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Proceedings of the 3rd European Workshop on Sea Buckthorn
EuroWorkS2014
Naantali, Finland, October 14-16, 2014

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Preface

Producing sea buckthorn of high quality asks skills and knowledge in every step of the food chain from the field to the consumer. The 3rd European Workshop on Sea Buckthorn (EuroWorkS2014) was held in Naantali, Finland on 14th to 16th of October 2014 under the theme "Producing Sea Buckthorn of High Quality". Conference concentrated on three topics that were recognized to be current under the theme: sea buckthorn fly, cultivation technology and standardization of sea buckthorn.

A special attention was paid to sea buckthorn fly because of its rapid and destructive invasion to Europe. Protective measurements need to be studied fast to get this new pest under control. Also long-term strategies are needed in order to continue efficient berry production, also without pesticides. Dr. Ljubov Shamanskaja has a long research experience with sea buckthorn fly in Barnaul, Russia, where the fly has been a problem over 20 years. Dr. Claudia Daniel has studied different control measuments for cherry fruit fly. It is a close relative of sea buckthorn fly and some of the protection methods may be adapted.

Since sea buckthorn is a relatively new crop in Europe its cultivation technology is still under investigation. Especially should be focused on more efficient harvesting method, new cultivars suitable to European climate, bigger yields by right cultivation methods and pest and disease control. Dr. Friedrich Höhne is concentrated on practical research in Germany, but he has a good knowledge of overall production situation in Europe. Entrepreneur Andrejs Bruvelis has a 30 years' experience of cultivation and nursery production so that his theories are worth to try and test in other countries, too.

International sea buckthorn society would like to create uniform standard for sea buckthorn and its products. What it could be was discussed during this conference. Dr. Jörg-Thomas Mörsel, a member of ISA standard group, and director and sea buckthorn grower David Eagle gave introduction to the challenging topic.

Number of presentations per workshop was restricted to two so that there was enough time for discussion and question resolving. Notes of the workshops were written down and delivered to the participants. In addition to six key note presentations also 16 posters were shown that introduced a wide range of new research. Altogether 67 research scientists, entrepreneurs and advisers from 12 European countries participated in the conference.

The other two European Seabuckthorn Conferences were held on 2010 in Postdam, Germany and on 2012 in Vilnius, Lithuania. Presentations of these conferences can be found from the home page of German Society for Sea Buckthorn and Wild Fruits www.sanddorn.net/EuroWorkS . This society is the creator of European Sea Buckthorn Conference and it coordinates its biannual event. Next meeting will be on 2016 in Latvia. EuroWorkS is organized alternate years with International Sea Buckthorn Conference. The organizer of international conferences is International Sea Buckthorn Association (ISA) and next meeting is held in India on November 2015.

The organizers of EuroWorkS2014 were MTT Agrifood Research Finland (the new name of organisation is Natural Resources Institute Finland) and German Society for Sea Buckthorn and Wild Fruits. Official conference homepage is www.sanddorn.net/EuroWorkS, where keynote presentations are available. Members of the organizing committee were Jörg-Thomas Mörsel, Sanna Kauppinen, Ekaterina Petruneva, Sylvia Thies, Ralf Godeck and Heikki Kallio. Conference was financed by MTT Agrifood Research Finland, German Society for Sea buckthorn and Wild Fruits and Federation of Finnish Learned Societies.

Hopefully these proceedings give you new thoughts and ideas to develop your own research and production and stimulate you to join sea buckthorn society to know more and share your thoughts with us.

May 2015
Editors

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Keynote Speaker Proceedings

Workshop I: Pests and diseases of sea buckthorn – our main concern is sea buckthorn fly

Bioecology of the sea-buckthorn fly (*Rhagoletis batava obscuriosa* Kol.) and pest control treatment in Altai

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Summary

The sea buckthorn fly is known as the main insect-pest of sea buckthorn on the territory of Russia. In Altai Krai, where sea buckthorn is the main industrial plant, crop losses from this insect-pest reach 100 % as larvae harm and destroy the pulp of fruit.

The sea buckthorn fly is a highly specialized insect-pest damaging only sea buckthorn berries. More than half of the varieties of Altay genotype are highly susceptible to the pest. Early varieties are damaged most strongly. We picked up only one pest–resistant form and some steady-state forms which can be cultivated without carrying out protective measures.

Population of the sea buckthorn fly changes considerably year by year and depends on biotic and abiotic environmental factors.

The short-term forecast of the sea buckthorn fly's productivity developed at our institute allows to refuse from carrying out spraying against the pests in certain years.

In the years of mass development of sea buckthorn flies, a reliable protection of crop is provided by protective measures directed against a harming phase – larvae. Spraying is carried out within the period of larvae hatching when their number reaches 5-12 %. Any delay in terms of protection promotes considerable losses of crop and deterioration of its quality by the main rate – the content of oil.

A method of decreasing the dose of chemical and biological pesticides by 2-10 times was developed, allowing the reduction of content of toxic remains in fruits and receiving a crop without remains of pesticides.

Key words

Sea buckthorn (SB), sea buckthorn fly (SB fly), short-term prognosis, agents, efficiency, productivity, quality of fruit.

Introduction

The sea buckthorn is the main culture in industrial horticulture in Siberia. Owing to the high ecological plasticity and the richest biochemical structure of the fruit sea buckthorn cultivation has been started in many countries of the world.

As a rule, insect-pests, among which the SB fly is the most harmful, follow SB in exploring new habitats.

On the territory of Russia crop losses because of this pest reaches 100 %. Universal distribution of SB flies in the midland of Russia became the main reason for withdrawing sea buckthorn berries cultivation from this region.

In Altai Krai, where the main industrial SB plantations are located, development of pest control measures began in 1960 and proceeds so far. The long-term practice of SB fly control showed that reliable crop protection is provided by chemical pesticides that does not guarantee receiving production without toxic remains (Goreeva, Prokofiev, 1996).This problem can be successfully solved due to cultivation of pest-resistant and relatively resistant varieties; however, the majority of varieties of the Altay genotype has high susceptibility to the pest (Shamanskaya, 2006).

Attempts to replace chemical pesticides by biological protection means (Kalvish, 1986; Hovalig, 2005), including a classical bio-method (Berger, Danilov, 1998), yet did not find wide application in practice.

Further research showed that using of biological product Phytoverm that was developed by the NBTs "Farmbiomed" (Moscow) against the SB fly, provides receiving eco-friendly yield (Shamanskaya, 2014). However, high cost of this agent is an obstacle for its use on big areas.

Development of economically and ecologically reasonable system of sea buckthorn protection against the sea buckthorn fly became the main objective of our further research.

Materials and methods

The test of the agents against the sea buckthorn fly was carried out in a production-scale experiment and a small plot experiment with the background of one - and double-fold spraying. The expected losses of crop were counted by the original technique (Shamanskaya, Usenko, 2007).

Sprayer OP-200 with the consumption rate of 1000 l/ha was used in production-scale experiment and knapsack sprayer "Quasar" with the consumption rate of 0.6-1 l/tree in the small plot experiment–. Control plants were nottreated. The experiment was repeated three times. The age of plants in the small plot experiment was 5 years, in the production-scale experiment 5-6 years. The total area of small plot experiment was 0,7 ha and the total area of production experiment was 6-7 ha.

The efficiency of treatment was determined by accounting the death of larvae in 100 damaged fruits while viewing them under the microscope of MBS-10.

The biochemical analysis of fruits was carried out by the standard techniques in biochemical laboratory of The Lisavenko Research Institute, the toxicological analysis was carried out in Central research-and-production veterinary radiological laboratory (Barnaul) by a method of gas-liquid chromatography. The remains of Aversectin C after treatment by biological agent Phytoverm were determined in NBTs "Farmbiomed" (Moscow) by a method of fluorescent highly effective liquid chromatography. Experimental data was processed by a method of dispersive analysis (Dospehov, 1973).

Results

The sea buckthorn fruit fly belongs to the order *Diptera,* family *Tophritidae.*On the first figure are presented the original pictures of imago, larvae and pupae of the sea buckthorn fly (Fig.1)

SB fly has a hidden lifestyle and a complex developmental circle. Puparium (false cocoon) overwinter under the canopy of damaged trees, mainly at the depth of 1-5 cm. Pupation of larvae occurs approximately one week before the beginning of fly flight.

The fly belongs to thermophile insects. The timing when the flight of adult insects starts depends on the temperature conditions in the previous weeks. Hatching of larvae lasts up to 6 weeks; the most active hatching lasts about a week and usually occurs in the first half of July.

Adult insects feed on sea buckthorn berries, piercing their skin by ovipositor. Flight, mating and oviposition occur only in warm weather. In 6-14 days after the beginning of flight, females start laying eggs, placing them one by one under the skin of fruit. Sometimes in one berry 2-3 eggs can be laid. The fertility of one female reaches 200 eggs and more.

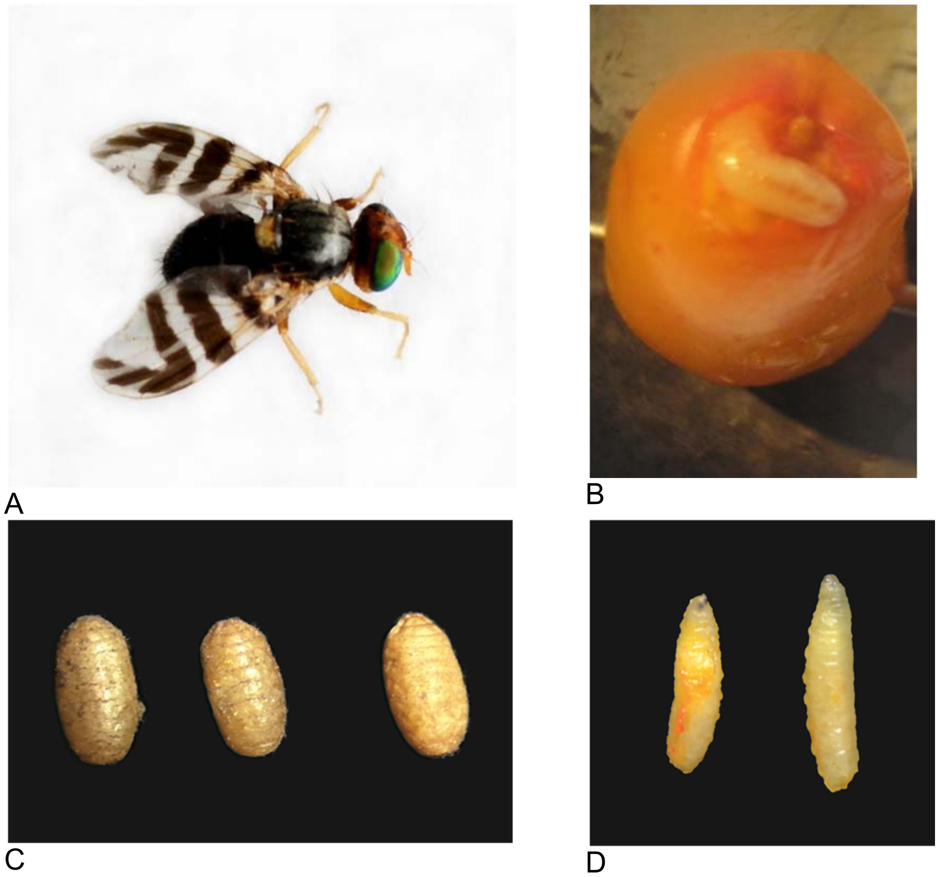

Figure 1. Sea buckthorn fly: A – imago, B – larva inside of berry, C- larvae before pupation, D – pupae

In the conditions of Altai Krai, larvae are the most harmful. Hatching begins in the first orsecond decade of July and ends in the second orthird decade of August. Larvae of the early age are usually small and transparent, later they have yellowish colour and reach 7 mm in length. On the forepart of their body two black chitin jaws (Maxillae) are situated and on the back-end a pair of light brown suction cups is presented which hold larvae on the surface of fruit. Within 3-4 weeks larvae feed on pulp of fruits, leaving the thin skin untouched. After destruction of one fruit, the larva gets over in the next one, damaging up to 50-100 % of fruits in one whorl (fig. 2).

The fruits occupied by larvae are softened, gradually deformed, darken and dried up without falling down (fig. 3).

Figure 2. Whorl damaged by larvae

A B

Figure 3. Character of fruits damaged by the sea buckthorn fly: A – in a period of active nutrition of larvae, B – after larvae leaving to pupation

SB fly is a highly specialized pest. It damages only sea buckthorn berries. The long-term assessment of sea buckthorn varieties of the Altay genotype showed different extent of their damage by the SB fly. Among them, 55 % are highly susceptible to the pest. They are, first of all, varieties of the early term of maturing. There is one form of a highly pest-resistant variety and some relatively resistant varieties which at a certain planting scheme can be cultivated without carrying out protective measures against the SB fly (Shamanskaya, 2014). The majority of relatively resistant varieties are small-fruited, red-fruited and late-ripening varieties (Fig. 4).

B
C
A

Figure 4. Varieties of sea buckthorn berries: A – highly-resistant to sea buckthorn fly,
B – relatively resistant red-fruited, C – relatively resistant late-ripening

Long-term observations have shown that the number of SB flies significantly changes year by year and depends on biotic and abiotic environmental factors. In certain years up to 70 % of puparium are catched by the parasite of fruit flies (*Opius rhagoleticollis*). In wet years up to 40 % of puparium can be damaged by diseases. Much greater influence on the change of SB fly population size in conditions of sharp and continental climate of Altai territory is caused by abiotic environmental factors. Population size of pests decreases in 2-6 times during long (2-3 weeks) flooding of sea buckthorn areas; due to the freezing of soil surface in the years with low snow cover; sharp cold events during the flight of adult insects and their additional nutrition, and also in conditions of abnormal heat while eggs are developing. Many larvae perish when they go into the soil to form pupae. We believe that not less than 90 % of the population perishes due to natural reasons.

Within 10 years we carried out observations of population dynamics of SB flies on the experiment site where pesticides were applied. Dynamics of adult insect flight was determined by the usage of yellow glutinous traps (fig. 5).

Figure 5. Yellow glutinous trap

Year2001 was the first year of monitoring under favourable conditions for pests and a lot of SB flies were noticed. (Fig. 6).

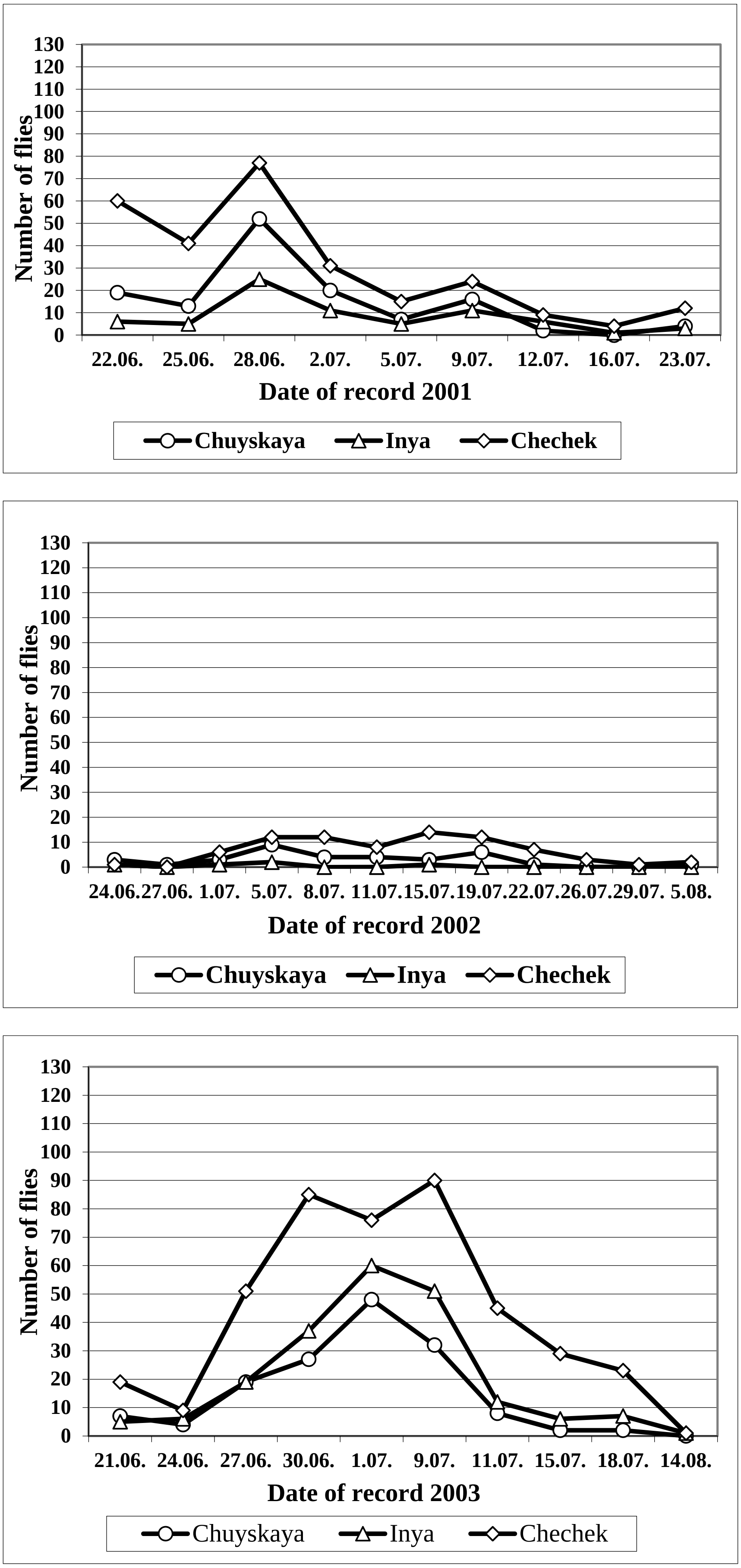

Figure 6. Dynamics of SB flies flight, 2001-2003.

In 2002 sharp temperature drops with the fall of air temperature down to 10-11^0 C during flight of adult insects led to reduction of pest population. The period of flight of adult insects was stretched and weak. In these conditions the fertility of females decreased sharply. Dry and hot weather at the end of spring – at the beginning of summer 2003, promoted an active reactivation of pupae, mass adult insect flight and substantial increase of pest population.

In 2004, population of SB flies remained high (Fig. 7).

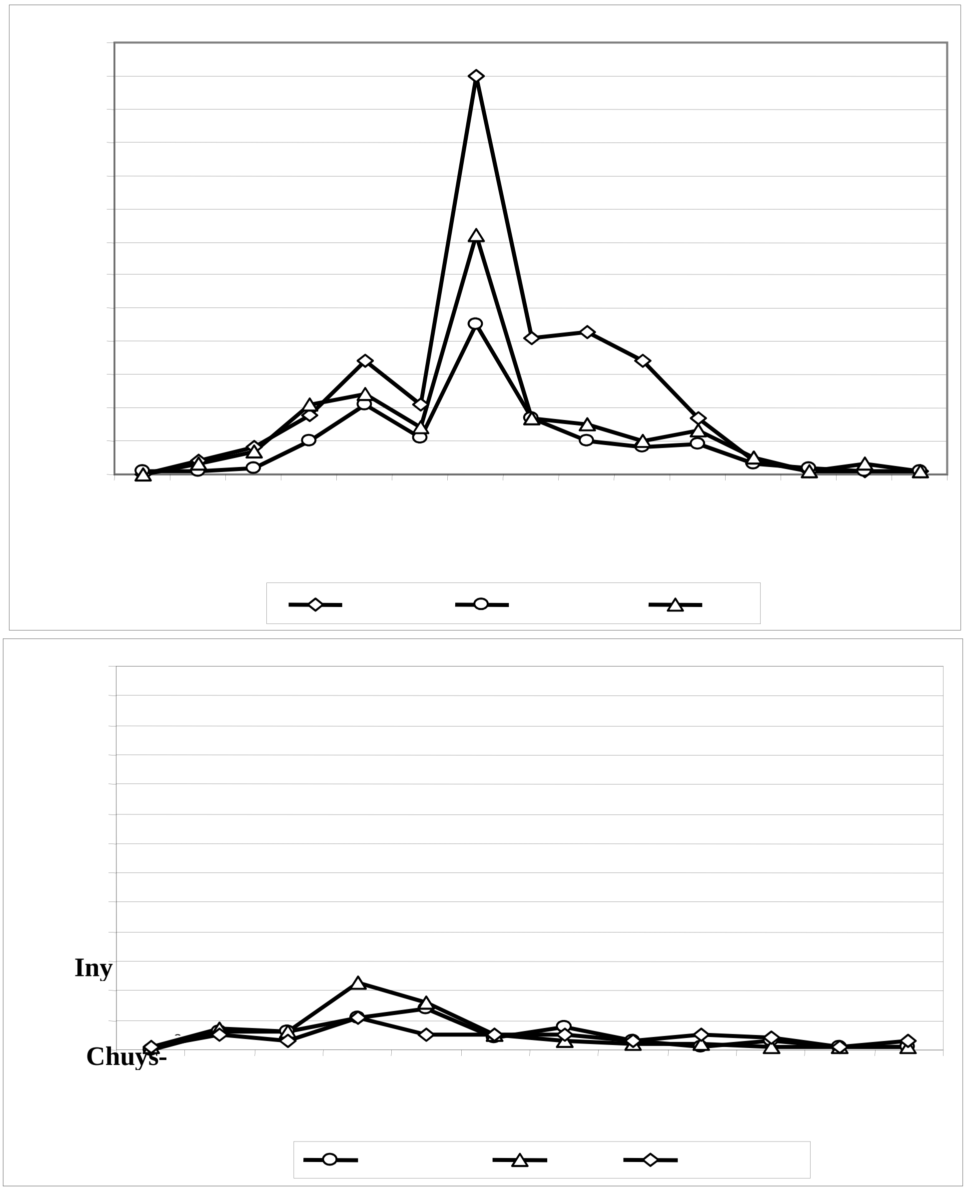

Figure 7. Dynamics of flight of SB flies, in 2004 and in 2006.

This was caused by hot and dry weather in spring and in the beginning of summer. The flight of adult insects began early – on the 10th of June, but it was suspended by pouring rains. In connection to this the timing of pest development was shifted for later period, but it did not reduce their harmfulness.

During the next 4 years (2005, 2006, 2007 and 2008) the population of SB fly in the experimental site considerably decreased, which is connected with the adverse weather conditions during the various periods of pest development. In fig. 7, decrease of SB fly population is presented by the example of year 2006.

In 2009, due to the favourable weather conditions, the increase of pest population was observed. In 2010 population of SB flies decreased again in connection with incessant pouring rains during the flight period of adult insects.

Despite considerable fluctuations of SB fly population, its harmfulness remains at the rather high level due to their ability for a long-term diapause. However, in certain years with a well-functioning forecast system of SB fly population it is possible to abandon spraying against the pest.

We developed and patented a short-term forecast of population of SB flies, based on the accounting of total number of adult insects on the yellow glutinous traps (Shamanskaya, Usenko, 2007) where the expected losses of yield are calculated with the use of equation of regression:

$Y = 0.45 \times X - 0.1496$, where
Y – the expected loss of yield, %
X – the total number of adult insects on the average on 1 trap
0.45 - regression factor
0.1496 - constant regression factor

The use of a short-term forecast of SB fly population in practice allows to abandon spraying against the pest on the area of tens hectares of industrial plantations in certain years. So, for example, in 2014 SB flies treatments were cancelled on the area of 92 ha.

The decrease of sea buckthorn fly population in 2014 was connected with the long influence of low temperatures in the reactivation period of pupae and the negative impact of abiotic stress – the air temperature increased up to +38 °C before larvae hatched from the eggs which led to their mass death.

When developing a short-term forecast for SB fly population, it is necessary to keep in mind that in the other regions the fertility of females can vary considerably in comparison with pest population in Altay , therefore in each case the adjustment in calculations of the expected losses of a yield is necessary.

SB flies move extremely actively from the old plantations to young plantations. In the first year of fruit-bearing highly susceptible varieties can already be damaged by 99,9 %. Thus, the varieties located in close proximity to the source of infection become populated most intensively. Our research findings showed that the most active settling of plants by the SB fly in 2004 was noticed on the variety Altayskaya (Fig. 8).

