NETTLE PAPER

OUR GOAL
TO REVERSE NETTLE IN A CROP AVAILABLE FOR HUMANS IN ALL WORLD

based on know-how

FROM TUBES THROUGH THE FIELD ON THE FORK

CONTENT

1. Opportunity

1.1 Burda´s farm and opportunity to use weedy stinging nettle

The farm cultivates on 4.5 ha agricultural soil in the status of ecological farming. Some from the fields are in the area where nettle (*Urtica dioica*, L.) weeding was trouble. Due to the restriction of usage of herbicides in ecological farming we were up for a challenge. How could these lands be beneficial? From agricultural historical data, we found out that nettle cultivation is possible (for textile usage especially). For example, during World War I, both Germany and Austria were running out of cotton. They chose the nettle as a suitable substitute. Furthermore, it is known, nettle is a suitable plant as well as for ecological cultivation or permaculture (Vogl and Hartl, 2003). Many scientists have already tried to cultivate nettles. The main benefits are:

1. there is data on good yield without nitrogen fertilization (Kohler *et al*., 1999)

2. the crop is grounded for several years (within 10-15 years it still achieves a satisfactory yield (Gatti *et al*., 2008)

3. green matter can be used in several ways (unsucked production to the food industry can be used in e.g. biogas, or used as a nutritionally full feed for livestock. This creates minimal losses as nettle is one of the low-cost plants)

Other advantages include:

• easy propagation and sustainable yields when using the principles of organic farming (Gatti *et al*., 2008)

• as a perennial crop, it requires lower inputs in terms of soil processing, which maintains soil fertility and its structure (e.g. protects the top layer of soil against erosion, crusting and excretion (Szewczuk *et al*.,2002)

• In addition to these effects, it also promotes biodiversity and acts as a food source for useful insects (e.g. water nymphs)

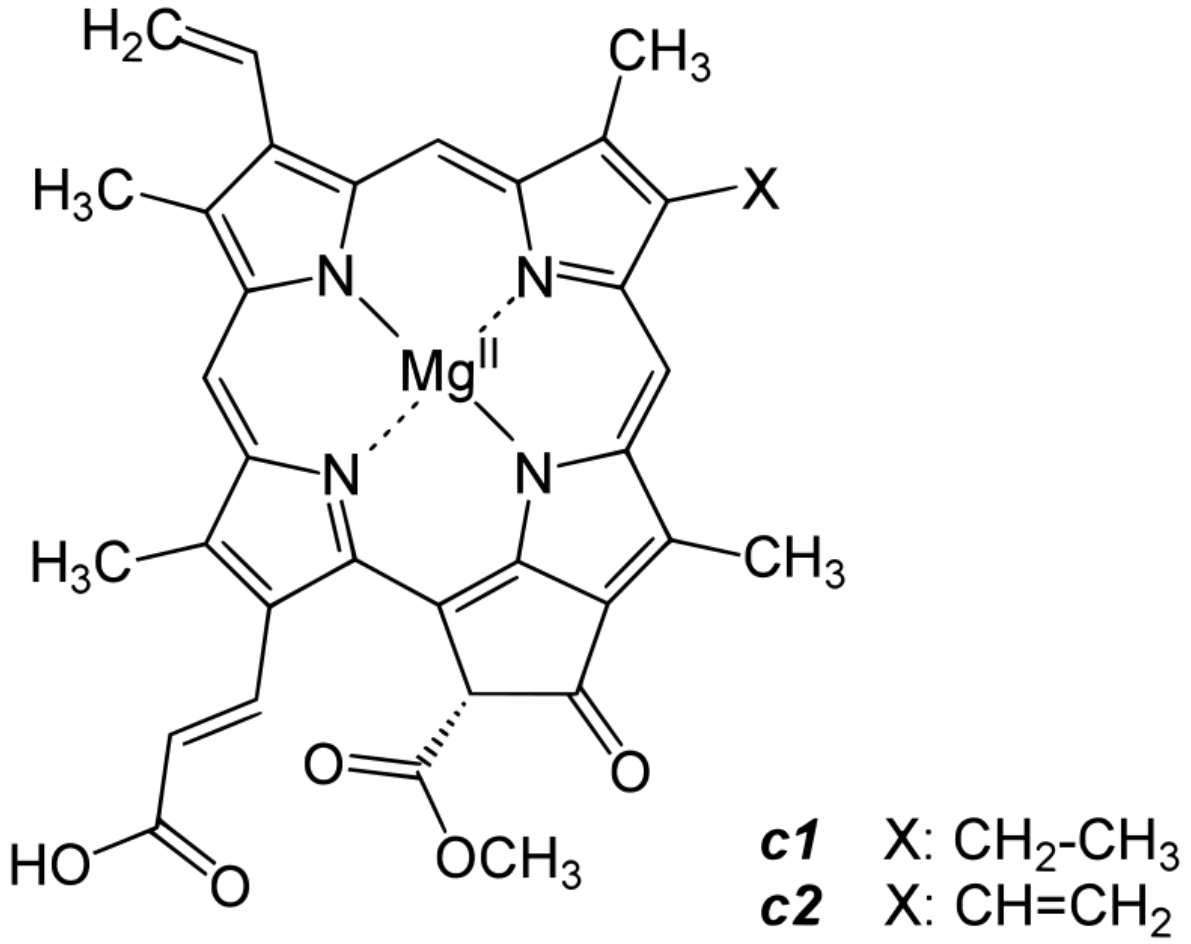
• is a fast-growing plant and therefore has an advantage over other weeds in the absorption of water and nutrients (Bacci *et al*.,2009), so the use of chemicals against weeds is not necessary

