

SOURCE: JOURNAL OF THE INTERNATIONAL HEMP ASSOCIATION 5 (2): 65, 68-74, 1998.

## Decimal code for growth stages of hemp (*Cannabis sativa* L.)

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### Abstract

A decimal code for hemp (*Cannabis sativa* L.) growth stages is presented. Life cycle is divided into four principal stages: germination and emergence, vegetation, flowering and seed formation, senescence. Each principal stage is subdivided into secondary stages. The practical use of the code is discussed with particular reference to different yield components.

### Introduction

An standardised scale for recording the growth stages of a crop is clearly useful for agronomists, physiologists, pathologists, breeders and, of course, also for farmers. Codes for development of important crops like maize, cereals, rape, pea, faba bean and potato have been described (Hanway 1963, Zadoks *et al.* 1974, Sylvester-Bradley *et al.* 1984, Knott 1987, Knott 1990 and Jefferies and Lawson 1991).

Although many authors have described the characteristics of growth and development of fibre hemp (*Cannabis sativa* L.), little attention has been given to a formal assessment and recording of these stages (Ceapoiu 1958, de Meijer *et al.* 1992, Slembrouck 1994, van der Werf *et al.* 1995a, Bócsa and Karus 1997, Clarke 1997, von Buttlar *et al.* 1997).

### Material and Methods

The general principles for a decimal code proposed by Zadoks *et al.* (1974) for cereals have been adjusted for fibre hemp.

### ***Principal growth stages***

Four principal stages describe the life cycle of a plant (Table 1) and are coded by their first digit of a four-digit code.

**Table 1. Principal growth stages of *Cannabis sativa* L. plants**

<b>1-digit code</b>	<b>Definition</b>
0	Germination and emergence
1	Vegetative stage
2	Flowering and seed formation
3	Senescence

### ***Secondary growth stages***

These stages are described by the second digit, which indicates the sex of the plant, the third and fourth digits which indicate the developmental stage of the plant (Table 2).

**Table 2. Definitions and codes of growth stages of *Cannabis sativa* L. plants**

Code	Definition	Remarks
<b>Germination and emergence</b>		
0000	Dry seed	
0001	Radicle apparent	
0002	Emergence of hypocotyl	
0003	Cotyledons unfolded	
<b>Vegetative stage</b> refers to main stem. Leaves are considered as unfolded when leaflets are at least one cm long.		
1002	1 <sup>st</sup> leaf pair	1 leaflet
1004	2 <sup>nd</sup> leaf pair	3 leaflets
1006	3 <sup>rd</sup> leaf pair	5 leaflets
1008	4 <sup>th</sup> leaf pair	7 leaflets
1010	5 <sup>th</sup> leaf pair	
:	:	:
10xx	n <sup>th</sup> leaf pair	xx = 2(n <sup>th</sup> leaf pair)
<b>Flowering and seed</b> formation refers to the main stem including branches.		
2000	GV point	Change of phyllotaxis on the main stem from opposite to alternate. Distance between petioles of alternate leaves at least 0.5 cm
2001	Flower primordia	Sex nearly indistinguishable
<b>DIOECIOUS PLANT</b>		
<b>male</b>		
2100	Flower formation	First closed staminate flowers
2101	Beginning of flowering	First opened staminate flowers
2102	Flowering	50% opened staminate flowers
2103	End of flowering	95% of staminate flowers open or withered
<b>female</b>		
2200	Flower formation	First pistillate flowers. Bract with no styles.
2201	Beginning of flowering	Styles of first female flowers
2202	Flowering	50% of bracts formed
2203	Beginning of seed maturity	First seeds hard
2204	Seed maturity	50% of seeds hard
2205	End of seed maturity	95% of seeds hard or shattered
<b>MONOECIOUS PLANT</b>		
2300	Female flower formation	First pistillate flowers. Perigonal bract with no pistils.
2301	Beginning of female flowering	First pistils visible
2302	Female flowering	50% of bracts formed
2303	Male flower formation	First closed staminate flowers
2304	Male flowering	Most staminate flowers open
2305	Beginning of seed maturity	First seeds hard
2306	Seed maturity	50% of seeds hard
2307	End of seed maturity	95% of seeds hard or shattered
<b>Senescence</b>		
3001	Leaf desiccation	Leaves dry
3002	Stem desiccation	Leaves dropped
3003	Stem decomposition	Bast fibres free