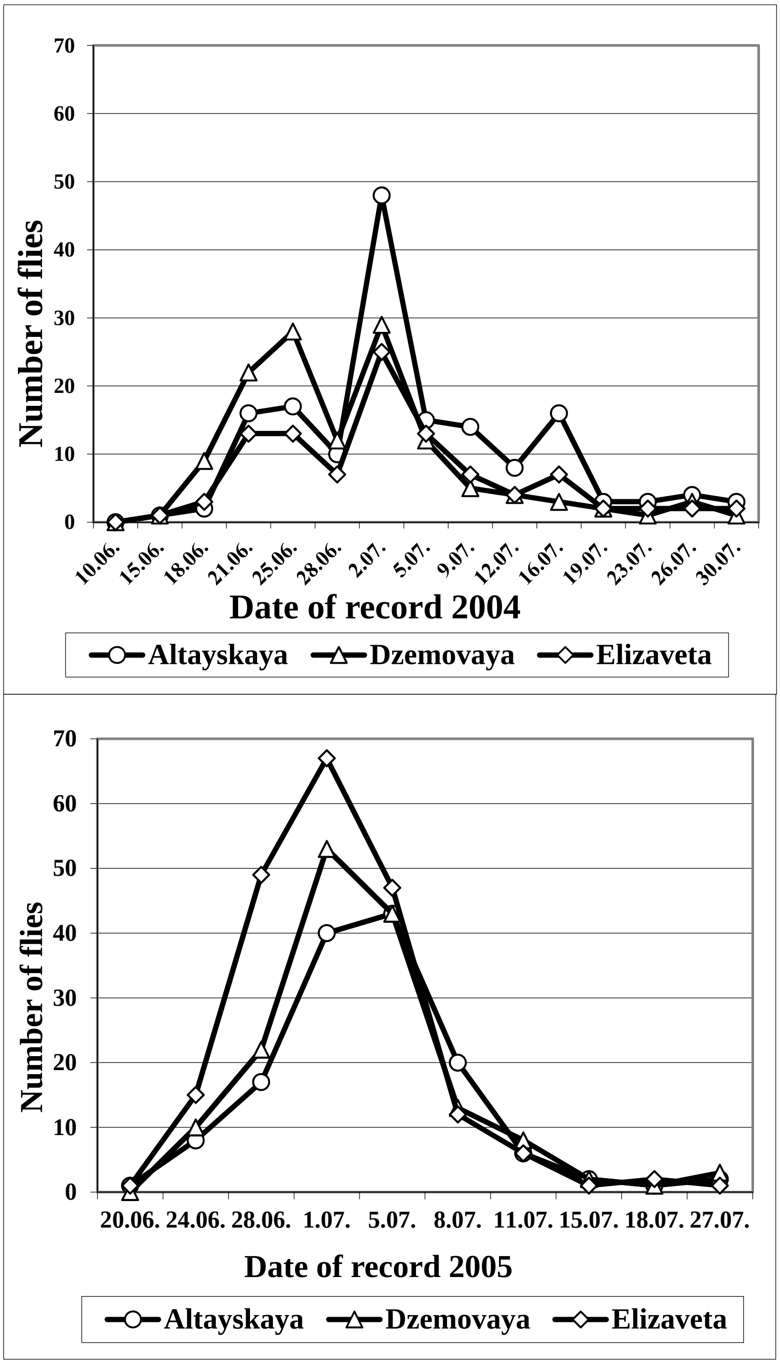

Figure 8. Dynamics of flight of adult insects when settling in a new sea buckthorn plantation

In the second year of fruit-bearing happened a redistribution of pests on the site and more intensive flight was noticed on the variety Elizaveta that is located at the maximum distance from the source of infection. During next years the variety Elizaveta remained leader in susceptibility to the sea buckthorn fly on this site.

The long-term practice of the sea buckthorn fly control showed that the reliable crop protection is provided by protective measures that are directed against a harming phase – larvae, with the use of chemical pesticides. Actellic (Pirimiphos-methyl) showed a good result among them. It reliably protects crop but does not guarantee production without toxic remains.

Further research work was directed on decreasing the dose of Actellic due to combined use with bio-supplements developed at our institute.

In case of low damage level caused by the sea buckthorn fly, with the expected loss of crop/yield 1.3-5 %, the efficiency of spraying at the level of 96-100 % is received by decreasing the agent dose from 1 litre to 100 ml /ha that is 10 times less (Table 1).

Table 1. Efficiency of a microdose of Actellic against sea buckthorn flies. Results of production experiment. Treatment 27.07.2007.

Treatment	Variety	Death of larvae, %
Without spraying - control	Chuyskaya Inya Zhemchuzhnitsa	19,3 25,4 18,6
Actellic - 1 l/ha (standard)	Chuyskaya	100
Actellic - 0,1 l/ha + bio-supplement	Chuyskaya Inya Zhemchuzhnitsa	96,0 100 100
$LSD_{0.5}$	-	3,6

In case of high damage where the expected loss of crop was 44 %, the total death of pests was received by decreasing Actellic dose from 1 litre to 400 ml that is 2.5 times less (Table 2).

According to the records of plant productivity in the second experiment, the productivity in control was 4,65 t/ha, and in Actellic spraying with total and lowered dose the productivity was more than 11 t/ha. The yield increase compared to control was 151 % and 149 %, respectively (Table 3).

Table 2. Efficiency of reduced doses of Actellic against sea buckthorn flies. Results of production experiment (variety Chuyskaya) on 30.07.2010.

Treatment	Death of larvae, %		
	2.-3.08.	9.-10.08.	19.08.
Without spraying - control	2,4	3,0	0,1
Actellic – 1 l/ha (standard)	93,0	100	100
Actellic – 0,5 l/ha + bio-supplement	85,0	97,0	100
Actellic – 0,4 l/ha + bio-supplement	82,0	95,0	100
Actellic – 0,3 l/ha + bio-supplement	70,7	82,5	96,2
Actellic – 0,2 l/ha + bio-supplement	59,2	62,0	84,8
$LSD_{0.5}$	0,98	0,86	0,84

Table 3. Productivity of sea buckthorn with reduced Actellic doses. Results of production experiment (variety Chuyskaya) from the year 2010.

Option	Productivity		Increase compared to control	
	kg/bush	t/ha	t/ha	%
Without spraying - control	3,94	4,65	-	-
Actellic– 1 l/ha (standard)	9,91	11,71	7,06	151
Actellic – 0,5 l/ha + bio-supplement	9,65	11,41	6,76	138
Actellic – 0,4 l/ha + bio-supplement	9,80	11,58	6,93	149
Actellic – 0,3 l/ha + bio-supplement	8,60	10,16	5,50	118
Actellic – 0,2 l/ha + bio-supplement	6,50	7,68	3,03	65
$LSD_{0.5}$	1,63	0,06	-	-

Results of biochemical analysis of fruit showed that combined use of reduced dose of Actellic and bio-supplement improved some indicators. The content of sugars, acids and the amount of carotenoids were higher in comparison with the full dose of the agent.

Decrease in dose of Actellic from 1 to 0,4 l/ ha in experiment in 2010 reduced the quantity of toxic remains in fruit in 1.4 times. In certain years this kind of treatment allows to receive production without toxic remains.

Among biological agents a good effect was shown by Phytoverm (1%), developed in Farmbiomed scientific and production association (Moscow) on the basis of not pathogenic soil fungus *Streptomyces avermetilis* strain VNIISKHM-54 or strain VNIISKHM-51. In the growth process the culture of primary producer forms a complex of "close to chemical"substances that are named avermectin, which have biological activity. Phytoverm does not pollute environment, it quickly dissipates in water and soil. Expectation time from the use on various cultures is 3 days, on the sea-buckthorn – 2 weeks.

A single Phytoverm spraying against SB fly provides a short-term protective effect; therefore for a reliable protection of crop the double treatment is needed.

Phytoverm is an expensive agent and it is an obstacle for its wide implementation into production chain. A combined use of Phytoverm with bio-supplement allows to halve the dose. The table 4 shows the efficiency of full and reduced dose of Phytoverm in the production experiment which in the first case was 100 % and in the second case was 97.7 %.

Productivity at full dose of Phytoverm was 9.92 t/ha, at the lowered – 9.45 t/ha. The increase of crop was respectively 162 % and 150 % (Table 5).

Table 4. Efficiency 1 %EC of Phytoverm against sea buckthorn flies.
Results of production experiment (variety Chuyskaya) in 2009.

Treatment	Number of sprayings	Death of larvae, %		
		28.7.	4.8.	11.8.
Without spraying - control	-	8,6	12,4	5,1
Actellic – 1 l/ha (standard)	1	76,2	100	100
Phytoverm – 3 l/ha	1	11,1	67,5	84,7
Phytoverm – 3 l/ha	2	-	89,6	100
Phytoverm – 1.5 l/ha+ bio-supplement	1	15.6	57,4	68,1
Phytoverm – 1.5 l/ha + bio-supplement	2	-	95,9	97,7
$LSD_{0.5}$	-	3,8	3,4	1,3

Table 5. Yield of sea buckthorn when used various Phytoverm doses. Results of production experiment (variety Chuyskaya) in 2009.

Treatment	Number of sprayings	Productivity		Crop increase	
		kg / bush	t/hectare	t/hectare	%
Without processing - control	-	3,2	3,78	-	-
Actellic – 1 l/ha (standard)	1	8,2	9,69	5,91	156
Phytoverm – 3 l/ha (standard)	1	8,4	9,21	5,43	143
Phytoverm – 3 l/ha	2	7,8	9,92	6,14	162
Phytoverm - 1,5 l/ha + bio-supplement	1	4,9	5,79	2,01	53
Phytoverm - 1,5 l/ha + bio-supplement	2	8,0	9,45	5,67	150
$LSD_{0.5}$	-	2,0	0,31	-	-

The biochemical analysis of fruits showed that combined use of Phytoverm with bio-supplement improves the quality of fruit according to the content of dry soluble substances, sugars, vitamin C and oil.

The toxicological analysis of fruit showed that the use of biological agent Phytoverm in sea buckthorn fly control allows cultivation of eco-friendly production.

The advantage of using biologically active supplement for decreasing a consumption rate of pesticides in years with arid weather conditions should be especially marked because one of its components increases the resistance of plants to the drought. For example in 2012 when there were arid weather conditions in a small plot experiment, the maximum increase of crop 138 -150 % in relation to control was received in treatments with bio-supplement (Table 6).

Table 6. Yield and weight of sea buckthorn berries (planting 2008) associated with different sprayingdoses. Results of production experiment (variety Chuyskaya) in 2012.

Experiment	No. of sprayings	Mass of 100 fruits g	Productivity		Increase of crop compared to control, %
			kg/bush	t/hectare	
Without spraying - control	-	50.1	1.5	1.8	-
Actellic – 0.1 % (standard)	1	51.0	3.2	3.9	116
Actellic – 0.04 % + bio-supplement	1	53.1	3.6	4.5	150
Phytoverm (1 % EC) – 0.3 %	2	51.4	2.6	3.2	77
Phytoverm (1 % EC)- 0.15 % + bio-supplement	2	54,5	3,5	4,3	138
$LSD_{0.5}$	-	0,7	0,8	0,98	-

Weight increase of fruit from 51.0 g (Actellic full dose) to 53.1 g (Actellic + bio-supplement) and from 51.4g (Phytoverm full dose) to 54.5 g (Phytoverm + bio-supplement) was observed.

Calculation of economic efficiency showed a low level of profitability of fruit production when using various sea buckthorn fly sprayings in 2012. It was connected with the high costs of manual harvesting and incomplete accession of plants into fruit-bearing phase. The highest profitability – 72.3 % was received when using Actellic in a complex with bio-supplement (Table 7).

Profitability increased by 11.5 % compared to the full rate of application of the agent. In case of double decrease of dose of expensive agent Phytoverm, the profitability of fruit production was 62.5 % which exceeds the ratio of full dose by 29.5 %-unit. The analysis of the received data showed that the use of full dose of Phytoverm is not economically justified, as profitability of fruit production was 1/3 less than in the control treatment.

The biochemical analysis of fruit showed the increase in content of carotenoids from 18.50 -18.97 mg/100g (use of full doses of the agent) to 20.08 – 22.10 mg/100 g (a combined use of reduced doses of Phytoverm and bio-supplement). The content of oil increased from 3.97-4.41 % to 4.59 – 4.93 %, respectively.

These results got confirmation in a production experiment where Actellic (0.4 l/ha) was used in a complex with bio-supplement. The experiment was carried out in the same year on the area of 28 hectares. In this case the amount of carotenoids increased from 17.94 mg /100 g to 20.14 mg/100 g when using lowered dose of Actellic and bio-supplement instead of full dose of Actellic, and the content of oil increased from 4.91 % to 5.64 %, respectively.

The toxicological analysis showed 4 times less toxic remains in fruits after combined use of Actellic with bio-supplement in a small plot experiment. In production-scale experiment toxic remains in fruits were not found.

Table 7. Economic efficiency of sea buckthorn fruit production associated with different spraying backgrounds.

Treatment	Yield, t/ha	Costs of 1 ha, thousand roubles		Prime cost, thousand rub/ha	Earnings thousand rub/ha	Profit, thousand rub/ha	Level of profitability, %
		total	protective treatments				
Without treatment - control	1,8	79,7	0	44,2	82,8	38,6	48,4
Actellic-1 l/ha (standard)	3,9	111,5	1,22	28,5	179,4	67,9	60,8
Actellic – 0.4 l/ha + bio-supplement.	4,5	120,1	0,49	26,6	207,0	86,9	72,3
Phytoverm - 6 l/ha	3,2	110,6	9,60	34,5	147,2	36,6	33,0
Phytoverm - 3 l/ha + bio-supplement.	4,3	122,3	4,80	28,4	198,8	76,5	62,5

During carrying out protective measures against a harming phase – larvae - it is very important to define optimum timing of spraying. This allows destroying the pest at early stage of its development and provides active regeneration of the damaged tissue and minimum loss of fruit weight. It is possible to destroy larvae at late stage of development, but the part of fruit is heavily deformed, its weight is decreased and that conducts to the considerable loss of crop (Fig. 9).

If the quantity of such fruits is 10% - the loss of crop is 3.5%, but if their quantity increase to 50 percent - losses of crop increase to 14% (Table 8).

The content of oil in damaged fruits decreases to 4.84%, in comparison with outwardly healthy fruits where content is 5.55%.

A B

Figure 9. Fruits of sea buckthorn, variety Inya: A – healthy, B – damaged by SB flies

Table 8. Change in yield weight at various amounts of damaged fruits. Variety Inya in 2009.

Indicators	Healthy fruit	Content of damaged fruit in yield weight, %										
		1	10	20	30	40	50	60	70	80	90	100
Weight of 100 fruits, g	92,7	92,5	89,5	86,7	84,0	81,8	79,8	73,5	70,3	67,1	63,9	60,5
% compared to control	-	99,7	96,5	93,5	90,6	88,2	86,0	79,2	75,8	72,3	68,9	65,2
Loss of yield, %	-	0,3	3,5	6,5	9,4	11,8	14,0	20,8	24,2	27,7	31,1	34,8

Conclusion

In the conditions of sharp and continental climate of Altai Krai population of SB flies varies considerably from year to year. Population forecast of pests developed by the Lisavenko Scientific and Research Institute allows abandoning application of pesticides on scores of hectares of SB industrial plantations in certain years.

During the days of mass development of sea buckthorn flies, combined use of insecticide Actellic and biological product Phytoverm with bio-supplement is ecologically reasonable and economically sound. Bio-supplement allows reducing the agents in 2-10 times compared to standard provides the maximum increase of crop by 138-150 % and improves the basic fruit quality index – carotenoid and oil content.

Combined use of bio-supplements and insecticide Actellic allows lowering the content of toxic remains in fruits by 1.4- 4 times, and in some cases to receive production without any remains of pesticides.

Application of biological pest control agent Phytoverm for crop protection allows getting crop without toxic remains. In the changing conditions of market economy the usage of this agent in complex with bio-supplements is economically justified.

The maximum safety of a crop is provided by the optimum time of spraying against a harming phase – the larvae, which number by the beginning of treatment should not exceed 5 – 12 % .

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Experiences of integrated management of European Cherry Fruit Fly (Rhagoletis cerasi) and how to utilize this knowledge for Sea Buckthorn Fly (Rhagoletis batava)

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Abstract

The genus *Rhagoletis* Loew includes about 65 known species distributed throughout Europe, Asia and America. Most species are oligophagous, attacking only a few closely related host plants. The European cherry fruit fly *R. cerasi* is the economically most important pest species in Europe. *R. batava* and *R. alternata* can also cause economic damage in Europe. In addition to these species, the American cherry fruit fly species *R. cingulata*, *R. indifferens* and *R. fausta*, as well as the apple maggot *R. pomonella*, the blueberry maggot *R. mendax*, and the walnut infesting species *R. completa* and *R. suavis* are pest insects of high economic importance. *R. cingulata* and *R.completa* were recently introduced to Europe and are currently spreading in Central Europe.

A lot of research was conducted on integrated control of the European cherry fruit fly *R. cerasi* during the past hundred years. For the monitoring of flight period of *R. cerasi* yellow sticky traps are used. This method was shown to give reliable results for the timing of application. However, an economic threshold cannot be determined with yellow sticky traps, because fruit infestation is also influenced by crop load and weather conditions during oviposition period of flies. Chemical control (Insecticides) of *R. cerasi* is currently impeded by the withdrawal of "old" organophoshporus compounds (Dimethoate) and the debate on side-effects of neonicotinoids.

Attract-and-kill strategies are available or under development: food baits (based on yeast hydrolysate and sugar) are mixed with the organic insecticides Spinosad or Neem. The baits attract the flies, stimulate feeding and thus increase uptake of the insecticide. However, the efficacy of attract-and-kill strategies strongly depends on the attractiveness of the bait which is influenced by climate conditions. Mass trapping by yellow sticky traps combined with baits is used for cherry fruit fly control in home-gardens. However, due to the high number of traps needed to achieve good control, this strategy is too expensive for commercial production.

The use of kaolin as mechanical barrier on the fruit surface to prevent oviposition was shown to be effective against *R. indifferens* and *R. mendax*. Because kaolin treatments leave white residues on fruit, this method is not used against cherry fruit fly. Recently, it was shown that oil products can also prevent oviposition by creating a slippery layer on the fruit surface. A biocontrol method using the entomopathogenic fungus *Beauveria bassiana* has been developed and is currently registered in serveral European countries (product Naturalis-L). This strategy is mainly used in organic production. Another biocontrol strategy could be the use of parasitoids. However, the use of larval parasitoids is hampered by the steadily increasing fruit size of cherries for fresh consumption: parasitoids are no longer able to reach the larvae in the center of the cherry fruit.

The use of crop netting to protect trees is the currently most widely used method of cherry fruit fly control. With the increasing number of dwarf tree cherry orchards covered against rain to avoid fruit splitting, it has become a viable, cost-effective method of cherry fruit fly control. However, for high standard trees this method is not suitable. In this situation, the use of nets for soil covering (to avoid hatching of flies from overwintering pupae in the soil) can be used.

Key words

Rhagoletis cerasi, biocontrol, *Beauveria bassiana*, insecticide, crop netting

Introduction

The European cherry fruit fly, *Rhagoletis cerasi* (L.) (Diptera: Tephritidae) is the most important pest of sweet cherries in Europe. Without insecticide treatment up to 100% of fruits can be infested. *R. cerasi* poses a challenge to cherry growers because the tolerance level of the market for damaged fruit is relatively low, with maximum 2% of infested fruits. Because fruit fly infested fruit cannot be sorted out, the whole lot is rejected if tolerance levels are exceeded. The disqualification of table cherries to distillery quality considerably reduces the market price, which causes serious financial losses.

The European cherry fruit fly belongs to the family of Tephritidae, which has world-wide distribution with about 4000 described species in about 500 genera. The genus *Rhagoletis* Loew includes about 65 known species. Most species are oligophagous, attacking only a few closely related host plants. In addition to *R. cerasi*, the American cherry fruit fly species *R. cingulata*, *R. indifferens* and *R. fausta*, as well as the apple maggot *R. pomonella*, the blueberry maggot *R. mendax*, and the walnut infesting species *R. completa* and *R. suavis* are pest insects of economic importance. Host plants of *R. cerasi* include various different *Prunus sp*. (Rosaceae; *P. cerasus*, *P. avium*, *P. serotina*, *P. mahaleb*) as well as *Lonicera sp.* (Caprifoliaceae; *L. xylosteum* and *L. tatarica*).

Life history characteristics of *R. cerasi*, like of other oligophagous Tephritid species, are best suited for exploiting resources that are predictable in time and space but are only available during a short period of the year. A close adaptation of their biology to the fruiting pattern of the host and precision in seasonal synchronisation are more important than high reproductive potential and high mobility. Hibernation occurs in the soil in the immediate vicinity of the hosts. Thus there is no need for dispersal flights. Adult emergence and life span are closely correlated with host plant phenology. Pupal carryover for two or more winters is used for “spreading the risk” of failure of the host plants to fruit in a particular year. There is only one generation each year and a long obligatory winter diapause. Relatively unspecific visual and odour stimuli are used to identify oviposition sites. Competition in the larval stages (contest type) is largely avoided by oviposition of only a single egg in each fruit and by the application of a host marking pheromone after oviposition, which ensures an adjustment of larval density to the carrying capacity of the host and maximizes dispersion over available food resources. The mating system of these species is usually resource-based: the males control the oviposition substrates and mating is often initiated by forced copulation without elaborate courtship behaviour.

The following paper summarizes the knowledge on biology, research and control of *R. cerasi*, points out general traits of all *Rhagoletis* species and suggests possible methods of control for the sea buckthorn fly *R. batava*.

Emergence and monitoring of flight period

Pupal development and adult emergence *of R.cerasi* is influenced by soil temperature in spring by temperature conditions during winter diapause as well as by the host plants from which the pupae originated and geographic provenance. The earliest attempts to develop a forecasting model for the eclosion time of flies were made in the 1930s. This model was revised and improved in the 1960s [1, 2] and 1970s [3, 4]. Knowledge of first fly appearance is important for a proper timing of control measures. For *R. cerasi*, beginning of the flight period can be determined using forecasting models based on soil temperature measured at a depth of 5 cm. Emergence starts at 430 degree days above the temperature threshold of 5°C [1, 5].

At the moment, a forecasting model predicting the emergence of adult flies in different regions in Switzerland is available for the farmers on the internet platform www.sopra-acw.admin.ch/ [6]. As *R.batava* also overwinters in the soil, hatching of adult flies is most likely also dependent on soil temperatures.

In absence of a forcasting model, depots of pupae in the soil can be used for precise monitoring of emergence [7]: larvae are collected from infested fruit, and buried outside in the soil. In the following spring, small cages are installed over the depots and emergence is daily counted.

An easier method to monitor the flight period, is the use of yellow sticky traps. For *R. cerasi*, , one or two traps per cherry variety should be placed on the southeast side of the tree canopy in full sun in mid-May prior to fly emergence and are examined twice a week. However, not all yellow traps are equally effective. For *R. cerasi,* traps with an high reflectance at 485 to 500 nm (yellow green region) and a secondary peak of reflectance at 365 nm (ultraviolet region) were most efficient [8, 9, 10]. For *R. batava*, most efficient trap colour needs to be identified. However, traps are not good indicators of the fruit infestation level. Depending on yield, weather conditions during oviposition and trap position, the economic threshold ranges between two and ten flies per trap. Treatment decisions should therefore be based on the expected yield and the infestation level in the previous year.

Pre-Oviposition period

Before oviposition, the adults go through a temperature-dependent maturation period of six to 13 days during which they need to feed on carbohydrates, proteins and water in order for the gonads to mature. Nutrients are obtained from bird droppings, honeydew, extrafloral nectaries, and bacterial colonies on leaf and fruit surfaces. This period is a suitable time span for most regulation methods.

Yellow sticky traps can be also used for mass trapping. However, in order for mass trapping strategies to be effective one to eight traps per tree (depending on tree size) are needed [11]. Traps are placed on the southeast side of the canopy. Because the traps should be hung in the upper part of the canopy, much labour is involved, thus making this strategy uneconomical for conventional cherry production. Nevertheless, mass trapping may still be the only option for controlling *R. cerasi* in home gardens, in which the application of insecticides is often impossible due to the lack of proper application equipment. Due to the lack of registered alternatives, yellow sticky traps are still widly used in organic cherry production throughout Europe. In addition to yellow surfaces, *Rhagoletis* flies also react to red or dark coloured spheres of approximately the same size as the host fruit [12]. For the apple maggot (*R. pomonella*) sticky spheres are used as traps. For cherry fruit fly, however, this strategy is unsuitable due to too small fruit/trap size.

As the flies are looking for food sources during the pre-oviposition period, food baits are an option to attract the flies. Food baits usually consist of yeast hydrolysate, and an ammonia releasing source (e.g. ammonium acetate). Trimethylamine (smell of long dead fish) and diaminobutane (rotting meat smell) were also shown to be effective. Food baits could either be used in combination with a yellow sticky trap, or in combination with an insecticide. Although some of the food baits tested in combination with yellow sticky traps were able to double the number of captured flies [13], none of the baits tested showed economic potential. However, the use of food baits in combination with insecticides showed promising results. The spinosad Product GF120 (Dow AgroSciences) is used in US and in dryer areas of Europe [14]. In regions with moist summer climate, the efficacy of GF120 was insufficient. Recently another bait (CombiProtec, Dedetec, Germany) was developed which shows better results in humid conditions. For *R. batava*, the application of insecticide-bait applications might be an option.

