acid, 16.25 ml of water and 24.4 ml of glacial acetic acid. After cooling down filter through a sintered glassfilter with a porosity number of 40.

2.3 Investigation of the essential oil

The essential oil was obtained by steam distillation. 13.3 g of the essential oil has been divided into a hydrocarbon terpene fraction (2.2 g) and a fraction containing the oxygen components (11.0 g) by means of column chromatography by eluting successively with petroleum-benzine (b.p. $< 40°$ C) and ether. These fractions and the essential oil were investigated by gaschromatography under the following conditions: column: length 2000 mm, diam. 4 mm, stainless steel; stationary phase: carbowax 20 M and SE 30; support: chromosorb W–AW (60–80 mesh); oven temperature: 80–200° C (carbowax 20 M) and 70–250° C (SE 30), 4°/min.; injectionblock: 250° C; katharometer: 250° C; flow: 60 ml He/min.; sample: about 2μl.

Some components of the essential oil (or the fractions) were isolated by means of preparative gaschromatography. The identification took place with IR-spectrometry (Unicam SP 1000 Infrared Spectrometer) by comparing the spectra with those of reference substances.

3. RESULTS AND DISCUSSION

The morphological determination of the mint-population showed that the distinguishing marks stood midway between those of *Mentha longifolia* and *Mentha suaveolens*. The most important differences between these *Mentha* species are:

a the shape of the leaves (smooth, oblong↔rugose, ovate or suborbicular)
b hair (branched trichomes absent↔plural branched trichomes present)
c corolla (lilac↔white or pink)

Our results distinctly pointed in the direction of a hybrid between *Mentha longifolia* and *Mentha suaveolens* that is *Mentha* × *niliaca*. The diploid chromosome number was 24.

The composition of the essential oil was: α-pinene (0.5%), camphene (0.1%), β–pinene (0.8%), sabinene (0.8%), limonene (1.6%), 1,8-cineole (2.5%), ocimene (0.3%), caryophyllene (6.2%), germacrene-D (3.8%), piperitone oxide

(74.7%), 1,2-epoxymenthylacetate (4.0%), piperitenone oxide (0.3%) and thymol (0.4%). Unidentified 4.0%, of which 0.9% sesquiterpene hydrocarbons.

Mentha × *niliaca* is known as a hybrid between *Mentha longifolia* and *Mentha suaveolens*, which is often fertile. The seedlings of our population resembled the orginal plants. Neither did the composition of the essential oil of the seedlings show important differences with the composition of the essential oil of the parents. The chromosome number of 2n = 24 was also found by HARLEY (1972) and OUWENEEL (1968). This chromosome number is characteristic for the parent plants *Mentha longifolia* and *Mentha suaveolens*. In the Netherlands these mint species only occur in the Chalk-, Löss- and the Fluviatile-district (Flora van Nederland, HEUKELS & VAN OOSTSTROOM 1973). Our population grows in the "Drents"-district. Therefore this population is probably an escape. Was this not the case the hybrid could have overgrown the parents, but in this region these parents were never found.

The composition of the essential oil is the same as that of the essential oil of *Mentha longifolia* 3 and *Mentha suaveolens* 6 as described in earlier papers (HENDRIKS 1971, HENDRIKS & VAN OS 1972)

Piperitone oxide and piperitenone oxide have often been mentioned as main constituents of the essential oil of these mint species (REITSEMA 1958, HANDA et al. 1964 and STICHER & FLÜCK 1968).

As is known from the results of the investigations of e.g. MURRAY (1960 a, b) and MURRAY & REITSEMA (1954) the composition of the essential oils of representatives of the genus *Mentha* is mainly influenced by genetical factors. This is schematically represented in *fig. 1*, which was discussed in more detail in other publications (HENDRIKS 1974, HENDRIKS et al. in the press).

A dominant allele R, not mentioned in *fig. 1*, causes the conversion ketone → corresponding alcohol as was found for the reduction menthone → menthol (MURRAY 1960a) and dihydrocarvone → dihydrocarveol (HEFENDEHL & MURRAY 1972). Therefore the investigated *Mentha* × *niliaca* population, with piperitone oxide as main constituent in its essential oil has the genotype cc aa PP rr. This could be confirmed by the fact that the composition of the essential oil of the seedlings did not differ from that of the original population.

Of *Mentha* × *niliaca* also dihydrocarvone containing forms are known (REITSEMA 1958). In that case the carvone-type of *Mentha longifolia* (LAWRENCE et al. 1972, and LAWRENCE, personal communication, 1972) and the dihydrocarvone-type of *Mentha suaveolens* could be (one of) the parents of these forms (HENDRIKS 1974).

