Selection and Production of Oregano Rich in Essential Oil and Carvacrol

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Abstract

There is an increasing interest in oregano essential oil and its component carvacrol for the use as a feed additive with antimicrobial properties, enhancing the health of poultry and pigs. This chapter describes the initial agronomic attempts (in the years 2001-2004) to acquire and develop *Origanum* strains rich in essential oil and carvacrol for optimal field production (of crop biomass and essential oil yield) under Dutch climatic conditions. Research activities to improve field production of oregano and efficient processing of this crop are briefly described.

INTRODUCTION

The Dutch company Ropapharm® has a growing demand for oregano essential oil with a minimum amount of 60% carvacrol for the production of feed additives, mainly used in pig and poultry feeds. Oregano oils, high in carvacrol, have strong antimicrobial activity (Ultee, 2000). Added in a specific additive and concentration to animal feeds it inhibits undesired bacteria (Salmonella, Clostridium, *E. coli*) in the gastro intestinal tract, reduces the risk of (or even stops) diarrhoea, thus improving the health and growth (weight gain/day) of the animals significantly (Günter and Bossow, 1998). Oregano oils are mainly sourced from different producers and middlemen based in Mediterranean countries. But most of the time their exact origin and the way they are produced is hardly documented. In order to secure the future supply of high quality oregano oils, from a reliable source, competitively grown and processed in a reproducible way under GAP/GMP conditions, PPO was asked to investigate the feasibility of oregano production in the Netherlands.

ASSUMPTIONS AND GOALS

Oregano is known to primarily be a Mediterranean herbal crop, consisting of many species and subspecies with huge intra- and inter-specific variation. The common opinion about establishing a viable production is to create clonal propagated chemotypes from individual selected plants (Putievsky et al., 1996). Considering the huge costs of vegetative propagation (in-vitro plants or cuttings), having in mind the aim of expanding the production acreage in a short period of time, we choose to develop improved selected populations and propagate them generatively. In this way seedlings can easily be raised from seeds and initial costs of production are limited. To make field production economically feasible, having in mind the average market price of the oil, we focused on producing 100 kg of essential oil per hectare, with a minimum of 60% carvacrol.

Apart from optimizing agricultural practices, large-scale efficient crop production finally has to be complied with efficient processing (harvesting and distilling) of the crop. For this we aimed on adapting the distilling techniques used in the US mint industry to our production conditions of oregano.

COLLECTING AND COMPARING DIFFERENT *ORIGANUM* ORIGINS

Collecting Different *Origanum* Origins

Most of the work on *Origanum*, done by different research groups all over the

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world, has been focused on the genetic resources, the huge variability in the genus *Origanum*, its difficult taxonomy, and the use of oregano as a spice crop. It has been summarised in the proceedings of the IPGRI International Workshop on Oregano, held in Italy in 1996 (Proceedings IPGRI workshop, 1996). Only recently, because of the increasing demand for oregano oils rich in carvacrol several initiatives (Greece, Germany, Israel and US) started focusing on the production of these specific oils. In *Origanum* the specie *vulgare*, with six different subspecies, is most important. There are purple and white flowering *Origanum* species, the first one being low in essential oil and carvacrol mainly being produced for culinary purposes. The white flowering *Origanum vulgare* spp. *hirtum* and *Origanum heracleoticum* are known to have high amounts of essential oil with carvacrol being the main constituent (Ietswaard, 1980). But even in these (sub-)species the amount of carvacrol varies according to the geographical areas where they are collected (chemotypes), and the time of harvesting (Kokkini, 1996). Being aware of the difficult taxonomy of the genus *Origanum*, the different names given to species and subspecies and the wide variety in their essential oil percentage and oil composition, for our experiments we had to find the right material to start with.

In 2001 we specifically asked some colleagues abroad and some seed companies for oregano seeds, most preferably from types high in oil and carvacrol.

Seeds from 11 different origins were received, all of them under scientific Latin or trivial names. In the greenhouse in April seedlings were grown from each of them. Mid-May a field trial was established in which the seedlings were planted. Each origin was planted in three replicates of small fields (1,5x5m, and plant spacing 30x40cm).

Results of Comparing the Different *Origanum* Origins

In 2001 the crops derived from these *origanum* origins basically only established themselves with a relatively poor growth. There was some flowering and in autumn, although there was only a small amount of biomass there, the crops were harvested. Fresh crop samples were steam distilled and it turned out that, apart from origin O1 (a purple one with a reasonable amount of carvacrol) only the five white flowering origins were high in the amount of essential oil and the percentage of carvacrol in the oil. Apart from Origin O11 (which originated from Chile) all origins survived the (average) Dutch winter (2001/2002) and a vigorous regrowth started in March/April.

In 2002 good crops developed producing nice yields in the first cut at the end of June.