Insecticide applications are also applied during the pre-oviposition period. Until recently, one application of Dimethoate has been the standard for controlling *R. cerasi* in Swiss sweet cherry production, because it is by far the most cost-efficient method. Since 2011, however, this product is no longer registered for use in fruit production in Switzerland because of problems of ecotoxicity and residues on harvested cherries. Two applications of Acetamiprid are currently recommended for cherry fruit fly control in Switzerland. The situation in many other European countries is comparable. However, implementation and transition periods differ between the countries. Mainly Neonicotinoids are currently used to control *R. cerasi.*

The life span of flies under field conditions is difficult to estimate and may range between four to seven weeks, which leads to a total flight period of seven to 11 weeks.

Mating

Mating of *R. cerasi* occurs on sunny days with temperatures above 15°C. Host fruit on sunny parts of the trees is used as a mating site. Mating is initiated when a female in search of an oviposition site lands on a fruit occupied by a male. It was shown that the males produce a highly species-specific pheromone which attracts females. However, contrary to the pheromones of many Lepidoptera, this pheromone seems not to have a long-range attraction [15]. Besides the pheromone, fly behaviour plays a major role in locating mating partners: due to their preference for host fruits in full sun, the flies aggregate in certain parts of the trees. In these circumstances, an elaborate long-range pheromone might be of minor importance [16]. It was even hypothesized that the pheromone might function primarily as an aphrodisiac [17]. Until now this pheromone is not fully identified and neither monitoring tools nor management strategies based on this pheromone are available for *R. cerasi* control. Same is true for other *Rhagoletis species*: pheromones are either not identified or not available for commercial use. Thus, the use of e pheromone does not seem an good option for control of *R.batava*. One to three copulations during a female's life span are considered to be necessary to maintain high egg fertility.

Another strategy to prevent offspring is the sterile insect technique. For cherry fruit fly control it was developed between 1960 and 1980 [18, 19, 20, 21, 22]. The major bottle-neck of this technique is the artificial rearing of the fly [23, 24, 25, 26]. Several points in the insect's biology complicate rearing: *R. cerasi* is univoltine, has an obligatory diapause of at least 150 days, and *R. cerasi* is monophagous with a strongly selective host choice. The lack of a suitable rearing method for producing enough sterile insects for mass releases prevented this strategy from being commercially introduced.

Dispersal flights

With the relative stability of the system, i.e. pests that overwinter beneath perennial hosts, there appears to be little impetus for adults to move long distances. Dispersal flights occur only in situations in which flies are deprived of suitable fruits for oviposition: when cherries are destroyed by frost or early harvest or when all fruits are already infested [27]. Driven by high oviposition pressure, the females leave their original tree [28], and the males follow a little later [27, 29]. The flies move from tree to tree until they find a suitable host [30]. Maximum distances of dispersal flights are difficult to evaluate experimentally and might range between 100 and 500 m [2, 30], in exceptional cases as far as 3 km [20]. Flight studies in the laboratory showed that flies are capable of flying several kilometres in 24 hours if no landing platforms were available. However, within orchards, 95% of the flies move only to neighbouring trees of later ripening varieties [2, 31], and from there on to *Lonicera sp*. bushes [27].

In order to prevent immigration of flies into orchards, net covering can be an option. Especially with the increasing number of dwarf tree orchards shielded from rain to prevent the large sized cherry varieties (>24 mm fruit diameter) from splitting, crop netting is a viable, cost-efficient strategy for protecting cherries from infestation [32]. Experiments using netting to cover the trees were conducted at the Palatinate Agricultural Service Centre (DLR Rheinpfalz, Germany [33]), at the Bavarian State Research Centre for Agriculture (LfL Bayern, Germany [34]) and at the Research Institute of Organic Agriculture (FiBL, Switzerland, Häseli, personal communication). The "Rantai K" net-type with a mesh size of 1.3 mm was used in all experiments. Netting should be installed before the beginning of the flight period and the netting should remain in place until the latest ripening cherry varieties are harvested.

Oviposition

Oviposition occurs around noon and during early afternoon on sunny days when temperatures rise above 16°C. Weather conditions during the oviposition period are considered to be crucial for the regulation of population densities: the high oviposition activity during long-lasting periods of fine weather can lead to extreme outbreaks of this pest. Both olfactory and visual cues are involved in the choice of suitable fruits for oviposition. However, the visual component appears to dominate. Females recognize the fruit by visual cues based on shape (spherical or hemispherical), size (2.5 to 10.3 mm diameter) and contrast-colour against the background (dark shape in front of lighter background) [17, 35]. Once a suitable fruit has been located, the female explores the surface structure (smoothness, softness and shape) by walking in circles on the surface and decides whether or not to oviposit [35]. The female pierces the fruit with its ovipositor and inserts a single egg just below the skin. After oviposition the females deposit a water-soluble host-marking pheromone by dragging the ovipositor around the fruit surface [36]. This pheromone prevents further ovipositions into the same fruit [37, 38]. The use of this pheromone was investigated in the 1970s [36, 38, 39]. In field experiments using naturally derived pheromone, an efficacy of 63 to 90% was observed [39]. High synthesis costs, however, prevented the use of this pheromone in commercial cherry growing. In addition, efficacy was low at high infestation levels and under rainy conditions. Moreover, about 10% of the trees had to remain untreated in order to provide unmarked fruits for oviposition [37].

Mechanical barriers are another option to prevent oviposition: Oviposition behaviour of cherry fruit flies is influenced by host fruit characteristics, such as texture [36], surface structure [35], and chemosensory stimuli [36]. Watching the flies during oviposition, gives the impression that flies need a lot of force to penetrate fruit skin. Altering physical properties of fruit surface can therefore prevent oviposition: It was shown that oil treatments prevent oviposition of *R. cerasi,* because the flies were not able to penetrate the slippery, oily skin with the ovipositor [13, 40]. For Apple maggot, *R. pomonella,* Kaolin clay is used as physical barrier with good success.Drawback of kaolin applications are the residues on harvested fruit. Therefore it is not an option for cherry fruit fly control. However, following up the idea of mechanical barriers seems promising also for *R. batava* especially for fruit needed for processing.

Egg and larval development

The duration of embryonic development mainly depends on temperature and ranges between two to ten days. After eclosion, the larvae immediately move towards the cherry pit in order to find protection from parasitoids and predators. Larval development lasts between 17 and 30 days, depending on the temperature and the maturity stage of the cherries. The larvae go through three instars, reaching a final size of approximately 6 mm. During their development, the larvae tunnel in the fruit, macerate the tissue and ingest the broken down pulp. Larvae develop better and faster in fruits with higher sugar content and lower acidity [41]. High populations of *R. cerasi* can be therefore observed in sweet cherry orchards, whereas sour cherries usually remain free from high infestations.

The infestation level can be estimated using the salt solution test [42]: 100 randomly picked cherries of each cherry variety are crushed until the pits are separated from the pulp. A saturated salt solution (350 g salt per litre water) is added. Floating larvae can be counted after 10 minutes. Control of larvae is only possible with systemic insecticides (Dimethoate), which are no longer registered in most European countries.

Pupation and overwintering

Around harvest, mature larvae bore exit holes through the fruit skin. Under field conditions, pupation usually occurs within three hours after entering the soil [29]. Most pupae are therefore found directly under the tree canopy, especially under the south and southeast parts of the tree, which is also where the highest fruit infestation levels are observed. Pupation depth is mainly influenced by soil type and usually ranges from 2 to 5 cm.

The cherry fruit fly is an univoltine species: the pupae remain in the soil until the following spring. Overwintering pupae enter diapause and require a chilling period before development can continue. Approximately 180 days at temperatures below 5°C are required for maximum emergence [2, 3, 43]. Pupal mortality during the nine to 10 months of diapause is high and is mainly attributed to unfavourable climatic conditions, predation and parasitation: usually only 5% to 15% [41] of the pupae emerge in the following year. A few individuals remain in diapause for an additional year or sometimes for several years [28, 29, 43]. This pupal carryover is a highly adaptive trait, ensuring that the population will not perish on account of failure of host plants to fruit in some years.

Because *R. cerasi* pupae spend more than 10 months per year in the soil and because the area of pupation is strictly limited to the surface directly under the canopy of infested trees [29], the possibility of soil treatments was appealing [44]. Covering the soil under the tree canopy with netting to prevent the hatching flies from reaching the fruit is another efficient management strategy. The netting can reduce fruit infestation by 91% [31, 45]. Because the flies can survive for a long time under the netting, it is advisable to bury the edges of the netting completely. This, however, leads to high labour costs. Moreover, expensive, fine-mesh netting (0.8 mm mesh width) is considered to be necessary, because young flies after emergence can easily get through nets with mesh widths of 1.3 mm. Nevertheless, this method could be an option for controlling *R. cerasi* in extensively managed standard tree orchards. This strategy could be also tested against *R. batava*.

Mortality and antagonists

In cherry production, harvest (and the consequent removal of larvae from the orchard) is considered to be one of the main mortality factors [41]. In addition, temperature and rain have a major impact on mortality. No literature is available on the effects of **viruses** or **bacteria** on *Rhagoletis sp.*.

The pathogenicity and virulence of different **entomopathogenic fungi** on different life stages of *R. cerasi* were also first evaluated in laboratory experiments. Adult flies were found to be the only life stage susceptible to fungus infection. *B. bassiana* ATCC 74040 showed a high virulence, the flies died during the pre-oviposition period. These results were the first evidence of the susceptibility of *R. cerasi* to infection with hyphomycetous fungi [46]. Field application strategies were therefore focused on adult flies using the fungus isolate *B. bassiana* ATCC 74040, which is formulated in the commercial product Naturalis-L (Intrachem Bio Italia). Repeated applications of Naturalis-L during the flight period of *R. cerasi* were shown to reduce the infestation level of fruits by 60-70% [47]. However, the application of Naturalis-L is considerably more expensive than the application of Dimethoate or Acetamiprid. The higher prices obtained for organically grown cherries might justify the higher input for pest control [13]. For good efficacy, four treatments of 0.25% Naturalis-L ($5x10^4$ CFU ml^{-1}) with 1000 l water per hectare should be applied at seven to ten day intervals. The first application should be made five to ten days after the beginning of the flight period. The time period between the last application and harvest should not exceed seven days. Other phytosanitary measures (early and complete harvest; removal of infested cherries) can further enhance the efficacy of Naturalis-L treatments. Because the use of fungicides can interfere with entomopathogenic fungi, close attention has to be paid to the whole pest management programme. The use of entomopathogenic fungi might also be a possibility for control for *R. batava.*

Laboratory studies have indicated promising results of **entomopathogenic nematodes** to control the third instar larvae of *R. cerasi* [48]. However, results of laboratory experiments conducted in the scope of the European COST 850 project were disappointing: in a screening of 18 different nematode strains, the highest mortality rates in third instar larvae were below 30% (observed after application of *Steinernema feltiae* at a concentration of $2x10^6$ infective juveniles per m^2 on soil, Grunder et al., data not published). Due to the limited time frame and the different spatial activity, the potential for entomopathogenic nematodes for controlling *R. cerasi* under field conditions was considered to be rather small.

Most Tephritid species are attacked by a complex of native **parasitoids** [49, 50]. For *R. cerasi*, 21 species of parasitoids (larval ectoparasitoids, larval endoparasitoids and puparium parasitoids) have been described [51]. No egg parasitoids of *R. cerasi* are mentioned in literature. In cherry production, however, the effectiveness of larval parasitoids is greatly impaired by the short ovipositor of parasitoid females, which cannot reach *R. cerasi* larvae in large cultivated cherries. Monaco [52] observed that 10 to 30% of *R. cerasi* larvae in wild cherries (*Prunus mahaleb*) are parasitized by *Opius magnus* Fischer (Hymenoptera: Braconidae), whereas no parasitization was observed in cultivated cherries. Similar observations were made by Haisch et al. [53] and Hoffmeister [15], who noted that *R. cerasi* individuals from *Lonicera sp.* generally show higher levels of parasitization than individuals from cultivated cherries: *O. magnus* [15] and *Halticoptera laevigata* Thoms. (Hymenoptera: Pteromalidae) [15, 54] have only been observed in individuals from *Lonicera sp*., whereas *Opius rhagleticolus* Sachtl. [15, 44] was also found in individuals from cherries – although in lower numbers. Contrary to these observations, Leski [2] showed *O. rhagleticolus* to be the principal parasitoid of cherry fruit flies in Poland. However, with parasitization rates of 22 to 32%, *O. rhagleticolus* could not control *R. cerasi* populations [2]. Pupal parasitation seems to be more important. *Phygadeuon wiesmanni* Sachtl. (Hymenoptera: Ichneumonidae) occurs throughout Central Europe [41, 55] and has been shown to be responsible for a pupal mortality rate as high as 72% [41]. Under bushes of *Lonicera sp*., however, the parasitation rates of pupae were found to be higher than under cherry trees [43]. A mass rearing and release of this parasitoid might lead to an effective control of *R. cerasi*. Until now only little effort was made towards this strategy. Other puparium parasitoids, such as *Phygadeuon elegans* Förster [55], *Gelis bremeri* Haberm. (Hymenoptera: Ichneumonidae) [2, 15, 43], *Polypeza försteri* Kieff. (Hymenoptera: Diapriidae) [56], and *Spilomicrus hemipterus* Marshall (Hymenoptera: Diapriidae) [15], were observed in lower numbers. Until now, no biocontrol strategies based on parasitoids of *R. cerasi* were evaluated under field conditions. Different parasitoids might attack *R. batava*. A monitoring of natural occurring larval and pupal parasitiods is necessary to select possible candidates for a biocontrol strategy.

R. cerasi is most likely to be attacked by **predators** only during the short time span after leaving the fruit and pupation or immediately after emergence. Ants (*Myrmica laevinodis*, Hymenoptera: Formicidae), carabid beetles (*Anisodactylus binotatus*, Coleoptera: Carabidae) or staphylinid beetles (*Paedrus litoralis,* Coleoptera: Staphylinidae) are of particular importance [41]. Boller [41] noted that up to 80% of larvae were destroyed by predators before pupation, and that ants seemed to be the most important enemy.

R. cerasi is not only associated with pathogenic microorganisms, but also with **symbiontic microorganisms**. Infestations by different strains of the endosymbiotic bacterium *Wolbachia* lead to an unidirectional cytoplasmatic incompatibility in *R. cerasi* [19, 57]. Because *Wolbachia* infestations can profoundly alter host reproduction, research on this topic might lead to new biocontrol approaches of *R. cerasi*.

Recommendation for cherry fruit fly control

Well-managed orchards are a prerequisite for the effective control of *R. cerasi*:

- Trees should be regularly pruned and trees height should be limited to 10 m to allow good coverage of spray applications and to facilitate an early and complete harvest of fruit.
- For new plantings of extensively managed standard trees, varieties suitable for mechanical harvest should be chosen to enable a quick harvest. Harvesting the cherries early and completely reduces the population level of *R. cerasi* by removing the larvae from the orchards before pupation.
- Infested fruits should not be dropped on the ground.
- If possible, early ripening cherry varieties should be chosen, because they are maturing before the majority of the flies are ready to oviposit.
- It is recommended not to cut the grass under the tree canopies until shortly before harvest. With a higher plant cover the soil temperatures remain low, which can delay fly emergence for about ten days .

Based on economic considerations, the following strategies for cherry fruit fly control are recommended.

- If still registered, one application if Dimethoate at the stage of colour change (green to yellow) of cherries is by far the most cost-efficient method.
- Alternatively, Neonicotinoid-products provide a good efficacy with reasonable costs.
- Crop netting with fine-mesh insect net (1.3 mm) to avoid immigration of flies into the orchard provides efficient control in intensively managed dwarf tree orchards covered by plastic or hail net.
- In organic cherry production in orchards without plastic cover or hail net, foliar applications of Naturalis-L (*B. bassiana*) are most suitable.
- The use of Spinosad-bait formulations (Spinosad GF120 or CombiProtec) is an option for control in areas with dry climate

- The use of yellow sticky traps is very expensive and only reasonable if no other regulation method is available.

Research options for *R. batava*

Research on *R. batava* is needed concerning several points:

- Testing and comparing different types of yellow traps, maybe in combination with baits
- Monitoring of flight period by yellow sticky traps (emergence time, duration) and development of a temperature based forecasting system
- Screening of host plant susceptibility (which varieties are less infested, which are the host plant traits responsible for infestation: sugar and acid content? ripening time? Colour?)
- Spinosad or neem-bait-applications: field efficacy trials using existing products and economic calculations
- Entomopathogenic fungi: field efficacy trials using existing products and economic calculations
- Netting over crop canopies or on the soil: field efficacy trials and economic calculations
- Mechanical barriers on fruit surface using oil or kaolin products: field efficacy trials using existing products: influence on infestation levels and on processing quality of berries
- Parasitoids: A monitoring of natural occurring larval and pupal parasitiods is necessary to select possible candidates for a biocontrol strategy.

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This conference proceeding is a shortened and adapted summary of Daniel & Grunder 2012 [58]

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Workshop II: Cultivation Technology in Europe – meeting the needs of growers

Overview of cultivation technologies and their challenges

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Abstract

The text will give an overview of the Sea buckthorn cultivation in Central Europe. The plant propagation, the cultivation, the assortment, diseases and pests, and harvesting methods are discussed.

Key words

Sea buckthorn, cultivation, diseases and pests, harvest

Introduction

Sea buckthorn is still a young fruit species in Europe. In Germany the first plantation was planted 34 years ago with 3 hectares. Now there is 600 ha of sea buckthorn cultivation in Germany. The cultivation is not easy and there are still many problems.

About the sea buckthorn cultivation in Europe, there are only a little information. Quite a lot of sea buckthorn is grown in the Baltic States. In Finland and Sweden sea buckthorn plantations are small. There are also plantations in Italy, France, Poland, Belarus and Romania, but statistics wasn't available.

Propagation of sea buckthorn plants

The seedling production is not very problematic. There are two methods - the propagation with hard-wood cuttings or green cuttings.

Hardwood cuttings have all the summer time to grow. They are already well rooted in the end of June. In November they will be nearly 1 meter high (Pictures 1 and 2). Approximately 80% can then be planted. Green cuttings usually need two years before they can be planted on the field.

Picture 1. Hardwood cuttings in Germany in the end of June.

Picture 2. Same cuttings in November.

Sea buckthorn varieties

The number of Sea buckthorn varieties for European climate is too low. In Germany there are now four varieties (Hergo, Askola, Habego, Leikora) + one new late ripening variety. The problem with German varieties is that they freeze to death at temperatures below -25°C. This means that they can't be grown safely in Northern Europe and in the Baltic countries.

The sea buckthorn cultivation in Germany is situated almost exclusively in the north-east area: in the federal states of Mecklenburg-Vorpommern, Brandenburg and Sachsen-Anhalt.

In Estonia only Russian varieties are in cultivation (Botanitscheskaya, Botanitschskaya Ljubitelskaya, Gibrid Pertsika, Otradnaya, Podarok Sadu, Trofimovskaya). Belorussian new varieties have not yet convinced in Estonia. In Sweden is the Russian variety `Botanitscheskaya Ljubitelskaya` the most widely cultivated variety. In Europe, new sea buckthorn varieties are wanted in several countries. In Finland, new frost-resistant varieties are searched. In Sweden new varieties are bred. In Romania there are new sea buckthorn varieties, but their cultivated area and their characteristics is so far unknown. This means that there are many new varieties in Sweden and Romania, but they are not yet tested.

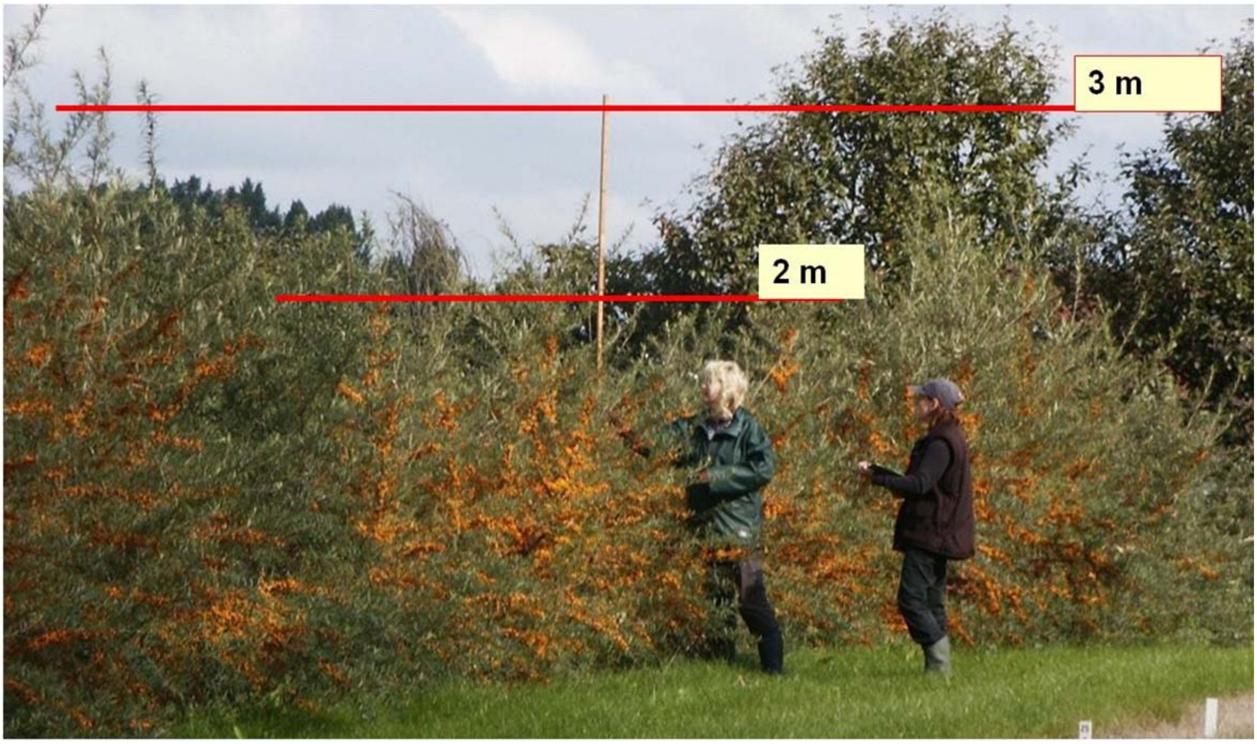
Also in Germany new varieties are searched. For example from the selection of alpine sea buckthorn clones subspecies *fluviatilis*.

A great common problem is the frost susceptibility of the male sea buckthorn varieties.

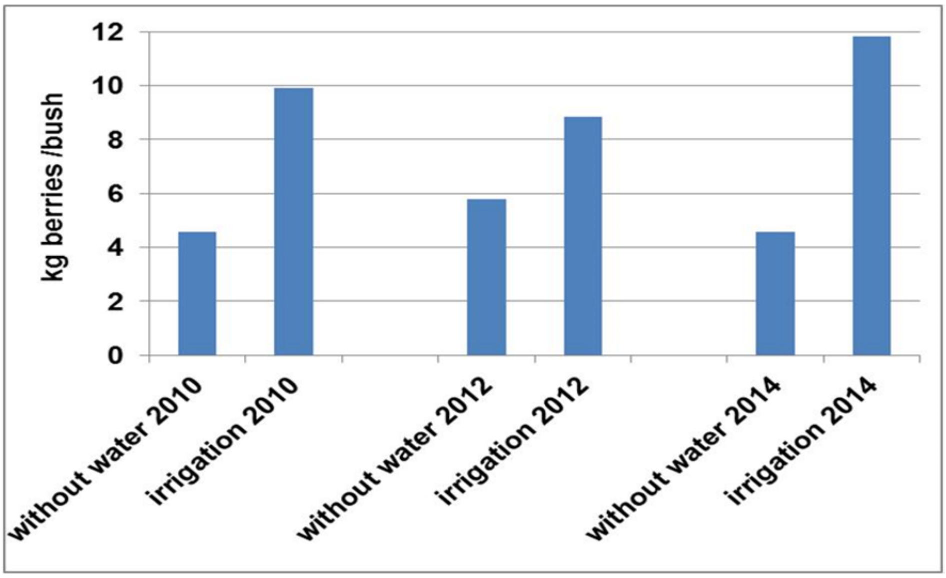
Cultivation of sea buckthorn

Sea buckthorn is relatively easy to grow. It grows well on sandy loams. But sea buckthorn also needs water in the soil. If there is too little water in the soil, sea buckthorn may wither and die, as seen in Ludwigslust in 2006. The effect of irrigation to the yield is proven to be significant.

In Gülzow, Germany, already in the third year after planting, in 2010, the growth differences between irrigated and non-irrigated plants were clearly visible at the beginning of harvest (Picture 3). The yield of the sea buckthorn berries from the bushes with irrigation was in average a twice as high as without irrigation (Picture 4).