### Germination and emergence (principal stage code 0)

After water intake (imbibition), the radicle appears (Figure 1A), the hypocotyl emerges and the cotyledons unfold above the soil (Figure 1B). The optimal temperature for germination is

24° C (Ceapoiu 1958), lower temperatures delay the emergence. The minimal temperature for germination is 0° C (van der Werf *et al.* 1995a). Germination usually takes three to seven days (Clarke 1997). In contrast to the subsequent leaves, cotyledons are sessile and have no serrate margin.

### **Vegetative stage (principal stage code 1)**

This stage is defined as the stage between emergence and generative development and is characterised by the growth of the stem and leaves. At the beginning of the vegetative stage the plant grows slowly. In this phase the plant usually forms up to five true leaf pairs and short internodes (Figure 2). Later, during the fast stem elongation, internodes are longer (Cepoiu 1958, Bócsa and Karus 1997). During the whole vegetative stage usually seven to twelve leaf pairs are formed. The first leaf pair consists of a single leaflet (Figures 1C and 2). The second leaf pair has three leaflets, the third leaf pair has five leaflets and so on, usually up to eleven leaflets (Clarke 1997). We consider a leaf as unfolded when its leaflets are at least one cm long.

Vegetative stage is defined by the number of fully developed leaves. Code 1002 is used for the first leaf pair, code 1004 for the second leaf pair and code 10xx for the n<sup>th</sup> leaf pair (xx = 2n). If the lower leaves have already been shed, it is necessary to count the nodes, taking into consideration that one node carries two leaves and that the first node belongs to the cotyledons.

Fibre hemp stems are usually not branched, but the stem can be ramified at low plant density. However, for the characterisation of the growth of hemp plants, branches are not taken into account for coding during the vegetative stage.

### **Flowering and seed formation (principal stage code 2)**

Depending on cultivar and day length, the change of phyllotaxis (leaf position) from opposite to alternate (Figure 3) indicates the induction of flowering („GV point“; Bócsa and Karus 1997). The first nearly indistinguishable flower primordia may appear and care has to be taken that they are not mistaken for branch primordia. Male primordia can be identified by their curved claw shape, soon followed by the differentiation of round pointed flower buds having five radial segments. The females are recognised by the enlargement of a symmetrical tubular calyx (Clarke 1997). Because the identification is difficult, for the coding system no sex differentiation of primordia is considered (Figure 4). In this phase the stem elongation slows down. The appearance of flower primordia, as well as the process of flowering proceeds from the base upwards to the top of the inflorescence (Clarke 1997).

After the GV point (code 2000) and the appearance of flower primordia (code 2001) have been reached, we distinguish the generative phase of male, female and monoecious plants by the second digit: '1' for male, '2' for female and '3' for monoecious plants. The third and fourth digits refer to the exact generative stage. Reproductive organs on branches are taken into consideration for the description of the flowering and the seed formation.

On dioecious male plants, staminate flowers usually appear about two weeks before the styles of female plants (Clarke 1997). The male inflorescence is branched and the hundreds of individual male flowers are in different stages of floral maturation (Figures 5A-B). We define the flower formation (code 2100) when the first still closed male flowers appear and the beginning of flowering (code 2101) when the first staminate flowers are open. The peak of the male flowering stage is reached when about 50% of the staminate flowers of one plant are open and pollen is released (code 2102). The end of flowering (code 2103) is reached when 90% of all the flowers are or have been opened.

On dioecious female plants, the appearance of the first perigonal bracts with no styles indicates that the female inflorescences will soon flower (code 2200). The inflorescence of female plants is leafy and compact. The female flower is hidden within the perigonal bract and is small, green and inconspicuous. At anthesis (pollen release) two styles protrude from each perigonal bract (Figure 6A). One female plant has numerous flowers in different developmental stages (Figures 6B-C). We define the beginning of the female flower formation (code 2201) when the first styles are visible, and the peak of this flowering stage is reached when 50% of the bracts within the inflorescence are formed, independent of whether styles are visible or not (code 2202; Figures 6A, 6D). After blooming and fertilisation, seeds (achenes) turn hard and seed shed begins (code 2203; Figure 6E). Single seeds mature in three to five weeks. Seed maturity is reached when 50% of the seeds are hard or have been shed (code 2204).