Fresh crop samples were steam distilled and the oils were analyzed on their carvacrol content. A second cut at the beginning of October, added small yields. Only from the white flowering origins, with high oil and carvacrol amounts at the first cut, fresh samples were steam distilled and checked on carvacrol again.

Table 1 gives an overview of the results (biomass, essential oil percentage, percentage of carvacrol in the oil and the amount of oil/ha) for the different *Origanum* origins compared in the second year of cultivation.

It is clear that the purple flowering *Origanum* origins produce higher biomass yields but have low essential oil percentages (and low carvacrol content of the oil) compare to the white flowering ones. The four white flowering origins O4, O6, O8 and O11 were acquired under the respective names *O. hirtum* (presumably *O. vulgare* spp. *hirtum*), *O. heracleoticum*, *O. vulgare* and *O. vulgare* spp. *hirtum*. Although there were huge individual plant variations within (and in between) these populations they were slightly distinguishable, O11 being the most uniform of all.

Main result of this 2002 comparative field trial, in which the crops were mechanically harvested and substantial crop samples (of a few kilos) were steam distilled, is that the four desired *Origanum* origins yielded 100 kg of essential oil per ha, with satisfying carvacrol levels (around 60%)! It strongly supported the idea to be able, even in the Netherlands, to establish a viable oregano oil production.

STARTING COMMERCIAL PRODUCTION AND SELECTION

With the acquired four well producing *Origanum* origins, the first step towards commercial production started. Based on these four origins a programme of individual plant selection was scheduled to improve the material, raising the oil and carvacrol amounts and narrowing the heterogeneity of the populations. Agro technical research was foreseen to provide the information for efficient (chemical) weed control, correct fertilization, and the optimum time of harvesting.

In 2003 all purple flowering origins, and the purple flowering individuals within the generally white origins, were removed. Seed was produced separately from each of the four left over origins. These seeds were used to start commercial production in 2004. For this a new project, including commercial partners was formulated. In the scope of this paper only some of the first experiences from the 2004 season can be mentioned here.

Planting

For field production seedling plants (with up to ten seeds per seedling sowed) were raised and machine planted in May, at 50cm wide rows and an in-row plant spacing of 33cm (50x33, which equals 60.000 plants/ha). From all original four *Origanum* origins an offspring, being a population, of 1 ha was planted at PPO. Although planting seedlings is expensive it is still the best option to establish an oregano crop. Vegetative propagation by planting cuttings or in-vitro plants would take too much time and is far too costly. Sowing directly in the field unfortunately is not yet an alternative. It requires too much seed and has a serious risk of production failures due to poor crop establishments.

Changes in the Accumulation of Essential Oil during Crop Development

As can be expected for a first year crop the oregano developed slowly but steady. The plants well established themselves and formed a limited amount of biomass. Most of the growth had a creepy character, but in June some erect development and even some early flowering started. To get an idea of possible changes in the oil percentage during development of oregano during its first year, crop samples of all populations were harvested four times (monthly during the growing season) and directly steam distilled.

Table 2 gives an overview of the results. It's obvious that the essential oil percentage (on FW-basis) increases significantly during the season. Populations O4 and O11 perform better compared to the other two.

Comparing the Quality Abroad

To compare the performance of the four populations elsewhere, seeds were provided to an Israeli colleague (N.Dudai, Newe-Ya'ar Research Center, Israel) At Newe-Ya'ar experimental station seedlings were raised, transplanted to the field and grown under different climatic conditions. After a few months of development crop samples were taken and analyzed. Table 3 gives the figures of the oil percentages (on a FW basis) and some of the main oil components.

It's obvious that the essential oil percentages are much higher under the Israeli climatic conditions. But, as in the Netherlands, the populations (offspring of the origins) O-4 and O-11 are higher in essential oil than the other two. In all four populations the carvacrol content of the oil is well over the desired 60%.

These first years of experimenting (2001-2004) with oregano shows that although the existing heterogeneity in (and in-between) the originally acquired origins and their offspring's they are slightly distinguishable, both in phenotype and development and with respect to their average quality (essential oil). Whatever their history, the four *Origanum* origins and offspring's seem to have the first characteristics of a population, waiting to be homogenized and improved by strict individual plant selection.

Start of the Selection Process

Apart from the production fields, in 2004 a specific selection field was established

in which individual plants from the four oregano offspring's were planted in a larger plant spacing (50x40) to be able to properly judge the development and appearance of every individual plant. Doing this made it even clearer that there's a huge variability between individual oregano plants within and in between the populations. An exciting experience and starting-point for effective strict selection! In this field individual plant selection primarily based on the plant phenotype started. Well developing, erectly growing, leafy, healthy (and if flowering in the first year) white flowering plants were marked. In 2005 the selection process in a second year crop, including analyzing the essential oil and the carvacrol content of the oil, will continue. Finally isolated seed production from the best individual plants will provide improved, much more uniform and high-quality, oregano populations.