Picture 3. Difference in plant height between irrigated (in the front and in the back of the picture) and non-irrigated (in the middle of the row) plants on field trial in Gülzow in 2010.



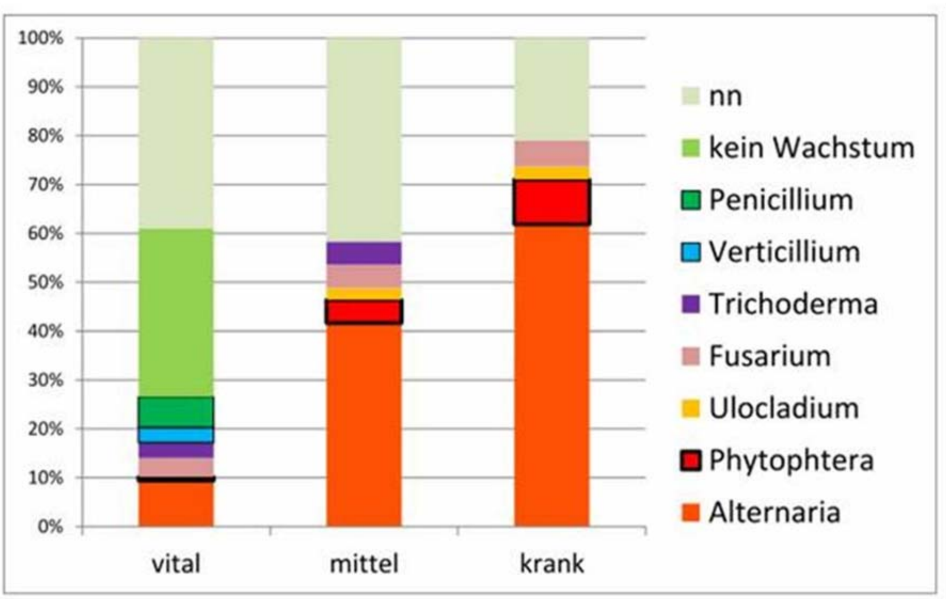
Picture 4. Influence of irrigation to the yield in field trial in Gülzow

Plant protection: diseases and pests

Even a few years ago was thought sea buckthorn is unproblematic in the cultivation. Today we know that there are many problems with the plant health.

In Germany *Verticillium* and other fungi are serious problems in some varieties, especially in Russian varieties. In 2005 we planted Russian sea buckthorn varieties. In 2007, many plants died to *Verticillium* and other fungi.

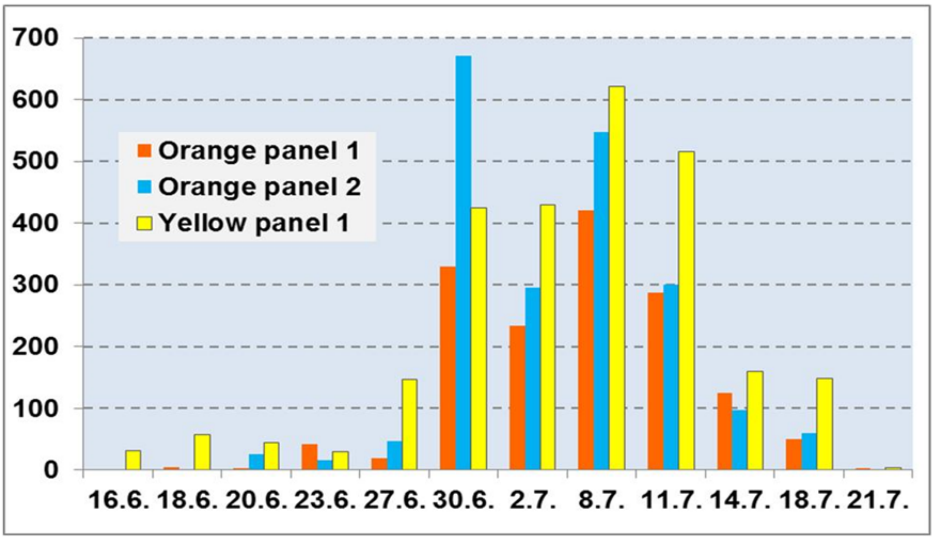
In the year 2012 sea buckthorn plants and soil from Gülzow were tested for fungal infection in ZALF Muencheberg. The question was what species of fungi colonize the sea buckthorn? Is *Verticillium* the principal fungus? Which species of *Verticillium* cause the damage? Healthy and diseased parts of the sick sea buckthorn branches were analyzed. The result was surprising: The fungus *Verticillium* was found only in 2% of samples (Picture 5). In the medium-damaged and diseased plant parts, Verticillium was not detectable. The more the plant parts were diseased, the more both *Alternaria* and *Phytophora* infection could be found.



Picture 5. Species of fungi that could be found from healthy and diseased parts of sick sea buckthorn plants in Gülzow. Vital= healthy, mittel= medium-damaged, krank=diseased plant part.

An extremely serious problem is the occurrence of sea buckthorn fruit fly (*Rhagoletis batava*) since 2013 in Germany. In 2012 no signs of fly could have been noticed. On 19th of August 2013 many maggots were found in frozen sea buckthorn berries. A few days later was the great damage seen in the plantation. In the soil under the sea buckthorn bushes many fly pupae were found. 100 fly pupae were put in gauze cages to observe when the first flies fly.

Also fly pupae was studied in the laboratory. In the laboratory of the Plant Protection Service Rostock the first sea buckthorn flies were seen in late April. During year 2014 also several yellow and orange sticky traps were hanged. The first flies were trapped on 16th of June (Picture 6). Main flight time of sea buckthorn fruit flies was from June 27th to July 14th in 2014.



Picture 6. Number of trapped sea buckthorn flies per day in Gülzow in 2014.

In the last years the bird starling (*Sturnus vulgaris*) has been a serious problem, especially for the early ripening varieties.

The starlings are not on the fields every year. The first time in 2008 the starlings ate the berries, and since 2012 this has happened each year. In 2014 the damage was especially bad.

Harvesting of sea buckthorn

We know a lot of harvest methods, but no one is perfect.

1. picking by hand,
2. Shaking off the fruit from the bush,
3. Cutting of the branches, freezing the branches with berries, shaking off the frozen berries.

Hand picking is laborious and costly. Only about 1 or 2 kg per hour can be picked. In Germany a harvesting machine was developed 30 years ago.The machine shakes the berries off from the beforehand cut branches. Unfortunately, only the variety `Hergo` is suitable for this method without freezing.

In Estonia a berry-shaker HK-2 was developed (Picture 7). That berry-shaker was purchased also to Germany. From 2007 to 2009 it was tested and the results were following:

- The berry-shaker is suitable for the harvesting of sea buckthorn berries. The optimum thickness of the branches is 1-2 cm.
- There is a big difference in variety suitability. Russian varieties suit well for shaking, German varieties don’t manage very well.
- The best result is obtained at almost ripe but still hard berries.
- By berry-shaker harvest of 20-30 kg / h can be reached.
- Work with the berry-shaker is hard physical work.
- The "Berry Shaker HK2" is no alternative for large-scale plantations in Germany.
- Applications are limited to small-scale cultivation, where the heavy physical work is not avoided.

Picture 7. Berry Shaker HK2 is developed in Estonia.

Picture 8. Harvester developed and manufactured by Kranemann, Germany.

In Germany has prevailed the method: cutting off the branches, freezing the branches with the berries and shaking off the frozen berries. The problem is that some of the berries remain on the shrub. However, it is necessary to leave leafy branches to the bush, because the plants have to assimilate another 2-3 months. If they don't have enough leaves for assimilation, many shrubs die during the winter.

A large farm in Saxony-Anhalt, in Quellendorf, has developed a harvester for sea buckthorn branches (Picture 8). Only one man harvest the sea buckthorn branches and moves them in a hall. In the hall the branches are crushed, frozen with nitrogen and then the berries are shaken down. The quality of the harvested berries is very good.

Summary

- The seedling production is not very problematic.
- The number of Sea buckthorn varieties is too low.
- For the sea buckthorn cultivation water is very important.
- There are many problems in plant protection. The most important are *Verticillium* and other fungi and sea buckthorn fruit fly.
- For harvesting, there are several methods, but no one is perfect.

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Experiences about sea buckthorn cultivation and harvesting in Latvia

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Abstract

A survey of the sea buckthorn cultivation history and management of orchards in Latvia is presented. There are several groups of sea buckthorn varieties, grown commercially in different regions of EU countries. Each group represents specific genotype adapted to certain climate. The influence of climatic conditions on the choice of sea buckthorn varieties grown in Baltic States is described. Among other orchard maintenance work a special attention is paid to supply of sea buckthorn plants with water and nutrients. Prospective sea buckthorn crop in Latvia can be harvested mostly on sites with controlled irrigation and fertilization. Functions of the sea buckthorn root system separate parts are studied to achieve the maximum effect of the irrigation and fertilization. Biological and economic benefits and disadvantages of the large and small plantations are evaluated. A separate chapter is given to represent some specific intelligent harvesting methods – vertical split and horizontal split of shrubs, as the primitive cut-harvesting is not suitable for varieties grown in Baltic States.

Key words

Varieties, irrigation, fertilization, roots, harvesting.

History, statistics and market situation

Sea buckthorn (SBT) is not a native plant in Latvia, though fossil pollen records indicate its presence in postglacial raw soils. Until 1980 only wild sea buckthorn seedlings from Kaliningrad region were grown in Latvia along the roadsides as windbreakers and have been planted for recultivation of used dolomite, sand and gravel pits. There were several trials of SBT introduction in Baltics of Russian continental varieties from Altai region. This group of varieties belong to ecotype *Hippophae rhamnoides ssp. mongolica*, They were not able to adapt to Baltic maritime climate with frequent winter thaws. In 1984 another introduction trial took place, using different group of varieties. As these varieties have been bred by crossings among *H.rhamnoides ssp. mongolica, H.rhamnoides ssp. rhamnoides* and *H.rhamnoides ssp. fluviatilis*, the adaptation was more successful mainly due to the presence of *H.rhamnoides ssp. rhamnoides* genes. Since then they are still grown in Latvia and Estonia.

There are about 350 ha of SBT orchards in Latvia, about 250 ha of them are in the age of harvesting. Average yield is 4 tons/ha. Harvesting is done by cutting off the whole branches. Until last year most of harvested fruits were consumed by local processing companies, producing different SBT drinks. On year 2014 situation was changed – several local and Estonian enterprises made a big demand of non-processed fresh fruit bearing branches. Gross price (fresh fruits on cut branches) rised from 1,00 EUR/kg in season 2013 to 1,60 EUR/kg in season 2014. Net price (cleaned IQF fruits) accordingly from 2,20 EUR/kg to 3,00 EUR/kg. The reason of market change is a rising demand of large juicy fruits. Some technically equipped local entrepreneurs buy small lots of non-processed fruit branches. After freezing, cleaning and blending fruits are bulk exported. Price was pushed up as well due to recent poor harvest of SBT in Baltic countries. Residues of SBT fruits by insecticides applied against the SBT fly in southern regions may as well influence the international market shares.

Choice of varieties

Active growing season in Latvia lasts 180 - 200 days; the sum of temperatures above +10^{0}C is 1700^{0}C – 2150^{0}C. Winter weather conditions in the Baltic States can change – even though the average temperature in winter is around – 6°C, the cold can reach even – 30°C, and spring-like warm weather can set in, with temperature staying above zero. Area along the Baltic Sea side is where winter temperatures often rise up above zero, therefore continental varieties adapted to unchangeable cold weather during dormancy are not winter hardy in our climate.

There are four existing environmental groups of SBT varieties, potentially suitable for commercial growing in the NE of Europe:

A. Selections of native SBT in Germany (Sirola, Leikora, Hergo, Askola, Dorana, Frugana, Orange Energy, Pollmix etc).

B. Selections of native SBT in Finland (Tytti, Terhi, Tarmo etc).

C. Crossings between continental Russian varieties with native SBT from southern coast of Baltic Sea (Botanicheskaya Ljubitelskaya, Prozrachnaya, Podarok Sadu, Marija, Tatjana, Lord etc).

D. Crossings between continental Russian varieties with native SBT from northern coast of Baltic Sea (successful trials since 2004, no commercial plantations yet).

Latvian climate allows grow all of them, however we have chosen C and D groups because of larger and more juicy fruits with better taste. Latvian final product market demands mostly SBT juice and drinks. We can get only 50-60% juice out of SBT varieties of A and B groups in comparison to 80-90% out of C and D groups. There are significantly less troublesome stellate hairs on the fruits of groups C and D. Juice made of C and D groups is not as acid and requires less sweeteners.

Planting and maintenance

SBT orchards are planted by one or two years old mostly bare root plants. Potted plants are not so popular for commercial plantings, as they are expensive and difficult to transport. Due to very late entry to dormancy bare root plants overwinter in the nursery, and they are planted out in next spring. Distance between plants within the rows is 2 m, between rows is 4 m. That results in 1250 plants/ha. Male plants are arranged in mixed or single rows in ratio of 1:8 to female plants. Grass growing between rows is mowed several times per season. Grass between plants within row is mowed only first two years after planting, when young plants are small. Both organic and mineral fertilizers are applied right under the plants in the area of fine roots, as space between rows is occupied mainly by propagative roots, which do not take up nutrients and water. Creeping rootstalks or “cable roots” are diageotropic or growing perpendicular to the force of gravity. They send out new shoots from their nodes. Some growers retain them within rows, as they are clones and can replace lost main plants. These roots do not feed the main plant, conversely they move water and dissolved nutrients from the main plant to the root sprouts. The evidence of this one-way streaming was observed in Canada, BC, Summerland, where system herbicide glyphosate was applied to eliminate weeds and SBT suckers. Suckers died without any visible influence on the main plants.

SBT, especially the varieties with juicy fruits tend to have a great water use. SBT grows best on sandy soils, where without irrigation they may experience severe water stress, which can result in loss of foliage, dieback of twigs, fruit fall and, in extreme cases, death. Water stressed plants will exhibit less cold tolerance than plants that have been well maintained. Stressed plants are more susceptible to the winter temperature changes and attacks of pests and diseases.

Optimum growth and sufficient yield can only be achieved if water is properly managed. Successful growing of juicy varieties of SBT is dependent upon farmers having sufficient access to water. We try to locate SBT plantations close to the surface water source – river, lake or large pond. Ground water extracted from wells can supply only small plantations. Perfect place for SBT plantation are ”regulatory” floodplains - the low, flat bottomlands adjacent to rivers and lakes within a reach of a 100-year flood. If the groundwater level holds on 80 -120 cm, plants have the amount of water they need, neither too much nor too little. Surface water in springtime must disappear before SBT buds open, as well the land should be sandy and well drained.

Distribution of rainfall is not even; therefore irrigation is needed on the most of growing sites. First two years after planting some growers deliver water in mobile tanks, pulled by tractors. When plants grow larger, this method is not effective anymore.

Localized drip or sprinkler irrigation is too expensive, if only 1250 plants/ha are planted. Cheaper alternative is a simple plastic pipe system, where main supply pipes are buried down in the soil, 6 outputs/ha are placed above the ground within rows, and connected garden hoses are moved around manually.

Size of plantations

There are three plantations in Latvia with size of 20 – 40 ha, but most of SBT orchards are 1-3 ha large. Two hectares could be considered as a minimum size for a small commercial growing and processing enterprise. It is large enough to use equipment efficiently and implement a continuous orchard renovation program, yet small enough that one person can take care of most of the work. In case of selling non-processed fruits on branches, the optimal size of plantation is 10-20 ha. Larger orchards can make more efficient use of machinery and equipment, but more hired labor, and thus more management skill is required. In Baltic states migrant workers would be needed in this case. Large sized orchards usually are poorly maintained, as a rule without irrigation, so their annual yield is well below the average and unpredictable.

To reduce production costs of water demanding juicy varieties it is advisable to place SBT orchards on well drained lowlands along the rivers, streams and lakes, out of flood reach. Such plots in Latvia are small sized.

The appropriate time of harvest is one of the most important factors affecting the quality of SBT fruits. Owners of small plantations have advantage to manage late harvesting, when fruits are completely ripe. This is very important for quality juice and oil producers.

Harvesting methods

For commercial production, a common harvesting technique is to remove an entire branch. It is done in various ways, which can be divided as follows:

Total cut

Upper part of bush is completely cut down. The whole tree disruption occurring after that is immense. Mainly loss of significant volumes of storage space and initiation of storage connectivity problems take place. By pruning of main dominant branches with fruits at the beginning of September, we destroy the greenwood primary pathways still in the period of active growth. Pruning the dominant branches can be done in March to rejuvenate old bushes. Then plants are still dormant and carbohydrates are stored down in the lower parts of trunk. By cutting the bush heavily during growing season plants get weaker. Total cut is not used in Latvia, as our SBT varieties do not tolerate this way of harvesting: plants recover only if not more than 50% of leaves are removed in case of late harvest, or not more than 30% in case of early harvest.

Lower cut

Deciding what to cut off and what to leave growing, branches that are marginal and had been barely hanging on for last seasons are chosen. “Blind cobs" with the berries are cut, since these will not continue to grow and will die and wither away by the end of the growing season. The problem is that the growth control pathway is taken over by the remaining dominant shoot, no new side shoots appear and the whole plant becomes like a tree with a single trunk. In this case amount of fruits in next years is reduced and located high above the ground, that causes necessity of radical cut down of the whole plant with following two “empty” years without harvest.

Vertical split
Bush is divided in two parts, leaving equal number of major branches on each side. Age difference between both parts is one year (picture 1). Harvesting is done by complete removing of one of the part, when it has reached the age of three years. Three years old branch holds six times more fruits than two years old one, therefore it is useful to skip the minor yield of the second year. Two years of harvest are followed by the third year of rejuvenation (picture 2)

Picture 1. Bush vertically divided in two parts with age difference of one year.

Advantages of this method: yielding part of the bush is located at easy accessible height for many years, bush is gradually rejuvenated, avoiding long interruptions between harvesting years.

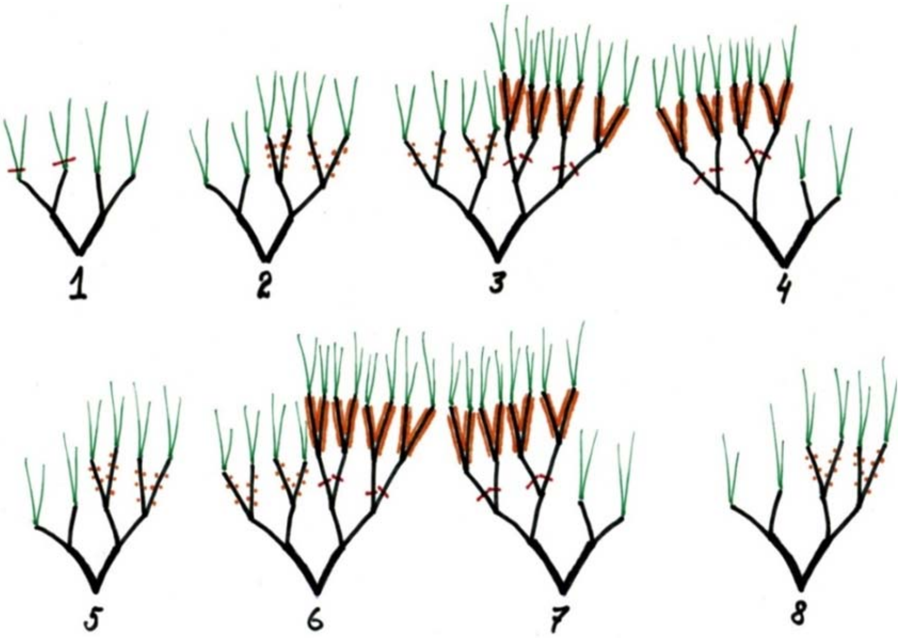
However, there are some preconditions to be taken into consideration. There are no active buds near the wound of three years old wood of SBT, as it contains only dormant growing points (adventitious buds). They will be released if there are carbohydrates available. Availability of carbohydrates depends mainly on two conditions:

- Sufficient growing factors are present (water, nutrients, light, temperature etc.).
- Removing time of the branch is optimal: The later in autumn the branch with fruits is removed, the more carbohydrates are stored around the forking point below the wound.

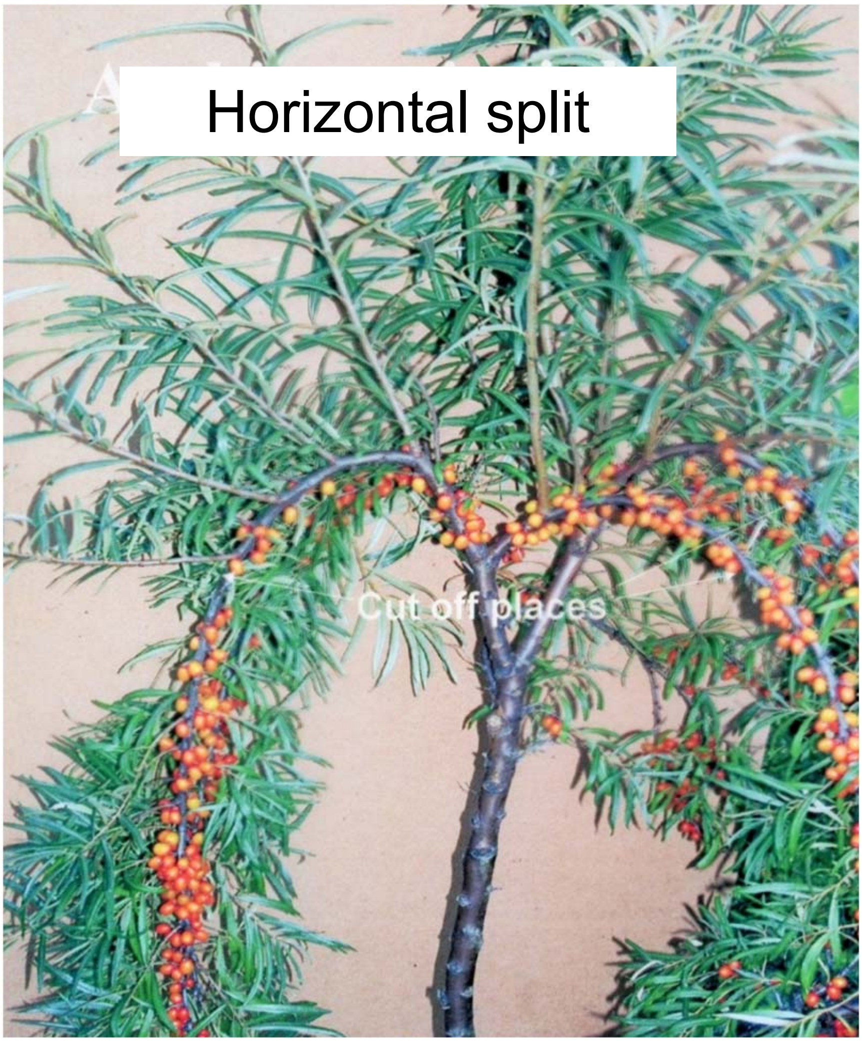
Therefore this method works well with healthy and strong growing plants, that are harvested late. Another condition is to remove all branches of the harvested side of bush. Any branch left will become the dominant growing point with active buds that subordinates all other growing points. It will keep adventitious buds dormant and no new shoots will appear on the harvested side. Trials of vertical split in Latvia have taken place for three years now, but more time for observations is needed.

Horizontal split
Annual dormant shoots are bent down, attaching their tops to the ground, thus creating “arches” (picture 3). This position initiates realignment of growth hormones – buds located on the top of arches become terminal and create the group of the next leading upward shoots. Bending is done in March. Downward directed parts of branches blossom and set fruits. In harvesting time they are pruned, leaving new leading shoots at the top of arch untouched (picture 4).

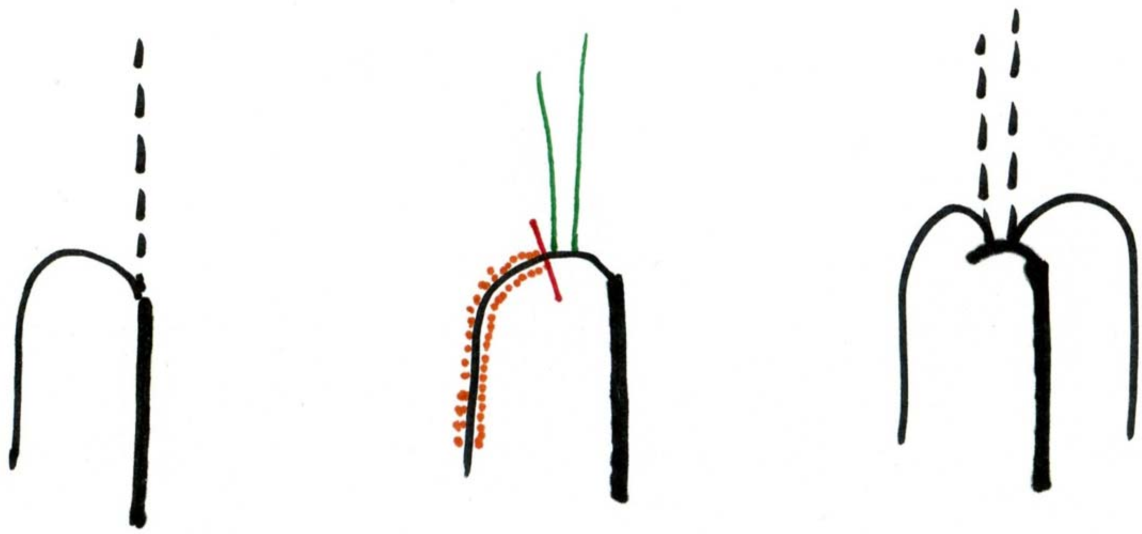
Next year the procedure is repeated with next new leading shoots. Thus we split up the whole plant in two zones – upper vegetative and lower generative zone (picture 5).