• the leaves are very rich in minerals and when they fall off, an important amount of nutrients are returned to the soil

1.2 Main benefits of stinging nettle

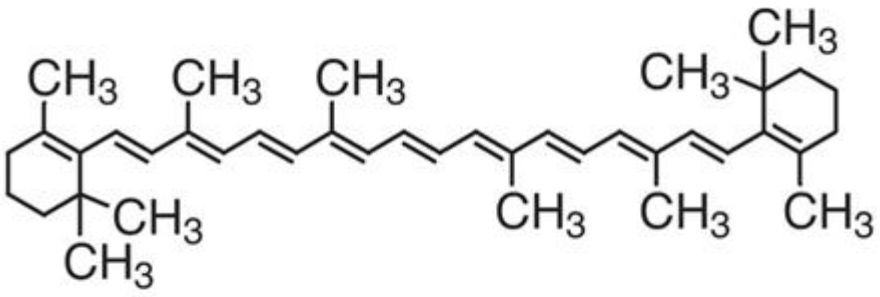
There are other reasons for growing nettle, it can be used whole - leaves, seeds, stem and root. In addition, many studies confirm very interesting compounds found mainly in nettle leaves, which have great potential for the food, pharmaceutical and cosmetic industries (tab. 1). Upton (2013) states that nettle has a high nutritional value and contains in its leaves: vitamins A, D,C, E, F, K and B-

complexes, proteins, calcium minerals, iron, potassium, manganese, choline, amines, antioxidants chlorophyll (picture 1) and 5-hydroxytryptophan.



Picture 1. Chemical structure of chlorophyll. There are several types of chlorophyll, but all share the chlorin magnesium ligand which forms the right side of this diagram. Chlorophyll stimulates the immune system or eliminates fungus in the body.

Carotenoids (which include vitamin A) are a nutritionally important component, and a total of 9 have been identified in nettle leaves, the largest proportion being β-carotene (picture 2), which is a precursor of vitamin A.



Picture 2. Chemical structure of β-carotene. It is a member of the carotenes, which are terpenoids (isoprenoids), synthesized biochemically from eight isoprene units and thus having 40 carbons. Among the carotenes, β-carotene is distinguished by having beta-rings at both ends of the molecule.

Guil-Guerrero *et al*., 2003 states that the total amount of carotenoids in fresh leaves is 29.6 mg 100^{-1}g dry weight. As is known, their fundamental contribution lies in antioxidant action. This means that it protects cells from damage from unstable oxygen molecules, so-called free radicals. Rafajlovska *et al*. (2001) states that extracts of nettle plants contain 6.8% palmitic, 1.1% stearic, 3.6% oleic, 20.2% linoleic and 12.4% linoleic acid. These fatty acids are an important component of lipids, and some of them are essential. They are important sources of energy for humans and animals too. Therefore, when they are metabolized, they yield large quantities of ATP.

A lot of studies have shown that *Urtica dioica* can be used in medicine. The aqueous and alcoholic extracts have been used for hundreds of years for the treatment of anaemia (Pinelli *et al*., 2008), rheumatism (Jaric´ *et al.*, 2007), gout and eczema (Orčic´ *et al*., 2014), and treatment of urinary, bladder and kidney problems (Orčic´ *et al*., 2014). Beneficial effects have also been reported on inflammation, hypoglycaemia, hypotension, benign prostatic hyperplasia, cardiovascular problems, arthritis, and allergic rhinitis (Upton, 2013). Furthermore, stinging nettle exhibits antioxidant, antimicrobial, antifungal, antiviral, and antiulcer activity (Upton, 2013). Nettles possess noticeable antimicrobial activity against Gram-positive and Gram-negative bacteria when compared with standard and strong antimicrobial compounds, such as miconazole nitrate, amoxicillin-clavulanic acid, ofloxacin and netilmicin (Gülçin *et al*., 2004). Different fractions of various *Urtica* species have been studied to determine their antimicrobial activity. The results indicate the great potential of this plant for the discovery of novel effective compounds.