Agro Technical Research

Along the introduction of oregano as a new crop in an efficient production system, some agro technical issues needed to be cleared. In 2004 field trials were established to check the possible use of different herbicides, and to find out the required amount of nitrogen fertilization needed for optimal crop production. During time an agro technical and economic evaluation will be executed to compare production of clonal propagated 'chemotypes' and generative propagated (improved) populations. Scientifically it is important to see how close, especially qualitatively, generative propagated improved oregano populations can get to determined oregano clones. Also the lifespan of oregano plantings under Dutch climatic conditions and agro technical treatments have to be determined since this has a tremendous influence on the production costs.

Proposed Harvesting and Distilling Systems

The proposed harvesting technique and distilling system will basically be copied from the system used in US mint farming, but will have to be adapted and optimized to our specific oregano production system. It is foreseen to start experimenting with this in 2005, the second year of commercial production in which the first significant oil yields will be expected.

Literature Cited

Ultee, A. Bactericidal action of carvacrol towards the food pathogen Bacillus cereus. A case study of a novel approach to mild food preservation, 2000. Thesis Wageningen University.

Günter, K.D. and Bossow. H. 1998. The effect of etheric oil from *Origanum vulgaris* (Ropadiar) in the feed ration of weaned pigs on their daily feed intake, daily gains and food utilization. Proc. 15th IPVS Congress, Birmingham, 223.

Putievsky, Eli, Dudai, Nativ and Ravid, Uzi. Cultivation selection and conservation of oregano species in Israel, Proceedings of the IPGRI international Workshop on Oregano, Italy, 1996: 102-109.

Oregano. Proceedings of the IPGRI International Workshop on Oregano, 8-12 May 1996, CIHEAM, Valenzano, Bari, Italy, editor; S. Padulosi.

Ietswaard, J.H. 1980. A taxonomic revision of the genus Origanum (labiatae). PhD thesis. Leiden Botanical Series 4. Leiden University Press, The Hague.

Kokkini, Taxonomy, diversity and distribution of Oreganum species, Proceedings of the IPGRI international Workshop on Oregano, Italy, 1996: 2-12.

Tables

Table 1. Fresh biomass yield, essential oil percentage, the amounts of carvacrol in the oil (only in first cut) and the essential oil yield of different *Origanum* origins compared in a field trial, PPO 2001-2002

		First cut 2002				Second cut 2002			Total
Origin and color of inflorescence		Fresh mass t/ha	Oil % FW	Oil kg/ha	Carvacrol %	Fresh mass t/ha	Oil % FW	Oil kg/ha	2002 oil kg/ha
O1	Hungary/purple	15,3	0,12	20,6	50,8	13,57	0,08	10,9	31,5
O2	Dutch seedcomp./purple	25,3	0,08	19,6	0,5	20,13	*	*	
O3	Dutch seedcomp./purple	24,3	0,07	17,6	1,2	19,27	*	*	
O4	Dutch seedcomp./white	19,7	0,41	**80,4**	**61,6**	12,0	**0,25**	32,4	**112,8**
O5	Germany/purple	22,7	0,07	16,6	2,6	19,4	*	*	
O6	Germany/white	20,7	0,38	**79,5**	**63,2**	9,63	**0,22**	21,2	**100,7**
O7	Finland/purple	19,8	0,09	17,8	1,5	16,3	*	*	
O8	Finland/white	18,8	0,33	**61,3**	**54,8**	7,9	**0,2**	15,8	**77,1**
O9	German seedcomp./purple	24,4	0,07	17,0	0,3	19,7	*	*	
O10	Chile/white	Did not survive during winter 2001-2002							
O11	US seedcomp./white	17,4	0,30	**53,9**	**57,7**	9,67	**0,3**	29,0	**82,9**

*Not determined

Table 2. Essential oil (percentage of FW) during crop development of four Oregano populations in their first growing season at PPO 2004.

Population	June-28	August-18	September-8
O-4	0,20	0,23	0,39
O-6	0,16	0,23	0,26
O-8	0,17	0,18	0,24
O-11	0,25	0,32	0.39

Table 3. Essential oil (percentage of FW) and main oil components of four Oregano populations in their first growing season in Israel, Newe-Ya'ar Research Center, 2004.

Pop.	**Oil%**	Para-cymene	G-Terpinene	Thymol	**Carvacrol**	Germacrene D
O-4	**0,72**	3,83	12,20	0,30	**68,28**	1,50
O-6	**0,53**	2,27	17,22	0,27	**65,09**	0,59
O-8	**0,45**	2,54	9,32	0,28	**76,35**	0,69
O-11	**0,61**	2,56	11,24	0,27	**75,24**	0,08