On monoecious plants male and female flowers are formed. The ratio of male : female flowers depends both on the cultivar and the individual plant. Flowering stage (code 2302) refers to female flowers and is similarly defined as for dioecious female plants. Male flowers usually appear during female bloom (codes 2303 and 2304) on the tips of female branches. Seed maturity is defined as for dioecious female plants (code 2306).

### **Senescence (principal stage code 3)**

After flowering of male, as well as after seed maturity of female or monoecious plants, leaves and stems start to dry (codes 3001 and 3002). Due to the frost in temperate regions,

the plant die and decomposition of the stem tissue causes the bast fibres to become free (code 3003).

### **Growth stages of a crop**

Definitions of developmental stages refer to individual plants. For agronomic purposes, however, it is necessary to be able to describe the growth stage of a field or a population (e.g., as 'a proportion at stage ...' or 'at a range of stages ... to ...'). The morphology of *Cannabis* plants varies depending on sex and growing conditions. The proportion of dioecious males, dioecious females and monoecious plants within a population depends on the cultivar. In a dioecious crop, male and female plants are usually present in about equal numbers. In a monoecious crop (e.g., French cultivars), depending on seed production, there are usually up to 30% male plants as well as a number of true-female plants (de Meijer 1995).

In order to identify the developmental stages of the crop, it is necessary to determine the stage of a sample of plants taken at random from the crop. For pea, faba bean and potato, a sample size of 20 plants is considered to represent the crop (Knott 1987 and 1990, Jefferies and Lawson 1991). But because of sex differences and self-thinning effect (Van der Werf et al. 1995b, I. Bócsa pers. comm. 1998), we propose a sample size of 50 to 100 plants for fibre hemp. For plot trials the sample size can be reduced to 30 plants.

In agreement with de Meijer *et al.* (1992) and de Meijer and Keizer (1994) the „limit of 50% of the plants“ was chosen for crop description (Table 3), e.g., male flowering is defined as the stage when 50% of the male plants have reached the code 2102. Flowering of dioecious female and monoecious is determined when 50% of plants in a population are in the stage of codes 2202 or 2302, seed maturity when 50% of seed-carrying plants reached codes 2204 or 2306.

**Table 3. Crop stages of *Cannabis sativa* L. plant populations**

<b>Plant code</b>	<b>Crop stage</b>	<b>Criterion</b>
0002	Emergence	50% of expected plants
2000	Flower induction	50% of all plants
2102	Dioecious male flowering	50% of all male plants
2202	Dioecious female flowering	50% of all female plants
2302	Monoecious flowering	50% of all monoecious plants
2204 or 2306	Seed maturity	50% of female or monoecious plants

### ***Practical use of a code for fibre hemp growth stages***

Depending on the use of the hemp crop, harvest time has an important effect on yield and quality of hemp products. For best fibre quality, harvest should be carried out during male flowering (code 2102) or during flowering of monoecious crops (code 2302; Bócsa and Karus 1997). Unfortunately, the European Union states that hemp harvesting - independent of utilisation - has to start after seed formation, i.e. code 2204 or 2306 (Hennink 1997, European Union 1998). This stage is the best time for seed harvest (Bócsa and Karus 1997). For the production of essential oil, the recommended stage is one to three weeks before seed maturity (codes 2203 and 2305; Meier and Mediavilla 1998). At this time the highest level of the secondary compounds (e.g., volatile compounds) is reached.

Another practical use of growth stages is fertilisation. In contrast to the custom in most countries, where all fertiliser are given at planting, Mediavilla *et al.* (1998), recommends for Switzerland to split the nitrogen fertilisation in order to reduce nitrogen loss. One rate is given at the emergence (code 0002) and the second rate when the third leaf pair has been formed (code 1006).