Tab. 1 Chemical composition of nettle leaf powders (Adhikari *et al*. 2016)

Parameter	**Content**
Moisture (%)	7.04±0.77
Crude protein (%)	**33.77±0.35**
Crude fiber (%)	9.08±0.14
Crude fat (%)	3.55±0.06
Total ash (%)	16.21±0.54
Carbohydrate (%)	37.39±0.72
Calcium (mg/100 g)	168.77±1.47
Iron (mg/100 g)	227.89±0.21
Tannins (%)	0.93±0.01
Polyphenols (mg GAE/g)	128.75±0.21
Carotenoids (μg/g, db)	3496.67±0.56
Caloric value (kcal/100 g)	307.24±0.13

Nettle provides humans and animals with nutrients and bioactive components, which support antimicrobial activity, immune enhancement and stress reduction. It is worth noting that theWorld Health Organization (WHO), in its monographs on 'Selected medicinal plants', describes *Urticae* as valuable herbs for many medicinal uses. The European Commission Directorate-General For Health and Food Safety showed that *Urtica* spp. fulfils the criteria of a foodstuff, as defined in Regulation (EC) No. 178/2002. This opinion is supported by the European Food Safety Authority(EFSA). It concluded that nettle has neither an immediate nor delayed harmful effect on human or animal health and has no negative effect on the environment.

2. Solution

2.1 Establishment of nettle field and testing food production

We established a testing cultivation nettle field. The first yield was about 9tha^{-1}. There was information about the cultivation of stinging nettle for the textile industry during the second war in Germany. In 1940 stinging nettle joined the wild plant breeding programme for fibre production at the Institute of Applied Botany in Hamburg, which managed to derive several clones with high fibre content and in this point of view, that could be considered an agricultural crop for this purpose. These clones are still kept in German and Austrian research institutions (Vogl and Hartl, 2003). At the start, we took this historical agrotechnical data and we tried to apply them to our conditions. However, the chemical composition of plants is affected by different factors, including the variety, genotype, climate, soil, vegetative stage of the plant, harvest time, storage, processing and treatment (Angela and Meireles, 2009). When and how nettles are harvested strongly determines the final product. For example, for fibre production, stinging nettles should be harvested when the seeds are mature or when the stalks reach 80% of the aboveground biomass, from the second year of planting. During the first year, the stalks are too thin, too ramified and have too many leaves. If the main product is to be the leaves, younger plants are harvested. The time of year for nettle harvesting depends on the purpose. Plants collected in April are used for fodder, medicine or chlorophyll production. Nettles harvested at the end of June are used for fibre production. The second harvest in September may be used for the collection of leaves (Di Virgilio *et al*., 2015). It seems that the quality and chemical composition of stinging nettle will be affected by epigenetic factors. It means we are able by suitably chosen agrotechnology to produce suitable plant material for food production in this way we can influence the taste of final products (eg. hydroponic or aquaponic culture, as well as indoor technology).

It is important to realize that a big advantage of nettle cultivation is that harvest could be used for plenty of usages: feed, fertilizer, mass into biogas, textile and last, not least food. Therefore, nettle is appropriate for a circular economy (more detail in the business plan).

After harvesting non-cultivated nettle (heterogeneous material), we started to test if it would be possible to make food products (pasta, lemonade etc.) based on different conditions of agrotechnology. After we fine-tuned agrotechnology and food technology, we found the taste, which is interesting and extra the nettle belongs to a superfood (Kriegel *et al*., 2018). Our nettle products perfectly fit a healthy lifestyle. Our pilot food products we released in the market and customers reacted positively. Based on this data we did market research and we revealed a gap - there are a few food products from nettle and all of them are not customer convenience (more details in a section Business plan). Therefore we take advantage. We found out we need to have nettle as a crop.

2.2 Clone preparation and reproduction

However, we did not have homogenous material due to the dioecy of nettle, which has a separate male and female sex. Closely, the stinging nettle (picture 3) is a perennial herbaceous plant belonging to the *Urticaceae* family.