Picture 2. Schematic illustration of annual cutting sequence by vertical split.



Picture 3. Principle of the horizontal split.



Picture 4. Schematic illustration of the horizontal split.

Picture 5. Bush divided in two zones – upper vegetative and lower generative zone.

Workshop III: Quality of Sea Buckthorn – can it be standardized?

Standardization of sea buckthorn - local and global aspects and demands

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Abstract

Sea buckthorn becomes a good in international trade. Standardization is a necessary mean to make exchange of final and semi-products possible and reliable. A main problem in developing an international sea buckthorn standard is the difference in social-economic systems in eastern and western world. Proposals for a pattern for standardization are discussed.

Key words

Sea buckthorn, standardization, legislation, quality indicators

Introduction

International trade with sea buckthorn (SBT) has been developed within the last ten years with great success. More and more not only berries but also semi-products are marketed and trade channels become more complex. There is a great demand in assuring quality and defining distinct parameters for SBT products. In 2013 International Sea buckthorn Association (ISA) launched a working group to make a proposal for SBT standards. First draft documents presented in 2014 indicate that there are tremendous differences in understanding what standardization means.

The problem

In general standardization means the unification of dimensions, types and procedure. In production it is used to unify products (typification) or / and define common sets of parameters (properties). Standardization is a result of work division and has its roots in the end of 19th century. In the beginning unification of dimensions was the leading force but soon exchange of goods led not only to unified measuring systems but also standardized properties.

Standardization is mainly a field of industries, industrial products and procedures. It is seldom used to define properties of naturally grown products. Different economic systems in western and eastern hemisphere developed in last 60 years a divergent understanding of the meaning. In western socioeconomic system (e.g. EC) standards are mostly a field of business community and this way does not have legal power (with some exemptions where standards get legal power by embedding them in legislation). In eastern economy (e.g. Russia, China) standards are widely used to define properties and technical rules for products and processes and have in practice partially legal power.

Standards in food industry are often used to grade products (e.g. class A, B of fruits or S, M, L for egg size) or for defining semi-products. The latter is mostly substituted by individual agreements between supplier and customer and based on specification of goods in trade.

Possible way to a solution

For the standardization of sea buckthorn a double sided strategy is proposed. On the one side basic properties of raw materials should be defined that are essential for processing. E.g. for products like

- berries
- frozen berries
- puree

Concerning such properties like

- amount of damaged fruits
- amount of impurities
- amount of sand
- fibers in product

And on the other hand a system for grading products by their application properties, like

- raw juice
- puree
- seed oil
- pulp oil

To define grading properties like

- acidity
- amount of individual vitamins
- amount of sugars
- levels of contaminants

The list of products and properties should be discussed and agreed on international ISA level.

Are quality standards necessary for sea buckthorn - a business perspective

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Abstract

Sea buckthorn is a plant producing fruit containing high levels of complex nutrients that competes within a developed market of internationally traded natural nutraceutical products. It has been grown as an agricultural crop in Europe since the 1970s. This is part of a global production that is largely Asian centered where investment in processing development and facilities has grown significantly since the year 2000. The health conscious consumer in Europe is spending 9 billion euro a year on nutritional supplements. Sea buckthorn as a crop is expanding in some EU member states, but cost structures and technical difficulties relating to harvesting need to be answered for production to grow.

Consumers in Europe demand quality. The European marketplace is highly regulated at all levels of the supply chain. These regulations are instigated both by the European Commission and by individual EU member states and impact on inter-state trade. The objective of regulation is to protect the consumer from poor quality, adulterated or dangerous products. These regulations apply to product manufactured within the EU member states and do not create a barrier to entry to product from non-EU countries.

Quality is measured by comparing things of a similar kind. This measurement formulates into standards which may be legally enforced or be a statement by a manufacturer to show the consumer that a product is both safe and suit the consumers' needs. Good regulation and standards can build trust and loyalty in consumers for products and services. Creating standards must build strong supply chains delivering value consistently to the consumer. Standards must return value in price premium so that costs and investment are recovered through the whole supply chain.

Supply chains are made up of businesses of varying size. Small and medium sized businesses are highly creative and innovative, but often have limited resources. It is the larger companies that will drive market growth but standards need to be set at realistic levels to be affordable by all members of the supply chain to achieve the objective of delivering consistent quality to the consumer.

There are very few unique products in the world. Standards set on product alone reduce differentiation between similar products made by different manufacturers. Consumers expect product quality but they chose between brands based on trust and association with the supplier or manufacturer. European sea buckthorn growers, processors and manufacturers have already invested in producing high quality. National identity, traceability, transparency, sustainability and environmental/ethical management are aspects that build trust, loyalty and competitive strength.

If global standards for sea buckthorn develop, Europe must respond in order to ensure that European sea buckthorn has a clear identity based on combined product and service standards. To grow and succeed it needs to communicate its strengths to the global market. European sea buckthorn must be defined as exceptional in product, range, service and value for money to become accepted as premium market leader.

Introduction

"Are quality standards necessary for sea buckthorn – a business perspective"? I come here as a grower, coming from a country where sea buckthorn is virtually unknown. The objective of my presentation is to introduce ideas for the workshops that we are about to have and inspire debate to reach positive conclusions for this part of the conference. It seems most appropriate that we are here in Finland where there are standards for sea buckthorn and so I hope local experience will drive some of this debate.

One of the issues of this subject is that everyone seems to have standards – maybe voluntary; product specification or regulatory but they are not being recognized. This subject is about standards being recognized and them being used as a marketing tool.

Where do you see the European sea buckthorn industry in ten years time?

This is a key because the world is changing and there is a thin line between survival and success in business.

Investment in sea buckthorn is growing in Asia and this is a competitive challenge to Europe. To compete in Europe, production costs must reduce; there needs to be a solution to affordable harvesting but of primary importance is market development. The European consumer must know what is exceptional about sea buckthorn and why European sea buckthorn is the best. As I see it, there are two potential aspects to this – nutrients and benefits. But I want to use a quote that I read last week from a German MEP – Peter Liese – (LEAZA): "I think it is a good thing that you cannot use claims if they are not approved by EFSA. But I don't think it is good that you can make a claim on a product that is not good quality".

When considering standards for botanicals we have to ensure that the indicators chosen must stand up to scrutiny as showing both individual quality and quality within the context of delivering benefit to real consumer need.

It is as important for the sea buckthorn industry to understand the consumer, as the consumer to understand sea buckthorn. Consumers are a dynamic mix of gender, age, lifestyle, perception and need. One needs to understand what people want and match product to their mindset – not just produce in the hope that there is someone out there that might buy it. Market development needs to be clever not expensive. This is particularly fundamental as supply chains are shortening and direct selling to the consumer is on the rise. In an era of economic downturn; growing consumer price sensitivity and the rise of value brands market development needs careful strategy to develop demand based on consumer need.

But the market will only develop if sea buckthorn can deliver what it needs. Identifying what sea buckthorn can deliver will define what sea buckthorn is to the consumer. Once linked to consumer need, then it will become attractive to investors for new product development.

Of course sea buckthorn is not a simple product as it falls into food, drink, cosmetic, and nutraceutical categories. Creating standards needs to consider the growth markets within this diversity and how standards can enhance market development.

Standards alone will not drive development but they can focus capability that investors can feel confident in. Standards could be seen as a catalyst to develop market awareness and the supply capacity of the sea buckthorn industry.

I see standards as a powerful tool to develop the market, grow the industry and create competitive advantage. In Europe we live and work in a world full of regulation - National and EU regulations cost time and expense to business. If sea buckthorn standards are to be created they must not add to these regulations. They should build a trusted image of business that delivers sea buckthorn to consumers; adds value to product and gives competitive advantage within the marketplace. Introducing standards will only work if business commits to them and believes in them. At the same time buyers and consumers must recognize standards as securing and providing added value for their needs.

Establishing standards

In establishing standards I suggest there are some practical issues that need considering - so standards must be:

- Understandable to provide clear information to markets and consumers to build trust.
- Credible through transparent reporting to build market and consumer confidence.
- Achievable by every link in the supply chain conforming to secure reliability in supply quality.

- Affordable, essential and most important – cost must never be a barrier to entry to a standard system for small companies.
- Flexible, to allow all business to be inclusive to accreditation so consumers see consistent quality management through the supply chain.

Establishing standards sounds easy but when one considers that the EU still has no consensus of opinion on nutritional profiles which should have been completed in 2009 then achieving consensus between sea buckthorn interests from around the world may be a real challenge.

The European Union has a population of over 500 million. The population is growing older and more health conscious. Euromonitor values the 2013 spend on nutritional supplements alone in this market at 7 billion euro. 2 billion euro was spent on botanical food supplements. Organic Monitor suggests the natural cosmetics market has a growth capacity for 5% globally. Retailers' shelves display both populist juices (orange/apple/mango) and high nutrient promoted fruits - cranberry, blueberry and pomegranate, even goji – but not sea buckthorn.

Nordic Natural Product Fair in Malmö in October 2014 promoted three sea buckthorn products. Is this driven by demand or entrepreneurial spirit? Either way, there needs to be more market awareness of the best characteristics of sea buckthorn to highlight the competitive advantage over other similar super fruits. I mention the term super fruit because although it has been used to over exaggerate information on the benefits that some fruit can give, it still describes sea buckthorn well. Interestingly the Nordic Fair catalogue shows one company seizing the title of the European Super fruit and giving it to Aronia.

A message that is clear, believable and defines difference

To be useful, markets and consumers need three things from information. One of the most essential considerations when setting standards is to ensure that consumer is not confused and believes in your system.

In Europe we have EFSA that is the European commission's attempt to provide consumers with guidance on health claims. But as we know botanicals have been systematically rejected through the EFSA system. Conversely it seems that the French and Italian governments are providing credibility to the BELFRIT list of botanicals that may deliver an alternative view on safety and efficacy. This challenge is good to provide an alternative view and opportunity to develop the botanical market as long as it provides that clear and believable message to consumers.

Confusion is not good. The internet has done sea buckthorn an injustice by describing it as a wonder plant. The natural highest vitamin C content of wild Himalayan sea buckthorn is not characteristic of all sea buckthorn. Multiple benefit claims are incredible, but not so credible to the consumer? 190 different nutrients in sea buckthorn is a mere number to consumers. Information must be relevant to them – and more importantly - it must be memorable. The internet has power to transmit information virally. Personal testimonials are more powerful than any marketing tool. That is why it is important that we should create simple messages that people remember and will pass onto their friends and colleagues.

Defining standards

Technically standards can be defined in two ways. Quantitative - define specific aspects within products or systems. They are numerical, objective and verifiable. This system is good because it is simple, conforms to international standards and is credible. But there is a downside: As manufacturers across the world conform to a standard – quality differential between products narrows. If this differential reduces then so does its value to the market. Similar quality of product will then have to compete on price not on quality. This one can already see with a company like Amazon. Low price may be good for consumers, but low income is bad for business as it reduces profit and the ability to invest in quality and innovation.

The alternative to the quantitative system is a qualitative one. It takes into account service as well as product. It could be deemed to be more subjective and less verifiable - but it has the advantage of telling your customer about you and your commitment to them. This is what makes the European sea buckthorn industry special to the European consumer.

Europe is a sophisticated marketplace. Premium markets value exceptional qualities. It is an unfortunate truth that almost all products are not unique. But what can be unique is the service that delivers them. To be an exceptional product every link in the supply chain must be committed to delivering exceptional service. These commitments can be quantified when producers are part of recognized codes of practice; quality assurance schemes; traceability schemes; trademarks and so on. This means of attracting market admiration is used by big players. A new example is the Sustainable Agriculture Initiative – Its code of conduct demands transparency and integrity, commitment to sustainable practices and promoting them to their supply chain stakeholders. Membership includes many household name brands across the world including Arla, Danone, Nestle, Pepsico, Kellog, Coco Cola and so on. It would be easy to say these companies need to improve their image, but they are creating these standards so that the market can judge them on the standards they set.

It is possible that across the European sea buckthorn industry many companies are already signed up to quality assurance schemes. These are all valid and show commitment but for sea buckthorn to make a name in the marketplace it must present a united industry with common standards. United should not compromise competition between business, nor the strength or identity of brands or national associations.

There should be consideration however towards building a European co-operative network to promote best practice to deliver quality.

Conclusion

The essence of good standards:

- They must be simple to create a memorable message to consumers.
- They must be flexible to allow for diversity in product and business so standards are represented within the whole sea buckthorn industry.
- They must be targeted to ensure they add value to products and services.
- They must be credible administered by regular monitoring and reporting to secure confidence in products and services from consumers and the market. This does not need an outside organisation and many systems are self-regulated.
- They must be relevant to market and consumer needs not focused on industry capability.
- They must be affordable so as not to be a barrier to entry but allow innovative small businesses to grow and add value to the supply chain.
- They must be understandable highlighting the characteristics of sea buckthorn to generate belief and trust in sea buckthorn product, services and the brands that supply them.

Lastly, they must be achievable so the whole supply chain is committed to providing defined quality products and services to their customers and consumers with total reliability.

A good system of standards will present a clear, authoritative message to the marketplace that the European sea buckthorn industry is committed to delivering quality to its consumers. Such a system would provide competitive advantage within a fast moving global economy.

Standardising taste is complex - technical descriptions regarding acid/ sweetness are good for business to business, but they might be misleading to consumers. Unfortunately consumers have become used to fraud, poor quality product and bad marketing. These are the reasons why we have regulations and regulators – and possibly to some extent EFSA. The horse meat scandal in 2013 was a desperately sad example of poor control over long supply chains and lack of commitment to consumers. The result is a sceptical consumer and media that love to make a bad story worse.

Standards are a means to prove to consumers that an industry is serious about what it does – and in the end it is the consumers that pay our bills. So I return to where I started –

“Where do you see the European Sea buckthorn industry in ten years time?” and if the answer is that it has developed and grown – can it do it without the guidance of quality standards?

Poster Proceedings

Phytosterols and Flavonols in Sea Buckthorn Leaves

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Abstract
Sea buckthorn leaves (SBT) form notable by-product during harvesting and post-harvest management of the berries. In this study, we characterized and determined phytosterols and flavonols in SBT leaf. Phytosterols are health promoting compounds best known for their cholesterol lowering properties. Flavonols are phenolic antioxidants with many potential health effects. Leaves of Finnish SBT cultivar 'Terhi' were collected in one berry farm located in north-west of Finland at three different growing states: (1) at the end of May after the blossom but before the fruit development, (2) at early July when the fruits were evolving, and (3) at the end of September when the fruits were already full-ripen. Crude fat was extracted from the leaf and saponified. Unsaponified lipids, including phytosterols, were silylated and determined by GC-MS. Flavonols were determined by HPLC-DAD after acid hydrolysation. The total phytosterol content of the leaf samples was high and varied between 208 mg/100 g (dw) and 432 mg/100g (dw). The content increased during the growing season. Sitosterol and amyrins were detected as major sterols in all samples. Small amount of δ5-avenasterol was detected only in young leaves. Isorhamnetin, kampeherol and quercetin were the flavonols detected in SBT leaf. The total flavonol content was high and rather stable varying from 562 mg/100g (dw) to 647 mg/100g (dw). The highest content was determined in July. Overall, this study confirmed that SBT leaf is a rich source of many bioactive compounds including phytosterols and flavonols.

Key words
Hippophae rhamnoides, sterols, amyrins, flavonoids, flavonols

Introduction
Sea buckthorn (SBT) is known and used by humans for centuries in medicine, cosmetics and food. Cultivation of SBT is rapidly increasing in Europe due to the demand for its berries rich in specific valuable oils, high content of different vitamins, minerals, phenolics and essential fatty acids. SBT leaves form notable by-product during harvesting and post-harvest management of the berries. Although the chemical composition of SBT berries is fairly well characterized the composition of leaves is still poorly known. Recently we showed that SBT leaf is extremely rich in many bioactive compounds including ascorbic acid, tocopherols, carotenoids, ellagitannins and proanthocyanidins, but their contents can vary significantly during the growing season (Hellström et al., 2013). In the present study, we continued the chemical characterization of SBT leaves focusing on phytosterols and flavonols.

Materials and methods
Leaves of Finnish SBT cultivar 'Terhi' were collected in one berry farm located in western cost of Finland at three different growing states: (1) at the end of May after the blossom but before the fruit development, (2) at early July when the fruits were evolving, and (3) at the end of September when the fruits were already full-ripen. Leaves were freeze-dried before chemical analyses. Crude fat was extracted from the leaf and saponified. Unsaponified lipids, including phytosterols, were silylated and determined by GC-MS. Flavonols were determined by HPLC-DAD after acid hydrolysation.

Results
The total phytosterol content of the leaf samples was high and varied between 208 mg/100 g (dw) and 432 mg/100g (dw). The content increased during the growing season (Figure 1). Sitosterol and amyrins

were detected as major sterols in all samples. Small amount of δ5-avenasterol was detected only in young leaves.

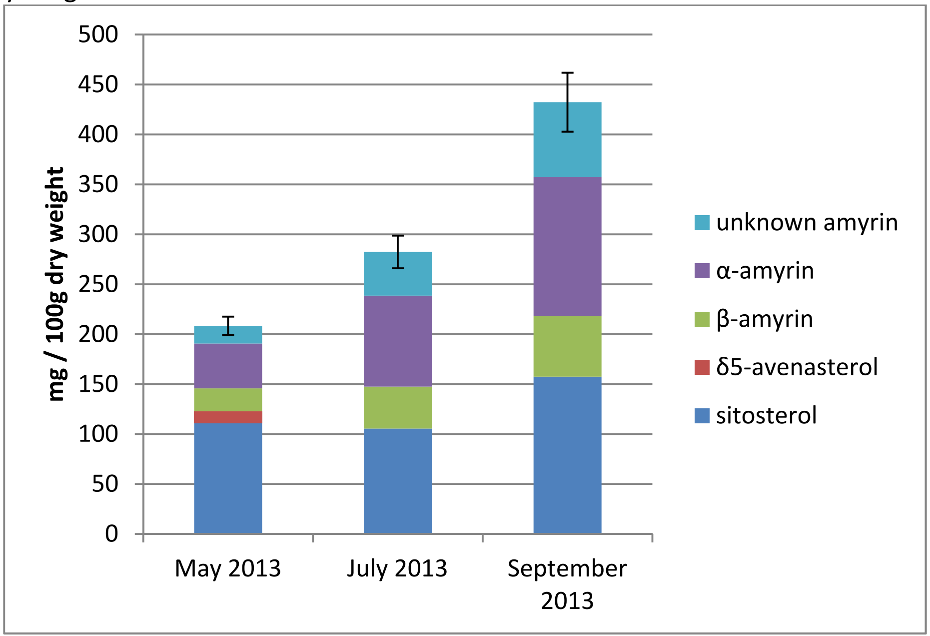

Figure 1. Phytosterols in SBT leaves.

Flavonols identified in SBT leaf included isorhamnetin, kaempherol and quercetin. The total flavonol content was very high and rather stable varying from 562 mg/100g (dw) to 647 mg/100g (dw). The highest content was determined in July (Figure 2).

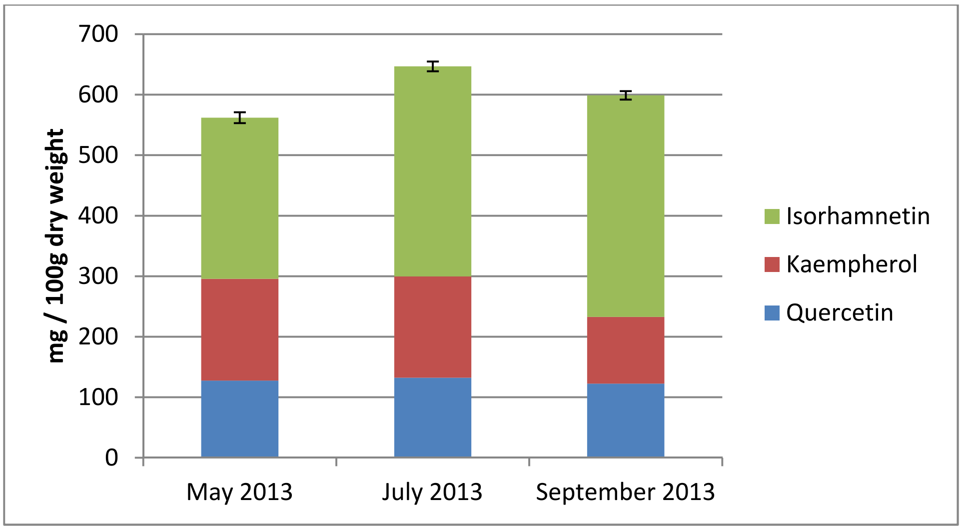

Figure 2. Flavonols in SBT leaves.

Discussion

Phytosterols and flavonols are well-known bioactive compounds with many health promoting properties. Dietary phytosterols are best known for their cholesterol-lowering effects while flavonols are phenolic antioxidants associated with multiply bioactivities. The high contents of these compounds in SBT leaf together with the previous data of its nutritionally promising chemical composition indicate that SBT leaf could be very attractive raw material for food/feed/nutraceutical industry.

Acknowledgements

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Applied research in Sea Buckthorn cultivation

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Abstract

The poster will give an overview of the Sea Buckthorn research in Mecklenburg-Vorpommern, Germany. Yield and health promoting compounds, optimization of the cultivation method, variety performance and disease resistance and biology of the sea buckthorn fruit fly are the important topics of research.

Key words

Sea buckthorn, cultivation method, variety performance, diseases and pests

Monitoring sea buckthorn fly in Mecklenburg-Vorpommern on 2014

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Abstract

The poster gives an overview of the monitoring for the occurrence of sea buckthorn fly in 2013 and 2014 in Mecklenburg-Vorpommern, Germany.

On August 18, 2013 maggots were noticed in sea buckthorn fruits for the first time. On August 22 damaged sea buckthorn berries were also seen.

In 2014 many sea buckthorn fruit flies were trapped on Rebell Yellow panels and Orange panels. The first flies were caught on 16th of June. Main flight time of sea buckthorn fruit flies was from June 27 to July 14.

Key words

Sea buckthorn, sea buckthorn fruit fly, occurrence in 2014

Introduction

The sea buckthorn is still a relatively new kind of fruit in cultivation in Germany. In 1980 the first sea buckthorn plantation of a size of 3 ha was planted in Ludwigslust. As a result by the year 1989 the sea buckthorn area was expanded to over 150 ha planted by several companies in the northern districts of the former GDR and in the district of Potsdam. In the 1990s a lot of plantations disappeared, but after approximately 15 years the sea buckthorn cultivation experienced a new upturn. According to German agricultural statistics (Stat. Bundesamt 2015) in 2014 seabuckthorn area was 671 ha (Stat. Bundesamt 2015). The main cultivation areas are Brandenburg, Mecklenburg-Vorpommern and Sachsen-Anhalt where more than 300 ha, approximately 200 ha and approximately 100 ha of sea buckthorn is cultivated.

Until now, sea buckthorn has been a relatively simple plant to cultivate. There haven't been serious problems in plant protection. Some fertilization has been given during planting and after that it has not been necessary to fertilize. This has been confirmed also by research (Höhne 2013). From the summer 2013 the situation dramatically changed. On 19th of August 2013 we found damaged berries in branches of some middle-early varieties in Gülzow. After freezing the branches maggots could be found (fig. 1).

Fig. 1. Maggots in sea buckthorn berries after the freezing on the 08/19/2013 in Gülzow (Photo Höhne)

Earlier nothing had been noticed during the harvest of the early varieties when cutting the fruit branches and removing the leaves before freezing. But during the subsequent days the damage was clearly visible in the bushes. Many berries had dried and with the exact glance one could discover small pin-size holes where from the maggots had left the fruits (fig. 2). The whole process went quite fast: from first finding of the maggots after the freezing to the clearly visible damage in the bush took no than a week (fig. 3).