For the determination of the cannabinoid content, in accordance with other authors, the inflorescences were collected at the beginning of the seed maturity (code 2203 and 2305; de Meijer *et al.* 1992). The appearance of diseases can be easily described by using a decimal code (McPartland 1996a, 1996b). For computer storage of data referring to the development of plant populations, e. g. for growth analysis, the decimal code is very useful.

### **Acknowledgements**

The help of the International Hemp Association is gratefully acknowledged. Part of this work was financially supported by several Cantons in the Northwest of Switzerland and the Institut Transfrontalier d'Application et Développement Agronomique (Colmar, France).

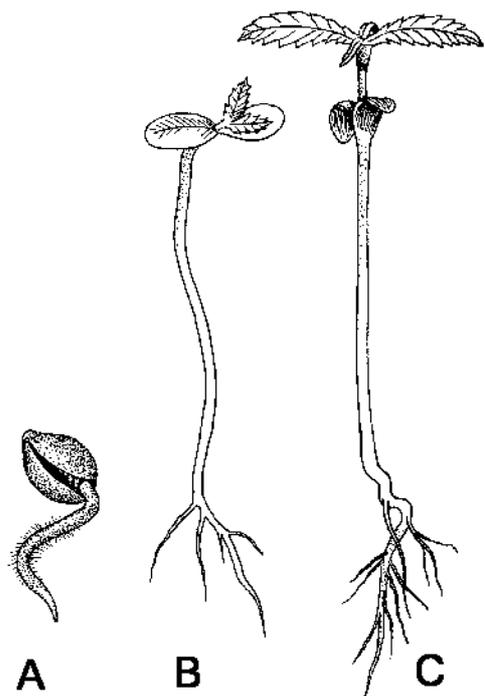
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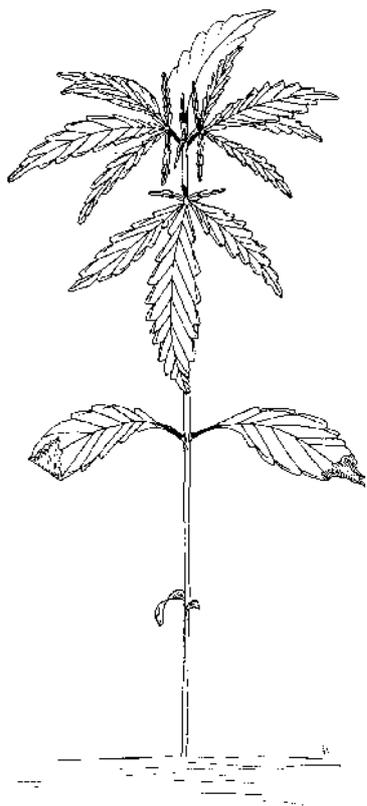
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**Figure 1.** A: Radicle apparent (code 0001), B: cotyledons unfolded (code 0003), C: first leaf pair (code 1002).



**Figure 2.** Third leaf pair (code 1006).



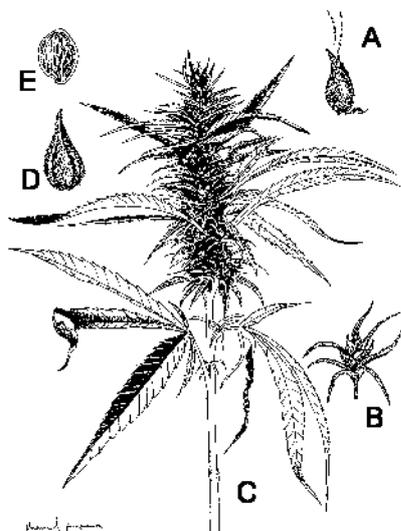
**Figure 3.** Change of phyllotaxis (code 2000).



**Figure 4.** Flower primordia (code 2001).



**Figure 5.** A: Staminate flowers open (code 2102), B: detail of a staminate male flower.



**Figure 6.** A: Pistillate female flower (stigmas, style, perigonal bract and stipule), B: spike, C: inflorescence, D: formed perigonal bract, E: hard seed.