Picture 3. Plant habitus of *Urtica dioica*

It is a well-known and common species, spread in temperate and tropical zones of Europe, Asia and America, adapted to a variety of climatic conditions. Stinging nettle is a perennial, monoecious plant, flowering and fruiting in the summertime. Its stems and leaves are covered by stinging trichomes containing a fluid that causes blistering when entering the skin (Bisht *et al*., 2012). Surprisingly, there are several subspecies for this species, probably due to polyploidization; most plants are predominantly tetraploid in nature (Rejlová *et al*., 2019). Genetic analyses point to a model of gender inheritance that is not bound to sex chromosomes and is predominantly Mendelistically controlled by one gene, however, there are exceptions to this model (Glawe and Jong, 2008). For this reason, homogenisation of nettle is difficult, however by using the in vitro method, it is possible. In vitro plant tissue culture is a collection of techniques used to maintain or grow plant cells, tissues or organs under sterile conditions on a nutrient culture medium of known composition. It is widely used to produce clones of a plant in a method known as micropropagation. Plant tissue culture is used widely in the plant sciences, forestry, and horticulture. For nettle applications, we can use:

- **the commercial production of plants used as potting, landscape, and florist subjects, which uses meristem and shoot culture to produce large numbers of identical individuals -clones - (already done)**
- to conserve rare or endangered nettle species
- a plant breeder may use tissue culture to screen cells rather than plants for advantageous characters, e.g. salt resistance/tolerance
- large-scale growth of plant cells in liquid culture in bioreactors for the production of valuable compounds, like plant-derived secondary metabolites and recombinant proteins used as biopharmaceuticals

- to cross distantly related species by protoplast fusion and regeneration of the novel hybrid
- to rapidly study the molecular basis for physiological, biochemical, and reproductive mechanisms in plants, for example in vitro selection for stress-tolerant plants
- for chromosome doubling and induction of polyploidy, for example, doubled haploids, tetraploids, and other forms of polyploids. This is usually achieved by the application of antimitotic agents such as colchicine or oryzalin.
- as a tissue for transformation, followed by either short-term testing of genetic constructs or regeneration of transgenic plants

Therefore we started a biotechnological approach in collaboration with Mendel University in Brno. Based on *in vitro* technology, our co-partner was able to generate genetic homogenous nettle called clones (picture 4). Because we have obtained knowledge about nettle habitus connected to food properties from field observation, we could provide appropriate candidates for micropropagation.

Picture 4. Nettle clones growing in vitro culture (Ing. Kamila Lónová, Department of Plant Biology, Mendel University in Brno)

After acclimatization, we obtained special clones to make our growth of nettle homogenous. Nowadays, we cultivate these clones in our field Vlčice (picture 5). We are able to trade these clones thanks to nettle nursery and as well as to offer consultancy regarding nettle cultivation.

P**icture 5. Nettle field in the Vlčice area**

Our main know-how is the production of 3 products: juice - spinach - cold syrup suitable for retail packaging as well. Our production process is specific in 3 ways of fermentation. We can preserve nettle with salt, fat, acid and freeze it. Moreover, we can produce as well as nettle extract for cosmetics and pharmacy. Furthermore, we processed the protocol for nettle explant, so we have opened the door for the breeding program of nettle by using a biotechnological approach, which makes our expansion easier.

3. Our vision

Our potential, we see in the combination of new disciplines of biotechnology, nanotechnology and blockchain. Our vision is to make quality nettle products that will be fitted for food, textile, cosmetics and pharmaceutical ware based on knowledge from genomics or nanotechnology and their tools. To realize our vision we need an economic tool making external markets available, therefore we take advantage of blockchain and token economy.

BIOTECHNOLOGY, NANOTECHNOLOGY AND OUR VISION
(FROM TUBES THROUGH THE FIELDS ON THE FORKS AND ON BODY)

Clones are a future for nettle farming due to obstacles with homogenization due to the dioecy of Urtica dioica. We plan to do deeper genomic analysis based on next-generation sequencing. We have already processed genome sequencing by an Illumina method (picture 6) to see basic differences between males and females (the cooperation with The Application Laboratory for Agricultural Research). Based on this data, we clearly see that transcriptome sequencing will be needed to reveal candidate genes playing a crucial role in metabolic pathways which are targeted. After identification of these genes, we will be able to produce plants with novel and extraordinary traits.

Target metabolic pathways for genetic engineering:

- phenolic pathways
- proteosynthesis
- metabolisms of flavonoids, tannins
- fatty acids synthesis
- metabolic pathways in trichomes

We would like to be a crucial player in the nettle products delivery in the world. To realize our vision we need an economic tool making external markets available, therefore we take advantage of blockchain and token economy. Among nettle food products you will find such products that even classic large food businesses or food retail companies need not be ashamed of. They are both conventional and organic products. Superfood, kitchen ingredients and semi-finished products, products for direct consumption originating from ecologically clean locations, of course, glutein-free and non-vacate products, vegetarian and vegan foods. Typical products of bakers, confectioners, liqueurs, brewers, milkmen, cheesemakers, butchers, smokers. Not only these nominal products can find an excellent basis or ingredient in the technologically well-processed and prepared nettle.

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