In fruits of later-ripening varieties we found still some maggots, partly up to 4 maggots in a fruit. The development of maggots into the pupae occurred quite fast and we could also measure the first pupae (fig. 4 and 5).

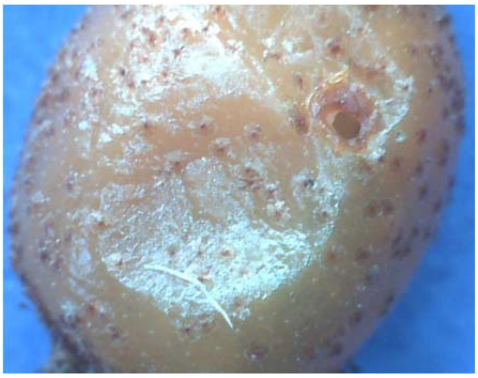

Fig. 2. Hole in injured sea buckthorn berry where the maggot has left the berry. (Photo Gießmann)

Fig. 3. Sea buckthorn variety 'Botanitscheskaja Ljubitelskaja' on 15th of Aug and on the 8th of Aug 2013 in Gülzow (Photos Höhne)

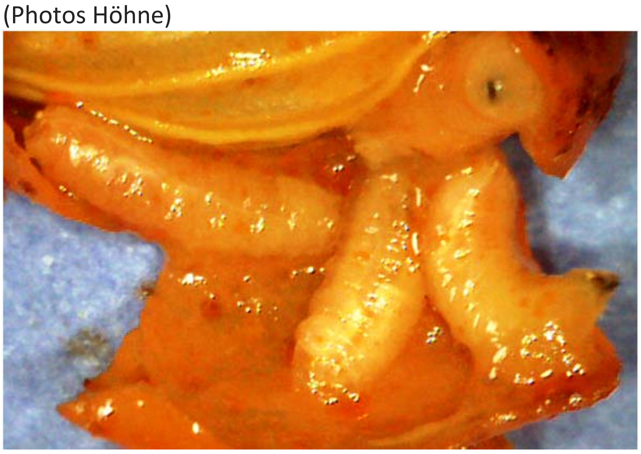

Fig. 4. Four maggots in a sea buckthorn fruit.
Fig. 5. Sea buckthorn fruit fly pupa (numbers = mm) (Photos Gießmann)

It was fast understood that only sea buckthorn fruit fly could have caused this damage. Unfortunately, in 2013 we could catch only one adult and we couldn't be absolutely sure. 14 days after the infestation in Gülzow we received pictures from Brandenburg, where similar damage was seen (Holz, in 2013).

Genetic finger code of sea buckthorn fruit fly

Pupae from Gülzow were sent through the Department of Plant Protection Service at LALLF to the State Office of Rural Development, Agriculture and Land Consolidation, Department of Phytopathology in Brandenburg.

There Dr. Marko Riedel examined pupae from Brandenburg (Glindow and Frankfurt/Oder) and our pupae from Gülzow by means of the sequence analysis COXI. These pupae showed an identical genetic structure (Riedel 2013). With the help of experts of genetic bank in the Dutch Naturalis Biodiversity centre these sequences were identified as *Rhagoletis batava*.

After the first publication about the damage of sea buckthorn (Höhne & Giessmann 2013) the Zoological State collections in Munich fly became attentive on sea buckthorn fly and asked for fly pupae sample.

The aim of the Munich researchers is to map all German animal species genetically and to make information available in an online library for experts. In the meantime, within the scope of the Germany-wide Barcoding project \"Barcoding fauna Germanica \" the pest was analysed there genetically. The Munich project is a part of the international Barcoding project iBol with its headquarter in Canada. Ambitious aim of the project is to map all animal species genetically worldwide (Natzer 2014).

Experimental design to monitoring the hatch of the sea buckthorn fruit fly

In September 2013 numerous sea buckthorn fly pupae were found directly under sea buckthorn bushes. They were accumulated in a layer of soil in a depth of 5 cm (fig. 6). We were surprised – in an area of approx. 2 m² we found more than 1,000 fly pupae.

To monitoring of the survival rate of fly pupae and the hatching time of the flies, we put in October 2013 several photo-eclectors and insect net cages, in each case equipped with 100 fly pupae, directly under the sea buckthorn plants (fig. 7).

Fig. 6. Sea buckthorn fruit fly pupae in the soil on 26th of Sep 2013 (Photo Höhne).

Fig. 7. Insect net cage with sea buckthorn fly pupae in Gülzow on 2nd of Oct 2013 (Photo Höhne)

The first hatch of the flies in 2014 under lab terms

At the end of March the first hatch could be observed in the lab of the Department of Plant Protection Service of the LALLF MV in Rostock and it was succeeded for the first time to take pictures of the sea buckthorn fruit fly.

Shortly after pictures also in Dessau sea buckthorn fruit fly had hatched of the pupae in the lab. These pupae were collected from sea buckthorn plantations in Quellendorf, Sachsen-Anhalt (Meyer 2014).

Dr. H.-J. Gießmann observed the fly hatch under the microscope and filmed it (fig. 9-12). Clearly the bladder-like pouch is to be seen on the head. With it, the fly break off the end of the puparium to emerge. By rhythmical contraction of this pouch (ptilinum) in the teamwork with the contraction of the abdomen the fly pushes bit by bit itself out from the puparium. In the soil this process runs indeed more quickly, because pressure from all directions is given by soil. A small tunnel from which the fly can leave the soil is then bored by the rhythmical contraction of the forehead bladder-like organ.

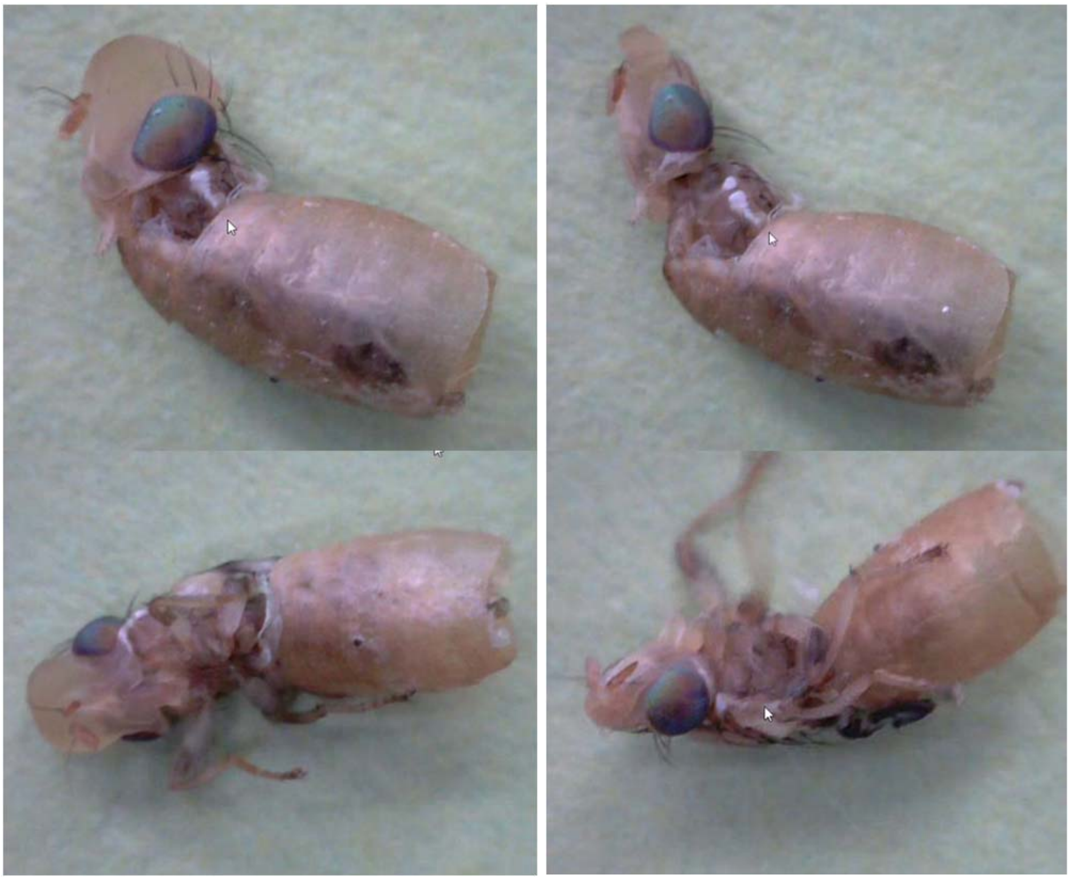

Fig. 9-12. Emergence of sea buckthorn fruit fly (Photos Gießmann)

Flies hatching in the field

Several cross-barrier sticky traps (yellow and orange) and photo-eclectors were installed for the monitoring of the flies hatching in Gülzow. Customary yellow traps that are used for the monitoring of cherry fruit fly and orange traps for the monitoring of the carrot fly were used. Orange traps were cut and could be thus used as a cross-barrier traps. These boards were put up in the net cages as well as in the field.

With the photo-eclectors we succeeded to catch untouched sea buckthorn fruit flies which were taken for measurements. The flies hatched, bored through soil, got into the pipe of the photo-eclector and drowned into alcohol and were preserved so (fig. 13, 14).

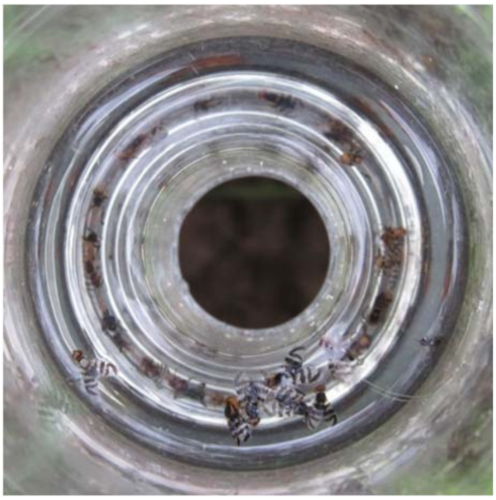

Fig. 13 and 14. Glass head box of the ground photo-eclector in profile and top view with caught sea buckthorn fruit flies (Photos Höhne)

Orange and yellow cross-barrier sticky traps caught flies differently according to the location. Three traps with the highest catch figures were hung up in the sea buckthorn row which had the strongest infestation in 2013. There the first flies were caught on 16th of June. During 10 days were 3 to 58 flies counted in each sticky traps.

By the 27th of June 147 were flies caught on a barrier sticky trap. Then the population exploded – during 14 days 234 to 670 sea buckthorn fruit flies were counted in each day. From the 14th of July the catches clearly decreased when 149 to 160 more flies were caught on a trap. From the 21st of July only a few flies were caught (fig.15 and 16)

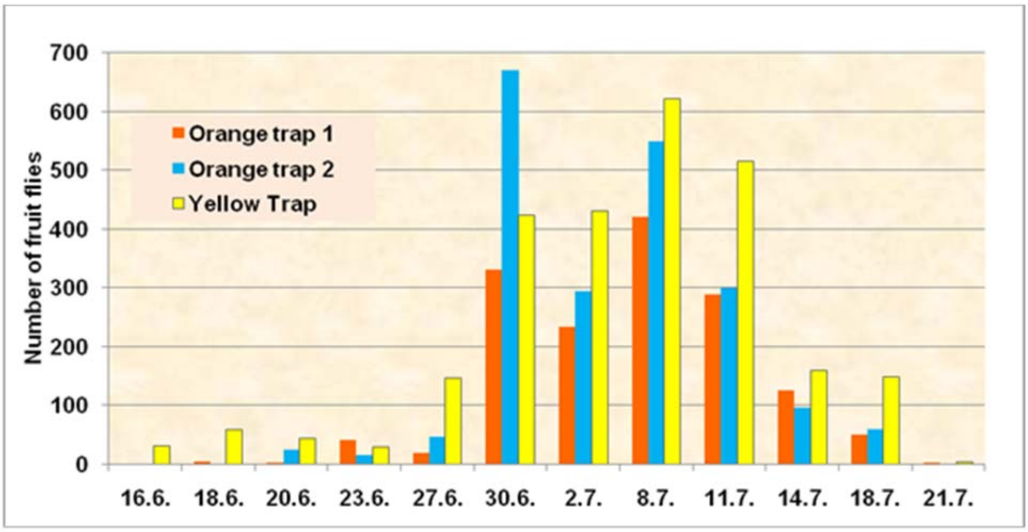

Fig. 15. The number of caught sea buckthorn flies on *cross-barrier sticky traps* per day in 2014 in Gülzow

Fig. 16. Yellow *cross-barrier sticky trap* covered with the sea buckthorn fruit flies at the beginning of July 2014 in Gülzow (Photo Höhne)

A similar slip beginning could be ascertained in Rostock in a net cage put up in outdoors which was equipped in October 2013 with 100 pupae. On the 18th of June there slipped the first flies. The flight altitude point lay between the 27th of June and the 1st of July. It was succeeded to observe how sea buckthorn fruit flies fed by the honeydew after hatching. Aphids were settled on ragwort (*Seneceo jacobaea*) and poppy (*Papaver rhoeas*) inside the cage.

On the 7th of July the first puncture sites were found in the skin of sea buckthorn berries and on 14th of July the first egg larvae were observed. The puncture sites had a diameter of 0.3 mm, as wide as the upper diameter of the female ovipositor. The first instar larvae themselves were 1 mm long and 0.33 mm wide (fig. 17-19)

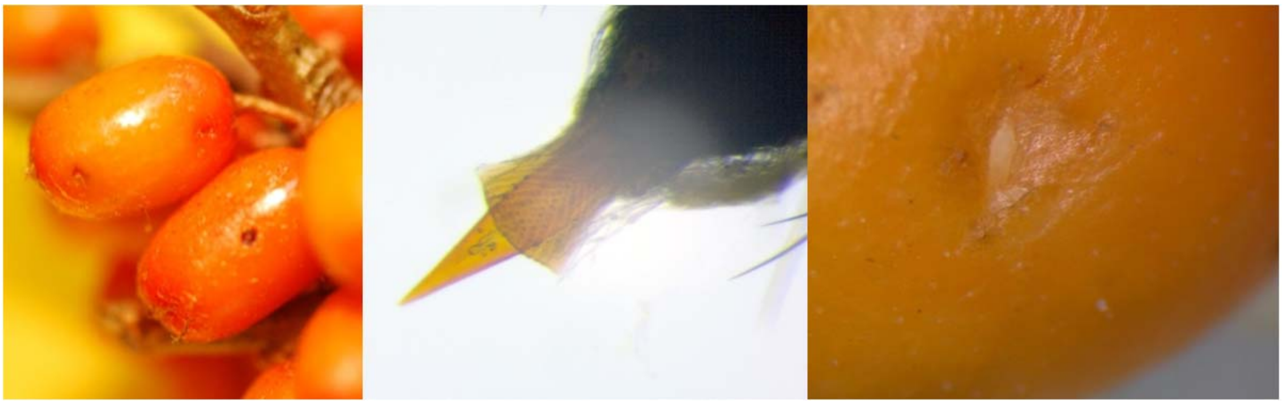

Fig. 17. Puncture site on the sea buckthorn berries, **fig. 18**. Ovipositor of sea buckthorn fruit fly (female). **Fig. 19**. Egg larva under the fruit skin of sea buckthorn berry. (Photos Kuhnke)

Fly size and characteristics

A bigger number of the flies caught in the eclectors were measured in the plant protection organization of Rostock. The average body length of female sea buckthorn fruit flies (fig. 20) was 5.51 mm and of males (fig. 21) 4.99 mm.

Fig. 20. Female sea buckthorn fruit fly
Sexes are well distinguished by the form of the abdomen.

Fig. 20. Male sea buckthorn fruit fly. (Photos: Kuhnke)

These values are about 20% bigger than dimension data of the flies in Russia (Shamanskaya 2014). These dimension differences were confirmed by Mrs. Dr. Shamanskaya personally, after she had seen caught sea buckthorn fruit fly from Gülzow on a barrier sticky trap in European Sea buckthorn Conference EuroWorkS 2014 in Finland. Whether the climatic conditions are the cause of the different dimensions, or whether there are different subspecies of the sea buckthorn fruit fly, is still unsure.

Prospect and further action

Sea buckthorn fruit flies are known to be damaging in sea buckthorn cultivation in Germany only for two years. We don’t know now for sure if they were here already before, them was only not noted. Dried berries at the branch tips were interpreted as over ripen or destroyed by bird, and nobody had looked after small holes in the dried berries.

For some reason they were able to increase explosively in 2013 (the long winter?). Not yet everywhere, but in locations with at least 10- year-old sea buckthorn cultivation and also sea buckthorn growing wild in Sachsen, Sachsen-Anhalt, Brandenburg and Mecklenburg-Vorpommern.

The high survival rate of sea buckthorn fly pupae is problematic. Under laboratory terms in Rostock 98% of the flies pupae survived. In the outdoor cages and eclectors in Rostock and Gülzow the survival rate was 56 to 75%.

Throughout Europe, for example in Lithuania and Belarus the sea buckthorn fruit fly is a big problem. Finland, Sweden and Estonia still seem to be free from the pest (Höhne 2015). Because sea buckthorn fruit fly is related very closely to the cherry fruit fly (Rhagoletis cerasi), information of similar biology and many control possibilities can be used for extensive investigations throughout Europe.

In Germany the plant protection product Mospilan is allowed against the cherry fruit fly. We have sprayed this agent against the sea buckthorn fruit fly and achieved a good success in Gülzow. Because, nevertheless, more than 90% of the sea buckthorn cultivation in Germany is organic production, extensive experiments and considerations are still needed to be able to produce sea buckthorn in Germany in the future.

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Assessment of mycorrhizal impact on photosynthetic processes on four varieties of sea buckthorn

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Abstract
Experiment was conducted on 2014 on bushes in their second year after planting at the Experimental Station in Lipnik near Stargard Szczeciński on light soil of good rye complex with low retention of useful water. The experiment was designed with a split-plot method in five repetitions. The aim of the presented study was to determine differences in photosynthetic activity between four cultivars of sea buckthorn (with mycorrhizl and without mycorrhiza): 'Askola', 'Habego', 'Leikora' and 'Hergo'. Mycorrhiza was applied in early spring according to agro-technical recommendations in the following combination: O-control (without mycorrhiza), M—with mycorrhiza. It was found that varieties had significant influence on photosynthetic activity of sea buckthorn bushes. Mycorrhization had a significant effect only on the assimilation.

Key words
mycorrhiza, photosynthesis, transpiration, stomatal conductivity, varieties

Introduction
Development and yield of plants is very strongly influenced by the process of photosynthesis, which is dependent on internal and external factors (Borowiak and Korszun 2011, Jaroszewska et.all 2009).

Materials and methods
A field experiment was conducted on 2014 at the Experimental Station Lipnik on acid brown soil. The soil is classified as quality class IVb, good rye complex, and for cultivation of light soils with low water retention. The differences in photosynthetic activity between four cultivars of sea buckthorn were evaluated (with mycorrhiza and without mycorrhiza): 'Askola', 'Habego', 'Leikora', 'Hergo'. The experiment was randomized block design in the system dependent (called a split-plot), in five replications. The research was conducted on the bushes in the second year after planting. The factor was mycorrhiza: O-control (without mycorrhiza), M- mycorrhiza sites. The mycorrhiza was applied in early spring, before mowing the vegetation, according to the agrotechnical recommendations. Measurements of photosynthetic activity of leaves were carried out at the beginning of the growing season in year 2014. The results of the photosynthetic processes in the sea buckthorn leaves were processed statistically with the application of an analysis of variance for multi-annual experiments, and the significance of differences with NIR of $_{0,05}$ was evaluated with Tuckey's test.

Results

Table 1.Photosynthetic activity of sea buckthorn bushes

Varities	E**	A	g_c	C_i	T_{leaf}
O-control	1,12	4,35	0,26	328	23,4
M-mycorrhiza	1,31	2,04	0,17	334	23,6
Askola	1,35	0,72	0,17	394	23,8
Habego	0,84	2,80	0,25	360	23,8
Hergo	1,45	3,10	0,20	321	23,2
Leikora	1,22	6,17	0,23	250	23,2
$LSD_{0,05\ for}$					
Varieties	0,61	1,32	n.i.	130	0,47
Mycorrhiza	n.i.	0,94	n.i	n.i.	n.i.

*n.i. – not significant difference,**intensity of transpiration (E) [mmol $H_2O \cdot m^{-2} \cdot s^{-1}$], the intensity of assimilation (A) [umol $CO_2 \cdot m^{-2} \cdot s^{-1}$], stomatal conductance (gc) [mmol $H_2O \cdot m^{-2} \cdot s^{-1}$], the concentration of CO_2 into the distance intercellular (Ci) [umol$CO_2 \cdot mol^{-1}$air], temperature (T) [^{0}C].

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Economics of Sea Buckthorn Production in Finland

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Abstract

Cultivation of sea buckthorn is a small sector of Finnish berry production. The share of sea buckthorn is only 2% of the total berry area of Finland. The area of sea buckthorn was 144 ha in year 2013, and the average area of sea buckthorn per farm in Finland was only 0.63 ha. Also, the economic value of sea buckthorn cultivation is very small, only 1% of the total market value of Finnish berry cultivation sector.

The economics of sea buckthorn production was analysed using farm models. Models were created so as to describe real farms and their production process. The models assumed certain farm facilities: fields, buildings, machinery and other production facilities. In the models the production process is described in detail, for example how many hours of labour each cultivation phase takes and how much fertilizer the grower uses.

Four different farm models were built. A small-scale sea buckthorn farm, where the cultivation area of sea buckthorn was 0.5 ha and a large-scale sea buckthorn farm, where the cultivation area was 10.00 ha. Both sizes of farm models were built to describe the conventional production method and the organic production method.

In the small-scale farm model, harvesting was done by cutting branches by hand. In the large-scale farm model, cutting and freezing machines were used. Small-scale farms had a yield of 5,000 kg/ha/year in conventional production, and 4,500 kg/ha/year in organic production. Large-scale farms had the same yield, 5,000 kg/ha in conventional and 4,500 kg/ha in organic production, but the yield was harvested only every third year. The average price of sea buckthorn was €/5.18kg (without VAT) in the small-scale farm and €4.35/kg (without VAT) in the large-scale farm.

Production in small-scale farms was not profitable. Profitability in organic production was slightly better than in conventional production, but the profitability ratio was still close to zero. A profitability ratio value of one or higher is the prerequisite for profitable production. Production in large-scale farms was not profitable either. The main reason for the poor profitability of small and large-scale farms was the low yield. Production would have been profitable in small-scale farms, if the yield had been over 6,500 kg/ha. In large-scale farms, harvesting every second year and a higher yield, over 4,600 kg/ha in organic and 6,300 kg/ha in conventional production methods, would have been needed to make production profitable.

Keywords

Farm, model, large-scale, organic, price, profitability, small-scale

Introduction

Cultivation of sea buckthorn is a small sector of Finnish berry production. The share of sea buckthorn is only 2% of the total berry area of Finland. The area of sea buckthorn was 144 ha in year 2013, and the average area of sea buckthorn per farm in Finland was only 0.63 ha. The economic value of sea buckthorn cultivation is very small, only 1% of the total market value of Finnish berry cultivation sector.

Current Finnish sea buckthorn production method is labour-intensive. Cutting and combining is done by hand. In the autumn, only part of the branches is cut and, therefore, harvesting can be done every year. The statistical yield level is low, only 750 kg/ha. The main reason for the low yields is unsuitable cultivars for Finnish climatic conditions.

However, sea buckthorn is a potential berry plant for Finnish berry cultivation sector. Firstly, sea buckthorn belongs to Finland's wild plants species, and it is therefore climatically suitable also for field production. Secondly, the Finnish berry processing industry already uses a substantial amount of sea buckthorn. Most of that berry material is now imported. In the future, a proportion of the imported berries might be replaced with Finnish berry material if only the production costs are able to decrease to a competitive level, or some additional value can be provided to the processing sector.

The aim of this study was to examine the production costs and profitability of the cultivation of sea buckthorn in Finland. Economic analyses were made both for current small-scale cultivation technology and for large-scale production technology which is not yet applied in Finland. A farm model approach was used.

Materials and methods

Two main approaches are employed to examine production costs of berry production. The first approach is to use real farm data, for example farm bookkeeping data. The other approach is to construct theoretical production cost models. In Finland, we unfortunately do not have any farm bookkeeping data of sea buckthorn growers, which makes it impossible to use real farm data. Secondly, we also wanted to examine the economics of the new production method, which is not yet applied in Finland. For those reasons, we decided to use a theoretical production cost and profitability model.

Our economic models were created so that they described real farms and their production process in as great a detail as possible. Models assumed certain farm facilities: fields, buildings, machinery, and other long-lasting production facilities. The production process was described graphically, how many hours of labour each cultivation phase takes, how much and what kind of fertilizers are used, etc. Interviews with two sea buckthorn growers and one expert were used as background information for the production process description.