In *fig. 2* the relationship between *Mentha* × *niliaca* from Oudemolen and supposed parents is rendered schematically as far as important for the composition of the essential oil.

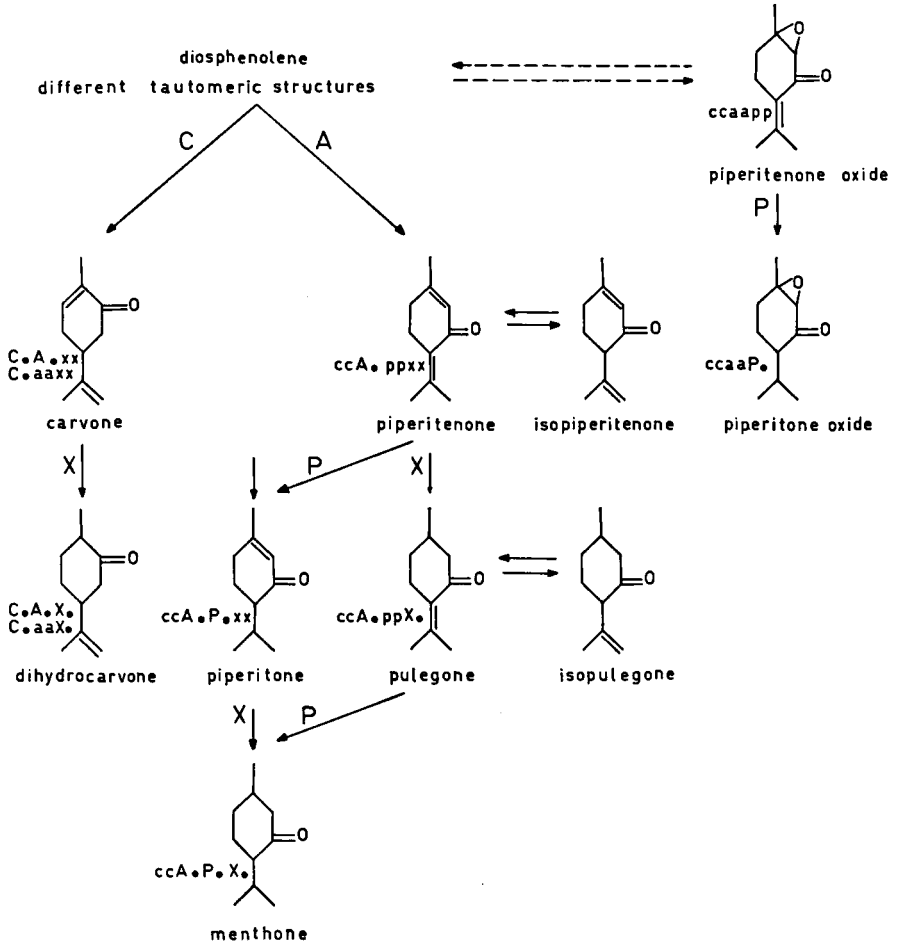

Fig. 1. Relationship between the genotype of representants of the genus *Mentha* and the ketones present in the essential oil.

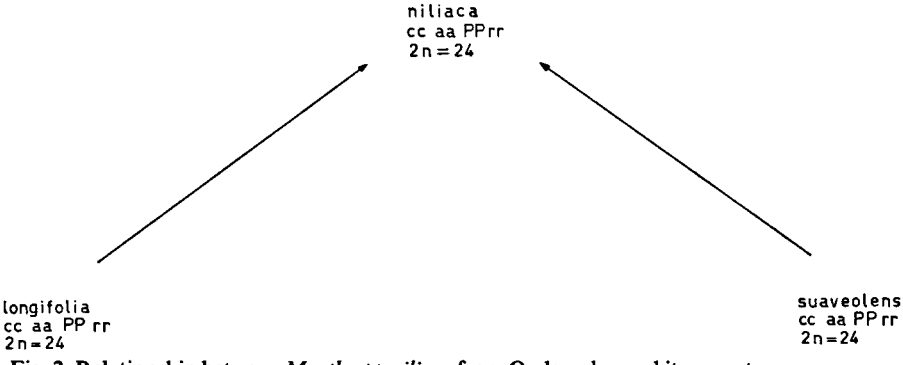

Fig. 2. Relationship between *Mentha* × *niliaca* from Oudemolen and its parents.

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