Two sizes of farm models were built. A small-scale farm model describes the current sea buckthorn cultivation practice. In the small-scale farm model, the sea buckthorn area was 0.5 ha. That farm also grows oats on a 13.0 ha area and had an ecological area of 1.5 ha to fulfil CAP requirements. The total field area of small-scale sea buckthorn farm was 15.0 ha. Part of the field area, 3.0 ha, was rented. The rest of the area was owned by the grower.

On a small-scale farm, harvesting was done by cutting only part of the branches by hand. That makes harvesting possible every year. The yearly yield was assumed to be 5,000 kg/ha when the conventional production method was used. The conventional production method means that chemical weed and pest controls were used, as well as regular commercial mineral fertilizers. The waste percentage was assumed to be 8%. That value describes the loss of berries during storage, for example sorting loss. Consequently, the sold yield per ha was 4,600 kg. The yield was stored in the farm’s own freezing containers and sold both to consumers and the processing industry.

Also, a small-scale organic farm model was built. In an organic model, the yearly yield was assumed to be 4,500 kg/ha and the waste percentage 8%. So the sold yield was 4,140 kg/ha. No chemical pest control was used, and only organic fertilizers were used. Otherwise, conventional and organic small-scale farm models were much the same.

The aim of this study was also to examine the economic prerequisites of large-scale sea buckthorn production technology in Finland. Large-scale technology means the use of cutting and freezing machinery, which are not yet applied in Finland. Such a cultivation technology is already used, for example, in Germany.

The area of sea buckthorn on this fictional large-scale sea buckthorn farm was 10.0 ha. The total area of the large-scale farm model was 30.0 ha, of which 5.0 ha was rented. The rest of the area, 25.0 ha, was owned by the grower. The large-scale sea buckthorn farm also grows oats using 18.0 ha and has 2.0 ha of ecological area. Cutting and freezing machines were owned together with three other farms, so the share of ownership was 0.25 in this large-scale farm model.

The yield in the large-scale farm model was the same in conventional production method as in the small-scale farm (5,000 kg/ha), but the harvesting was done only every third year. The corresponding yield in the large-scale organic farm model was 4,500 kg/ha every third year. The waste percentage was the same, 8%, as in the small-scale farm models. The freezing storage service was bought from another company. Only a small proportion of the berries was stored on the farm using its own freezing container. Berries were mainly sold to the processing industry and only a small proportion of berries were sold directly to consumers.

Total cultivation time for sea buckthorn plantation was assumed to be 18 years. First three years are preliminary cultivation years, when no proper yield is got. After those three years, there are 15 cropping years until the plantation is removed.

The average price of sea buckthorn was €5.18/kg (without value added tax, VAT) in the small-scale conventional production method farm model, and €4.35/kg (without VAT) in the large-scale farm. In organic farm models, average prices were €6.21/kg and €5.40/kg, respectively. On small-scale farms, a larger proportion of the berries was sold using consumer packets, which increased the average price. Parameters of farm models are described in Table 1.

Models were carried out in Excel format. The fundamental idea of these production cost and profitability calculations were that they should be freely available, easily accessible, and in such a format that they can be modified in program which is commonly used. Farm model calculations can be found on the internet service called Taloustohtori (EconomicDoctor in English), and there in a subsection Laskelmakirjasto (CalculationLibrary in English). The internet address is www.luke.fi/taloustohtori -> Laskelma-Kirjasto -> Marjat -> Tyrni. Calculations are so far only in Finnish.

Table 1. Parameter values of farm models

		Small-scale conventional	Large-scale conventional	Small-scale organic	Large-scale organic
Sea buckthorn area	ha	0.5	10.0	0.5	10.0
Oats area	ha	13.0	18.0	13.0	18.0
Ecological area	ha	1.5	2.0	1.5	2.0
Total field area	ha	15.0	30.0	15.0	30.0
, of which is rented	ha	3.0	5.0	3.0	5.0
Yield	kg/ha	5,000	5,000	4,500	4,500
Waste percentage	%	8	8	8	8
Yield sold per year	kg/ha	4,600	1,533	4,140	1,380
Price of yield without VAT	€/kg	5.18	4.35	6.21	5.40

Results

Production cost includes all the costs of production: variable and fixed costs, as well as the wages claim of entrepreneurs, and interest claim. Wages and interest claim are computational costs and do not cause money transfers. Fixed costs were calculated using commonly used methods of cost accounting. Costs of buildings and machinery consisted of depreciation costs using straight-line depreciation, capital costs using 5% portion of purchase-value, maintenance costs, and insurance costs. Maintenance costs were machine- and building-based, and varied between 1 to 5 % of purchase-value. Insurance costs were assumed to be 0.2 % of the purchase value. Production costs were computed without VAT.

Production costs varied between €7.88 and €9.19/kg. Production costs were slightly lower in the conventional production farm models than in organic. Production costs of large-scale farm models were lower than in small-scale. Costs per ha were approximately three times higher in small-scale farms than in large-scale farms (Table 2).

In small-scale farms, the share of costs of building and machinery were the biggest cost factor. The second largest cost factor was labour costs. Instead, in large-scale farms material costs were the largest cost factor, the second largest was the cost of buildings and materials (Picture 1).

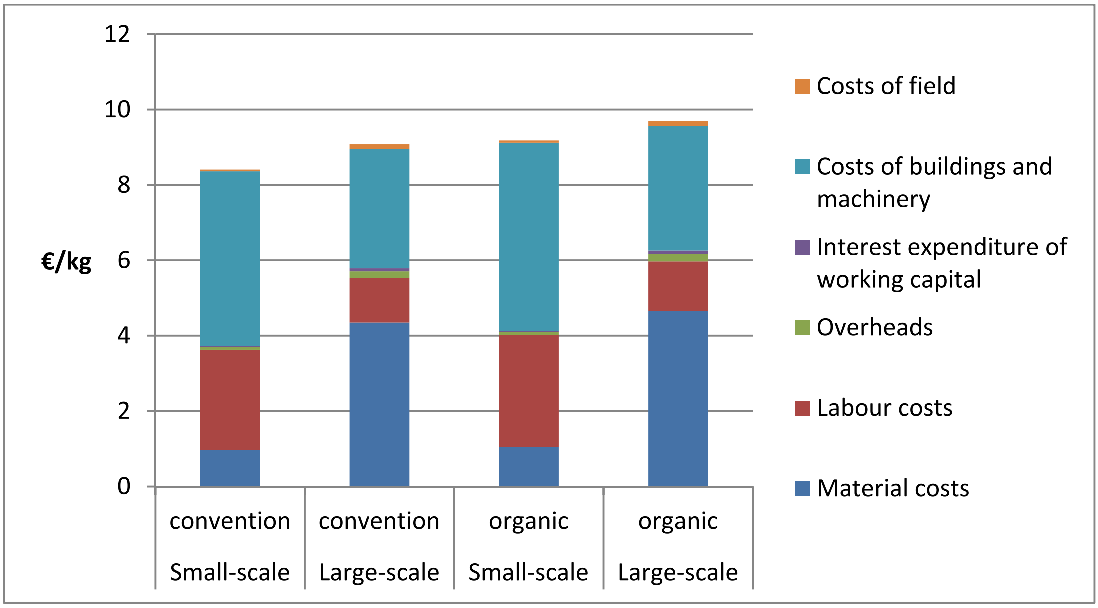
Table 2. Production costs of farm models without VAT.

	Small-scale conventional		Large-scale conventional		Small-scale organic		Large-scale organic	
	€/ha	€/kg	€/ha	€/kg	€/ha	€/kg	€/ha	€/kg
Material costs	4,418	0.96	4,844	3.16	4,357	1.05	4,599	3.33
Labour costs	12,292	2.67	1,815	1.18	12,290	2.97	1,813	1.31
Overheads	331	0.07	282	0.18	331	0.08	282	0.20
Interest expenditure of working capital	155	0.03	92	0.06	154	0.03	88	0.06
Costs of buildings and machinery	21,311	4.63	4,849	3.16	20,675	4.99	4,550	3.30
Costs of field	243	0.05	193	0.13	243	0.06	193	0.14
Production costs	**38,751**	**8.42**	**12,075**	**7.88**	**38,051**	**9.19**	**13,684**	**8.35**

Turnover consists of two sources: the selling income from berries and subsidies. In Finland, farms get an area-based subsidy to the sea buckhorn area. The subsidy level varies regionally. In the calculations we used the subsidy level of the main sea buckthorn cultivation area, Ostrobothnia, which belongs to subsidy area C1.

Small-scale farms have about a three times higher turnover per hectare than large-scale farms. That comes from the different harvesting density. In small-scale farms, harvesting is done every year, and in large-scale farms only every third year.

The economic results of farms are expressed using the same key numbers as in the MTT bookkeeping results. Almost all farm models have a negative gross return. Only small-scale organic farm model have slightly positive gross return. All farm models have a negative operating margin and entrepreneurs' profit. Farm family income and hence the profitability ratio had a positive value only in an small-scale organic farm model. Profitability of organic farm models was better than conventional production farm models. A profitability ratio value of one or higher is the prerequisite for profitable production. Consequently, none of the farm models were profitable.



Picture 1. Cost structure of farm models.

Table 3. Profitability key numbers.

	Small-scale convention		Large-scale convention		Small-scale organic		Large-scale organic	
	€/ha	€/kg	€/ha	€/kg	€/ha	€/kg	€/ha	€/kg
Turnover								
Selling income	23,805	5.18	6,670	4.35	25,709	6,21	7,452	5.40
Subsidies	1,108	0.24	1,108	0.72	1,249	0,30	1,108	0.80
Turnover in total	*24,913*	*5.42*	*7,778*	*5.07*	*26,958*	*6,51*	*8,560*	*6.20*
Gross return	-219	-0.05	-2,422	-1.58	2,088	0,50	-1,303	-0.94
Operating margin	-9,287	-2.02	-4,764	-3.11	-6,680	-1,61	-3,501	-2.54
Entrepreneurs profit	-13,768	-2.99	-6,100	-3.98	-11,022	-2,66	-4,767	-3.45
Family farm income	-1,550	-0.34	-3,323	-2.17	1,055	0,25	-2,062	-1.49
Profitability ratio	-0.13		-1.20		0.09		-0.76	

Discussion

Finland is a high-cost country. All the costs of production are high, especially labour costs. So, on the cost-side it is very difficult to point out factors which alone have a major effect on production costs. The main way to reduce cost per produced yield kilo is to obtain a higher yield. In our farm models, quite moderate yield levels (4,500 – 5,000 kg/ha) were used. Moderate means that theoretically it is possible to obtain much higher yields. But, on the other hand, our moderate yields were extremely high if one compares those yields to the statistical mean value of real farms in Finland (750 kg/ha). The yield has to be over 6,500 kg/ha, before the production in small-scale farms is profitable. In large-scale farms, the prerequisite for profitable production is that harvesting can be done every second year. Even then the yield has to be over 6,300 kg/ha in conventional production methods, and 4,600 kg/ha in organic production methods. Consequently, in large-scale models the main reason for weak profitability is that the yield is too infrequent, only every third year.

The use of cutting and harvesting machines reduced the share of labour costs as expected. Also, the share of the cost of buildings and machinery was smaller in large-scale farms than in small-scale farms. The explanation for this can be found both from co-ownership of cutting and freezing machinery and the use of a freezing service. The rental cost of the freezing service is categorized as material costs, which leads for a large cost share of material costs in the large-scale farm models.

Profitability in organic production was slightly better than in conventional production. So far, sea buckthorn is quite easy to produce using organic cultivation methods. Hence, in choosing organic production one can improve profitability without any major changes in production technology.

Acknowledgements

The work was a part of the “Two super berries” project, which was funded by Rural Development Programme for Mainland Finland managed by Ostro-Bothnia ELY-centre. Local farmers and municipalities of the province have also financed this project.

Sensory contribution of ethyl-β-D-glucoside isolated from sea buckthorn juice

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Abstract
Sea buckthorn (*Hippophaë rhamnoides*) berries are known to be very acidic, sour and astringent [1, 2]. Berries contain also various sugar alcohols and alkylated sugars and the contents vary during ripening [3]. Our previous study demonstrated how ethyl-β-D-glucoside (EG) content may effect on pleasantness of sea buckthorn juice [4]. In this study, we isolated the alkylated sugar from the juice of cultivar 'Tytti' and we studied its contribution to sourness, bitterness or astringencies (puckering or mouth-drying) in a water solution.

Sugar compounds of the juice were first isolated from acids using applied method of Tiitinen et al. [1] with anion exchange column chromatography. In the second phase, EG was isolated from other sugars with column chromatography using a modified method of Nurminen et al. [5]. In both columns the sugars including EG was eluted with water and the whole process was carried out in food-grade conditions. The final purity of the fraction was assessed with gas chromatography [1] and with spectrophotometer. The resulting EG-rich fraction was diluted to water (0.50g/100mL). The sensory panel (n=26) was trained to evaluate the four attributes using generic Labelled Magnitude Scale (gLMS). The two samples (water solution of EG and water) were evaluated in two sessions in randomized order and in controlled sensory laboratory conditions.

The final fraction contained 87 % of EG, 5 % glucose and 8 % quebrachitol. Additionally, it may have contained traces of water-soluble phenolic compounds. Due to low-yielding and time consuming isolation process the purity was compromised. In the end, only a 0.5 mL sample was applied on an assessors tongue in the two sessions. The fraction in water was significantly more bitter than water ($n=26\times2$; t-test, $p<0.05$) and did not contribute to other attributes. Additionally, in open-ended descriptions the panelists described the fraction as "pungent" and "sharp". This study further indicates that EG has a significant role in the sensory profile of sea buckthorn. Bitterness in foods can often be accepted as negative features. Therefore EG can be one of the factors contributing negatively to pleasantness [4]. Better understanding of its sensory contribution and interactions with other berry components may aid to mask its possibly negative properties. Additional research with more purified EG in up-scale process is needed to verify the preliminary results of this study.

Key words
Bitterness, Ethyl-β-D-glucoside, Sea buckthorn, Sensory evaluation

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Anti-inflammatory activity of Sea Buckthorn leaf extracts

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Abstract

Anti-inflammatory food compounds are considered to protect against type 2 diabetes and cardiovascular and neurodegenerative diseases. We reported earlier that water extracts from SBT leaves inhibited strongly radical production of activated of human granulocytes. Now we tested efficacy of alcoholic extracts made from sea buckthorn (SBT) leaves on free radical production in resting state human phagocytes, during priming with *Escherichia coli* lipopolysaccharide (LPS) and during respiratory burst (RB) that was triggered with zymosan (Z) or serum treated zymosan (OZ) particles. Both superoxide radical and and myeloperoxidase enzyme (MPO) derived radicals were measure as lucigenin and luminol chemiluminescence (CL) emissions, respectively.

In resting phagocytes all SBT leaf extracts reduced spontaneous basal level production of both superoxide radical and MPO derived radicals in dose dependent pattern. In resting cells the IC_{50} values for superoxide anion levels were almost ten times smaller (4-10 µg/ml, w/v) than those for MPO derived radicals (30-50 µg/ml). However, during LPS-priming and Z and OZ induced RB the magnitude of inhibition of both radical production systems were not essentially different. During LPS -priming the IC_{50} varied between 4 and 10µg/ml and during RB between 20 and 60 µg/ml depending on SBT preparation and the nature of activator.

The release of superoxide radical and MPO into blood circulation by distorted leukocyte function has in time being a crucial role in the development of atherosclerosis, metabolic syndrome and related diseases. These results suggest that compounds in SBT leaf ethanol extracts inhibit these deleterious processes and may have beneficial effects for health.

Key words

Sea buckthorn leaf, anti-inflammatory, leukocyte, granulocyte, superoxide, myeloperoxidase, type 2 diabetes, vascular disease

Introduction

Consumption of polyphenol-rich berries, fruits and vegetables reduce incidence of atherosclerosis, age-related neurological disorders and type II diabetes. Inflammatory phagocytes play a central role in all stages of development of these diseases. Primed monocytes and activated macrophages are a major source of pro-inflammatory cytokines and oxidative stress in arterial walls. Granulocytes, as the most abundant phagocytes, are the main source of oxidative stress during the inflammatory process. Anti-inflammatory food compounds are considered to inhibit development of above mentioned diseases. SBT leaf extracts are reported to possess anti-inflammatory properties in animal model studies. We reported that water extracts from SBT leaves inhibited strongly RB activity of human granulocytes (Marnila et al. 2014). Now we tested efficacy of SBT leaf alcoholic extracts on free radical production in resting state human granulocytes, during priming and RB.

Materials and methods

Extracts: Leaves of Finnish SBT cultivar 'Terhi' were collected in a berry farm in North-West Finland at three different growing states: (1) at the end of May after the blossom before the fruit development, (2) at early July when the fruits were evolving, and (3) at the end of September when the fruits were already fully ripened. Extracts were prepared by mixing 10 g of freeze-dried leaves with 100 mL of 99.6% ethanol and refluxing the mixture for 30 min. The extracts were freeze dried and the powders stored at -20°C.

Reagents: Stock solutions of 10mM luminol (5-amino-2,3-dihydro-1,4-pthalazinedione; ICN Biomedicals) and 10mM lucigenin (*N,N*′-Dimethyl-9,9′-biacridinium dinitrate, Sigma-Aldrich) were made in 0.2 M sodium borate buffer (pH 9.0). Zymosan (cell wall preparation from *Saccharomyces cerevisiae*) was boiled and suspended in HBSS buffer (Hank's balanced salt solution, without phenol red; pH 7.4) and stored at -20°C. Serum opsonized zymosan (OZ) was prepared by incubating Z with 50% serum and 50% HBSS (v/v) for 60min at 37°C, then washing and suspending in HBSS (4 mg/ml) and storing at -80°C. LPS stock was prepared from *Escherichia coli* 055:B5 (LPS; Sigma-Aldrich), dissolved in HBSS buffer and stored at -20°C.

Free radical measurements: 5mg of SBT extract samples were dissolved into 100µl of EtOH and the pH was adjusted to 7.4 using Na-borate buffer (pH 9.0) and HBSS-buffer (pH 7.4) to give final concentrations of 3.45 – 833µg/ml (w/v). For protocol steps see Figure 1. Briefly, peripheral EDTA-blood samples were drawn from adult healthy volunteers. Leukocytes were isolated and suspended into HBSS-buffer. Lucigenin or luminol was added (final conc. 0.5 mM). Luminol CL is dependent on PMO activity. Lucigenin CL is a measure of superoxide anion production. Leukocytes were first incubated with SBT leaf extracts (3.45 – 833µg/ml, w/v) for 10 min at 37°C. The basal radical production was measured as CL emission using luminometer (Luminoskan EL-1, Thermo Scientific, Finland). Then leukocytes were primed to inflammatory cells by adding *Eschericia coli* LPS (final conc. 250ng/ml) and incubated for 35 min. The radical production was measured. Then phagocyte RB reaction was induced either with Z or OZ (300µg/ml) (Figure 1). The RB was measured kinetically for 1 hour and 20 min at 37°C. The CL peak values were regarded as the RB activity. In the last RB measurement the peak time from adding Z or OZ was used as a measure of the RB reaction velocity.

Results

Basal radical production: The extracts did not evoke any notable cell activation in resting phagocytes. The SBT leaf extracts reduced dose dependently the spontaneous basal level production of both superoxide radical and MPO -derived radicals of resting phagocytes. The inhibition of superoxide anion levels were stronger than inhibition of MPO -derived radicals seen as almost ten times smaller IC_{50} values in superoxide anion production (4-10 µg/ml, w/v) than in MPO –activity (30-50 µg/ml) (see Fig.2 and 3).

LPS priming: During priming with LPS the basal radical production increased 60 – 200 times higher than in non-primed cells (4.0 - 7.73 rlu). In priming process the extracts clearly reduced the free radical production. IC_{50} concentrations were 4-10µg/ml depending on preparation and concentration over 100µg/ml abolished over 95% of radical production.

Respiratory burst: In LPS –primed cells the RB reaction velocity was faster than in non-primed controls. This was seen as 6-7 min shorter peak time (P<0.001) and 2-4 times higher CL in early phase of the reaction at 5 min time point than in non-primed controls (P<0.001) (data not shown). Also the maximum CL response was 15-30 % higher than in non-primed cells (P<0.05). These changes are similar as described in literature after experimental priming *in vitro*. SBT leaf ethanol extracts inhibited similarly both superoxide radical production and MPO derived radical production. IC_{50} concentrations varied between 20 and 60 µg/ml depending on SBT preparation and the nature of activator used to trigger the RB (see Fig. 4).

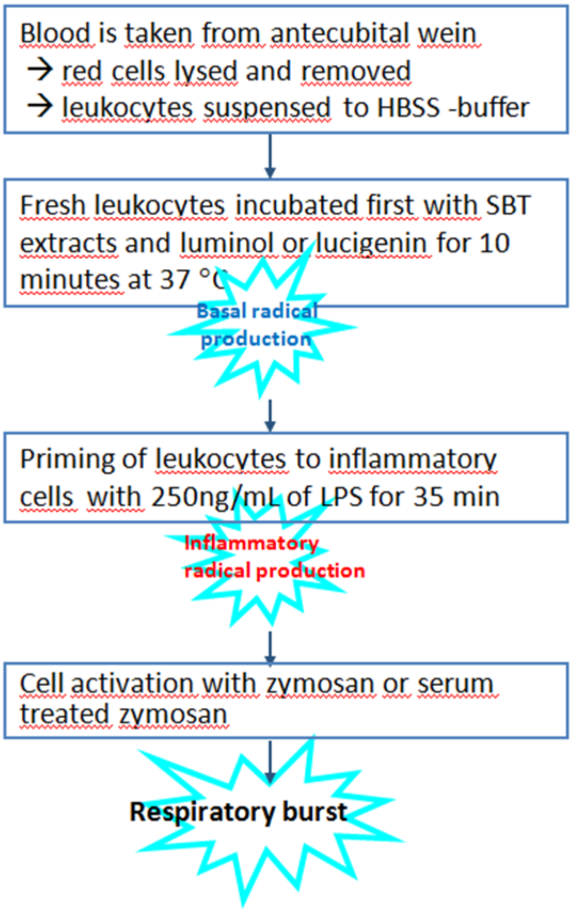

Figure 1. Flow chart of the experimental procedure.

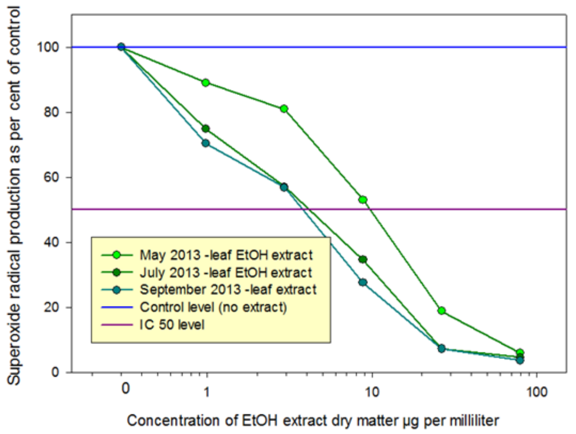

Figure 2. Effects of SBT leaf ethanol extracts on basal level superoxide production of resting state human phagocytic cells *in vitro*.

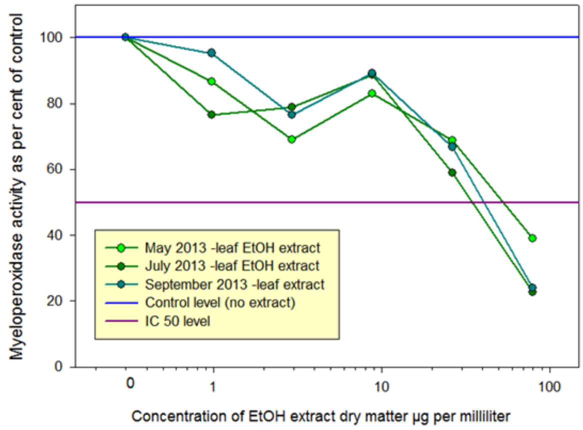

Figure 3. Effects of SBT leaf ethanol extracts on basal level superoxide production (upper figure) and on MPO -activity (lower fig.) of resting state human phagocytic cells *in vitro*.

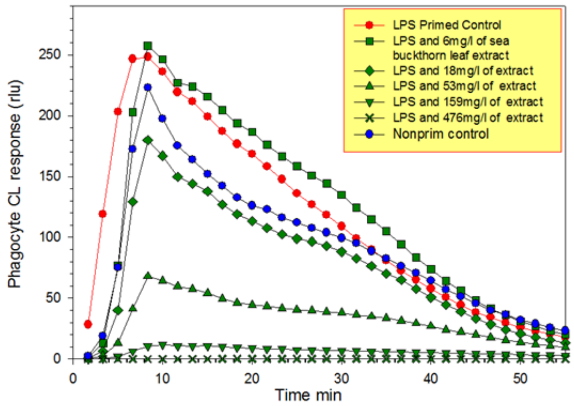

Figure 4. The effect of SBT leaf (July 2013) ethanol extract on MPO-reaction kinetecs during phagocyte RB. The cells were activated with OZ and RB was measured as luminol enhanced CL emission. Two smallest concentrations reduced the LPS priming effect and higher ones inhibited strongly the MPO –dependent radical production.

Discussion

Overactive and distorted function of phagocytes contribute to oxidative stress by increasing release of superoxide radical, MPO and inflammatory mediators into blood circulation. The MPO -dependent radical production of primed resting cells was less than 0.5% of that in OZ induced respiratory burst. However, since MPO secreted to blood circulation attaches endothelia and as its activity is a constant process it in time being has a crucial role in the development of oxidative damage contributing to development of widespread diseases like atherosclerosis and metabolic syndrome. Our results suggest that compounds in SBT leaf ethanol extracts inhibit this deleterious process and on this basis may have beneficial effects also *in vivo* with regard to cardiovascular disease, age-related neurological disorders and type II diabetes.

Further research will provide detailed information on the composition and relative abundance of bioactive compounds of SBT leaves (Hellström et al. 2014). Identification of these compounds and their mode of action will in future help to address the very real public health issues by providing nutritional solutions for improving health. We expect that identification of the active compounds will also offer an opportunity for developing novel nutraceutical and pharmaceutical products in future.

Acknowledgements

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Major phenolic compounds in processed sea buckthorn leaves

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Abstract

Sea buckthorn (Hippophae rhamnoides L.) is increasingly being cultivated because of its valuable fruits which are rich in several bioactive compounds. However, also the leaves of sea buckthorn plants are of interest since they have a high content of eg. ascorbic acid and phenolic compounds. In this pilot study leaves of the sea buckthorn cultivar 'Ljubitelskaja' were processed in different ways to mimic production of tea leaves. Thus, sea buckthorn leaves were dried, steamed and fermented for preparation of herbal, green and black sea buckthorn "tea" leaves, respectively. Total phenolic compounds were investigated in hot water extracts and in ethanol extracts. The content of catechin, epigallocatechin, gallic acid, hydrolyzable tannins I-III, isorhamnetin-3-O-glucoside, isorhamnetin-3-O-rutinoside, kaempferol, kaempferol-3-O-glucoside, procyanidin dimer aglycones, procyanidin monomer glycosides, quercetin, quercetin-3-O-glucoside and rutin were investigated only in hot water extracts.

Ethanol extracts had significantly higher contents of total phenolic compounds compared to the water extracts, and the highest content was noticed when herbal (only dried) sea buckthorn leaves were extracted. By hot water the highest content of total phenolic compounds was instead obtained from the green (steamed and dried) sea buckthorn leaves. The content of specific phenolic compounds differed depending on the processing method of the leaves, and on the method for extraction. The sea buckthorn infusion of fermented (and dried) sea buckthorn leaves had the most pleasant taste compared to the infusion of green sea buckthorn leaves. In the infusion of fermented sea buckthorn leaves the grass-like flavour was almost absent and the bitterness was even less than in the infusion of green sea buckthorn leaves.

Key words

Catechin, gallic acid, isorhamnetin, kaempferol, tannins, tea

Introduction

Sea buckthorn (*Hippophae rhamnoides* L.) is increasingly being investigated as a valuable multipurpose Euroasian plant species because of its good adaptation to marginal soils and harsh climates, and because of its high content of different bioactive compounds (Ruan et al. 2013). Despite that sea buckthorn leaves are known to be a rich source of ascorbic acid and phenolic compounds, and also used for tea brewing, there are only few reports available on specific phenolic compounds in sea buckthorn leaves (Heinäaho et al. 2006, Kumar et al. 2013, Xing 2003, Morgenstern et al. 2014). No report has been found on the effect of different post-harvest treatments of sea buckthorn leaves on the content of phenolic compounds in the extracts of processed leaves. Thus, the aim of this study was to investigate the influence of different processing methods on the content of phenolic compounds of three different sea buckthorn leaf tea preparations: herbal, green, and black tea, extracted by hot water and ethanol.

Materials and methods

Fresh leaves were sampled from annual shoots of the sea buckthorn cultivar `Ljubitelskaja` in the middle of August. Leaves were prepared as green sea buckthorn tea (GST), black sea buckthorn tea (BST) and herbal sea buckthorn tea (HST). For preparation of GST and BST, immediately after harvest fresh leaves were spread on paper towels for 3 h at room temperature. For preparation of GST leaves were then steamed for a few seconds over boiling water, rolled for 30 min and dried for 6 h at 50 °C. For preparation of BST leaves were rolled for 30 min, wrapped up in 1 cm layers in warm (about 45 °C) and wet paper towels, fermented in a perforated plastic bag for 5 h at room temperature, and then dried for 60 min at 85 °C. For preparation of HST leaves were cut in strips of 5 to 10 mm directly after harvest and then dried for 6 h at 50 °C in a convection oven.

Phenolic compounds were extracted from intact sea buckthorn tea leaf preparations by hot water (2 min, 90 °C) and from finely ground samples by 50% EtOH.

The content of total phenolic compounds was analyzed by spectrophotometer using Folin-Ciocalteu's reagent and specific phenolic compounds was analyzed by HPLC-MS as described by Morgenstern et al (2014).

Results

The resulting sea buckthorn tea leaf preparations following processing of fresh leaves is shown in Figure 1. From the figure it is obvious that the different treatments changed the color and structure of the leaf preparations.

Figure 1. Fresh and processed sea buckthorn leaves, from left to right: steamed and dried (GST), fresh, dried (HST), fermented (BST).

Significantly more total phenolic compounds were extracted by 50% EtOH from HST than from GST and BST (Figure 2). By hot water the highest amount was instead obtained from GST and HST yielded the significantly lowest amount of phenolic compounds.

The content of specific phenolic compounds differed depending on the processing method of the leaves, and on the method for extraction. Quercetin and epigallocatechin were not present in detectable levels in any extracts of GST, BST or HST despite that in a previous study (Morgenstern et al. 2014) it was present in the leaves of the same sea buckthorn cultivar ('Ljublitelskaja') sampled in July the same year (Table 1). In general GST yielded the highest amount of water extractable specific phenolic compounds with a few exceptions. The content of catechin was significantly higher in HST and the content of gallic acid was higher in BST, however this was not significant.

There were also visible differences in colour between the tea brews of GST, BST and HST. The colour of the GST hot water extract was brown, the BST extract was dark brown and the colour of the HST extract was bronze. The tea brews also varied in their taste. The HST brew had a grass-like taste and became bitter. The taste of the GST brew was more like the taste of herbal tea brew, and the bitterness and the grass-like flavour was less pronounced than of the HST brew. The BST brew had the most pleasant taste. The grass-like flavour was almost absent and the bitterness was even less than in GST. The general taste was little stronger and more aromatic than the taste of GST and HST.

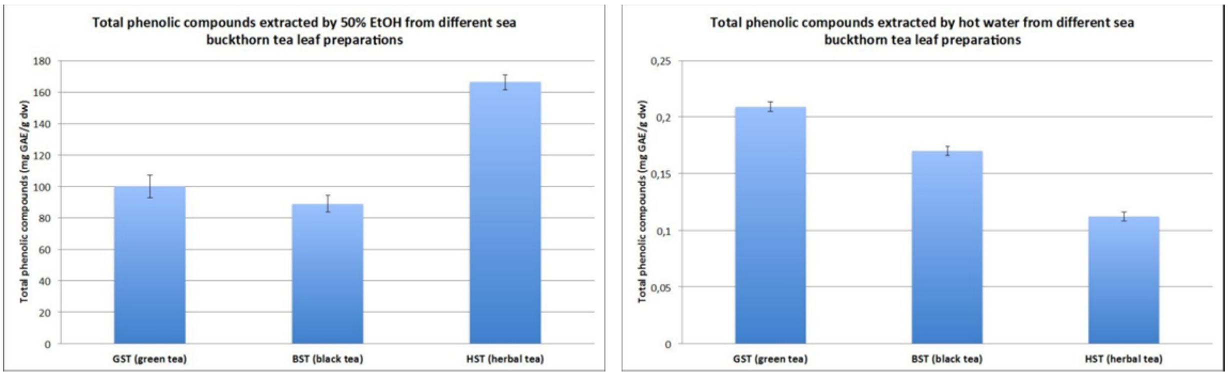

Figure 2. Total phenolic compounds extracted by 50% EtOH of finely ground samples or by hot water from entire leaf samples of different sea buckthorn tea leaf preparations (Average value and standard deviation, n = 3).

Table 1. Content of specific phenolic compounds of three preparations of sea buckthorn leaf tea: green sea buckthorn tea (GST), black sea buckthorn tea (GST), and herbal sea buckthorn tea (GST) when extracted by hot water (values sharing the same letter in a row are not significantly different at $p < 0.05$, n=3, dw = dry weight)

	Content (average ± standard deviation, µg/g dw)		
Phenolic compound	GST	BST	HST
Catechin	101 ± 2.9[a]	13 ± 0.3[b]	175 ± 2.9[c]
Epigallocatechin	0 ± 0	0 ± 0	0 ± 0
Gallic acid	968 ± 229.1[a]	1287 ± 411.3[a]	422 ± 86.4[a]
Hydrolyzable tannin I	2108 ± 402.0[a]	584 ± 28.0[b]	1743 ± 163.5[a]
Hydrolyzable tannin II	92 ± 14.1[a]	19 ± 0.7[b]	35 ± 1.7[b]
Hydrolyzable tannin III	234 ± 1.3[a]	115 ± 3.7[b]	190 ± 6.4[c]
Isorhamnetin-3-*O*-glucoside	55 ± 1.4[a]	41 ± 1.8[b]	19 ± 1.0[c]
Isorhamnetin-3-*O*-rutinoside	229 ± 9.3[a]	205 ± 8.2[b]	110 ± 2.1[c]
Kaempferol	12 ± 2.9[a]	5 ± 0.3[b]	4 ± 0.2[b]
Kaempferol-3-*O*-glucoside	52 ± 22.4[a]	51 ± 10.3[a]	50 ± 10.0[a]
Procyanidin dimer aglycone	0 ± 0	0 ± 0	18 ± 0.6
Procyanidin monomer glycoside	120 ± 5.0[a]	111 ± 3.5[a]	47 ± 3.1[b]
Qercetin	0 ± 0	0 ± 0	0 ± 0
Quercetin-3-*O*-glucoside	46 ± 5.5[a]	40 ± 5.0[a]	32 ± 6.4[a]
Rutin	215 ± 70.3[a]	223 ± 57.5[a]	102 ± 1.8[a]

Discussion

The green colour of fresh sea buckhorn leaves was almost completely preserved in the HST preparation whereas GST and BST lost their green colour to a large extent. This was especially noticeable for BST, which had an even darker colour than GST. The change in structure and colour of the sea buckthorn leaves following processing is similar to the change that occurs when leaves of the tea plant (*Camellia sinensis*) are being processed.

The fact that 50 % EtOH extracts of finely ground samples yielded more than 400 times the amount of phenolic compounds than hot water extracts of intact leaves shows that sea buckthorn leaves are indeed a valuable source of beneficial phenolic compounds and that grinding may considerably improve extractability.

Total content of phenolic compounds and content of specific phenolic compounds differed between GST, BST and HST due to the different post-harvest processing treatments. This may be ascribed to redox processes in combination with thermal heating causing activation and deactivation of enzymes in the plant material during the production of green, black and herbal tea.

The content of catechin in the hot water extracts of the three sea buckthorn tea preparations BST, GST and HST in this study was 13, 101 and 175 µg/g dry weight, respectively. These values are considerably lower compared to the average catechin contents reported for water extracts of green (240 µg/g dry weight) and black (1670 µg/g dry weight) tea (Peterson et al. 2005). Catechin content was highest in HST, when leaves were only cut and dried, and decreased during processing of GST and BST. This is likely a result of higher enzyme activity in BST during fermentation process when apparently the catechin was converted to more complex proanthocyanidins (condensed tannins). These results differ from ordinary tea production where the black tea usually has the highest content of catechins.

In conclusion, sea buckthorn leaves are very rich in beneficial phenolic compounds, however hot water is only able to extract a minor part of the total amount in the leaves. Processing affects the extractability and is also a possible way to affect the composition of specific phenolic compounds in extracts.

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Emerging sea buckthorn diseases in Latvia and associated pathogens

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Abstract

Despite the importance of sea buckthorn as an agricultural plant species, the research on diseases is limited. In general, only a few diseases have been recorded, such as canker caused by *Stigmina* sp., wilt (*Verticillium* spp.), bud bacteriosis (*Pseudomonas syringae*) and dry shrink disease (*Fusarium* spp.). During the recent years concerns of growers have raised on diseases spreading in sea buckthorn plantations in Latvia. As a response to grower concerns, the research was initiated on identification and characterization of sea buckthorn diseases prevailing in Latvia. In order to identify the causes of observed problems, surveys and samplings were performed from June to September. The samples from branches and trunks with diverse symptoms were collected. Fungi and bacteria were isolated in pure cultures, and the potential pathogens were preserved. Identification of fungi was carried out by means of morphological characters and molecular methods. Presumptive identification of bacterial isolates was done based on colony morphology and biochemical tests. Observed damages and symptoms during the surveys, identity and significance of the associated pathogens are discussed.

Chemical composition of individual morphological parts of the sea buckthorn fruit (Hippophaë rhamnoides L.)

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Abstract

Seabuckthorn (*Hippophae rhamnoides* L.) fruits are a rich source of lipo- and hydrophilic biologically active substances, such as: organic acids, vitamins, sterols, phenolic compounds and characteristic fatty acids. Since knowledge of the chemical composition of individual parts of the morphological sea buckthorn fruit will improve technological management of the entire fruit, the aim of the study was to carry out a detailed chemical analysis of individual morphological parts of the sea buckthorn fruit.

The sea buckthorn fruit is composed 79.80±4.18% of pulp, 10.32±3.08% of skin and 9.88±1.87% of seed. On the basis of the analyses performed, it was found that the amount of raw fibre in sea buckthorn skin accounted for 6.59±0.32% DM. It was found that the main component of this fibre was a neutral fraction, the so-called neutral detergent fibre (NDF), which accounted for 24.29 ± 0.87 % of the raw fibre mass in sea-thorn fruit skin. Additionally, the acid detergent fibre (ADF) fraction was found in the skin in the amount of 13.4 ± 0.25 %, as well as structural fibre, the so-called acid detergent lignin (ADL) in the amount of 6.62 ± 0.15%**.** The cellular walls of the skin contained 12.65±0.18 % of protein and 29.76±1.55% of lipids. Cell walls, forming an integral part of the pulp and accounting for about 7.72% DM, were built primarily of fibre, an amount of which, expressed as raw fibre, accounted for 4.68 ± 0.18% DM. It was found that the main component of this fibre was the neutral detergent fibre fraction (NDF), which accounted for 22.29 ± 1.12 % DM of the sea buckthorn fruit pulp. It was also found that the pulp of the sea buckthorn fruit contained an ADF fibre fraction of 13.49 ± 0.25 % and an ADL structural fibre of 5.31 ± 0.15%. Following an analysis of the chemical composition of sea buckthorn pulp, it can be claimed that the main component of the sea buckthorn fruit pulp are sugars (6.33±1.99% FM) and fat (4.46±0.95%). Carbohydrates contained in the seed of the sea buckthorn fruit are represented by monosaccharides (5.32±0.56), starch (4.92±0.89% DM) and other forms of polysaccharides expressed as fibre, accounting for 12.99±0.32 % DM. The main component of this fibre is the NDF fraction - 50.48 ± 1.23 % DM. Additionally, it was found that the seeds of the sea buckthorn fruit contained an ADF fraction of 25.08 ± 0.96 % and ADL structural fibre accounting for 14.54 ± 0.41% DM of seeds. It was established that sea buckthorn fruit seeds also contained 29.34±1.14 % of protein and 3.00±1.32% of lipids.

Key words

chemical analysis, fruit, morphological parts, varieties

Introduction

Sea buckthorn (*Hippophae rhamnoides* L.) fruits are a rich source of lipo- and hydrophilic biologically active substances, such as: organic acids, vitamins, sterols, phenolic compounds and characteristic fatty acids. Since knowledge of the chemical composition of individual parts of the morphological sea-buckthorn fruit will improve technological management of the entire fruit, the aim of the study was to carry out a detailed chemical analysis of individual morphological parts of the sea-buckthorn fruit.

Materials and methods

The experiment was set up at the experimental station of University of Warmia and Mazury in Olsztyn (Poland). Five varieties of sea buckthorn ('Podarok Sadu', 'Botaniczeskaya', 'Otrodnaya', 'Trofimovskaya' and wild Baltic form) were studied. Chemical analysis was performed in three replications. The following were determined: total lipids according to the method of Folch (AOAC method 1974), raw fiber – all the fractions of fiber were determined by the FIBRETEC 2010 SYSTEM, total sugars - according to standard PN-90/A-75101/07 by protein-spectrophotometry with Bradford reagent. The result was subjected to analysis of variance using the „STATISTICA 10" software. Significant differences were determined by Duncan test at the level of significance of 0.05.

Results

Table 1. Basic chemical composition of sea buckthorn seeds

Discriminant	**% dm.**
Dry matter	90,50±1,24
Total sugars (as invert sugar*)*	5,32±0,56
Starch	4,92±0,89
Protein	29,34±1,14
Lipids	13,00±1,32
Raw fiber	12,99± 0,32
Ash	2,37 ±0,21

Table.2. Chemical composition of sea buckthorn fruit peel in a dry weight.

Discriminant	
Amount in % fresh matter	
Dry matter	22,19±3,95
Extract	14,50±0,95
Amount in % dry matter	
Total saccharides	18,16±4,30
Starch	traces
Protein	12,65 ± 0,18
Lipids	29,76±1,55
Raw fiber	6,59± 0,32
Ash	3,60±0,04
Pectin	0,48±0,03

Table 3. Basic average chemical composition of pulp.

Discriminant	**Pulp**	
	Fresh	**Dry matter**
Dry matter (%)	12,89±3,00	100
Extract (%)	8,25±1,98	64,00±8,20
Total sugars (%)	6,33±1,99	48,95±8,20
Reducing sugars (%)	1,69± ,20	13,11±3,20
Pectin (%)	0,19±1,20	1,47±0,09
Protein (%)	0,67±1,20	5,21 ± 0,22
Mineral composition(%)	0,28±1,20	2,17±1,20
Total lipids (%)	4,46±0,95	34,63±2,95
Raw fiber (%)	0,60±0,10	4,68 ± 0,22
Starch %	traces	traces

Conclusions

1. The sea buckthorn fruit is composed 79.80±4.18 % of pulp, 10.32±3.08 % of skin and 9.88±1.87 % of seed.
2. The amount of raw fibre in sea buckthorn skin accounted for 6.59±0.32 % DM. It was found that the main component of this fibre was a neutral fraction, the so-called neutral detergent fibre (NDF), which accounted for 24.29 ±0.87 % of the raw fibre mass in sea buckthorn fruit skin.
3. Additionally, the acid detergent fibre (ADF) fraction was found in the skin in the amount of 13.4 ± 0.25 %, as well as structural fibre, the so-called acid detergent lignin (ADL) in the amount of 6.62 ± 0.15%**.**
4. The cellular walls of the skin contained 12.65±0.18 % of protein and 29.76±1.55% of lipids. Cell walls, forming an integral part of the pulp and accounting for about 7.72% DM, were built primarily of fibre, an amount of which, expressed as raw fibre, accounted for 4.68 ± 0.18% DM.
5. The main component of this fibre was the neutral detergent fibre fraction (NDF), which accounted for 22.29 ± 1.12 % DM of the sea buckthorn fruit pulp. It was also found that the pulp of the sea buckthorn fruit contained an ADF fibre fraction of 13.49 ± 0.25 % and an ADL structural fibre of 5.31 ± 0.15%.
6. The main component of the sea buckthorn fruit pulp are sugars (6.33±1.99% FM) and fat (4.46±0.95%).
7. Carbohydrates contained in the seed of the sea buckthorn fruit are represented by monosaccharides (5.32±0.56), starch (4.92±0.89% DM) and other forms of polysaccharides expressed as fibre, accounting for 12.99±0.32 % DM. The main component of this fibre is the NDF fraction - 50.48 ± 1.23 % DM.
8. The seeds of the sea buckthorn fruit contained an ADF fraction of 25.08 ± 0.96 % and ADL structural fibre accounting for 14.54 ± 0.41% DM of seeds. It was established that sea buckthorn fruit seeds also contained 29.34±1.14 % of protein and 3.00±1.32% of lipids.

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Sea buckthorn pests and diseases in Belarus

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Abstract

Results of fitosanitary monitoring of sea buckthorn plantation at the Institute for Fruit Growing showed that the most harmful pest appeared to be seabuckthorn fly (*Rhagoletis batava obscuriosa Kol).* The pest is capable to damage 21.5-87.2 % of the harvest depending on the variety. No varieties resistant to sea buckthorn fly were revealed. Green sea buckthorn aphid (*Capitophorus hippophaes* Walk.) and sea buckthorn psylla (*Psylla hippophaes* Frst.) are also widely-spread pests. In certain years wood leopard moth (*Zeuzera pyrina* L.), common lackey (*Malacosoma neustria* L.) and European leafroller (*Cacoecia rosana* L.) were observed.

The most widely-spread sea buckthorn disease is a wilt (pathogens - fungi from genus *Verticillium* Nees. and *Fusarium* Link.). The disease caused 100 % death of the plants in some varieties.

As a result of resistance estimation to the wilt 42 varieties and hybrids were divided into 4 class – relatively resistant, weakly susceptible, medium susceptible, highly susceptible. Weakly susceptible varieties were dominant – they accounted for 46 % of the varieties. 14,3 % of the varieties ('Desert maslichnyi', 'Zolotoi klyuchik', 'Yolochka', 'Mendeleevskaya', 'Syurpriz Baltiki', hybrid 11-28-00) were relatively resistant to the wilt. In certain years "endomycosis" was harmful to the fruits. The fruit damage degree was 7-75%.

Key words

Hippophaë rhamnoides, specialized pests species, "endomycosis" of fruits, wilt, resistance, variety

Introduction

A certain species composition of pests and diseases is typical for each region of sea buckthorn cultivation. It is variable in course of time because of cultivar introduction and change of climate conditions and cultivation technologies. Data on a species composition, domination structure of phytophages and phytopathogens in sea buckthorn plantings in Belarus have fragmentary character (Garanovich 1992, Shalkevich 2001, Garanovich *et al.* 2009). Increase of prevalence and injuriousness of diseases and pests is observed in different countries (Singh V. *et al.* 2008, Shamanskaya 2009, Kauppinen 2013). A decisive role in prevention of crop shortage and fruit quality deterioration belongs to measures confining number and development of phytophages and phytopathogens. Selection of resistant varieties is one of them.

The purpose of this research was to specify the species composition and domination structure of phytophages and phytopathogens in sea buckthorn plantations and to reveal resistant varieties against the most harmful pests and diseases.

Materials and methods

The research was carried out at the Institute for Fruit Growing within 1995-2014 where sea buckthorn trees of 42 varieties and promising hybrids of a various genetic and geographical origin are situated. The plants were planted at the experimental fields in 1992, 1996, 2006 and 2008. The amount of plants in each variety was 6-20. The planting scheme was 4 × 2 m (1250 plants / per ha).

The records of diseases and pests were carried out by annual route investigation during the vegetative period by the methods developed in M.A.Lisavenko Research Institute of Horticulture for Siberia (Orel 1999). Development of diseases was estimated by the formula generally accepted in phytopathology. Grouping of varieties for wilt and "endomycosis" resistance were carried out by the methods of N.I.Vavilov Research Institute of Plant Industry (Khokhryakova *et al.*, 1972). Distribution of varieties and hybrids on susceptibility to sea buckthorn fly was carried out according to the List of Descriptors for the species *Hippophae rhamnoides* L. (St.-Petersburg, 1993).

Results and discussion

As a result of investigation there was revealed the damage of sea buckthorn plants by green sea buckthorn aphid, sea buckthorn psylla, wood leopard moth, common lackey, European leafroller and sea buckthorn fly. Sea buckthorn fly is the most dangerous pest in the regions of natural and cultural areas. In Belarus this pest was noticed in 2010 for the first time and that was caused by stable increase of daily average air temperatures up to + 19 °C in the period from the 2nd decade of June till the second decade of August. In subsequent years its number has increased (Figure 1).

The outbreak of pest and increment of its number and injuriousness within the last four years has been noted in industrial and experimental sea buckthorn plantings in some other European countries like Poland, Lithuania and Germany.No variety not damaged by the seabuckthorn fly has been found. The pest is capable to damage up to 21.5-87.2 % of the harvest depending on a variety. The varieties were divided into 4 classes by the degree of pest resistance (Table 1).

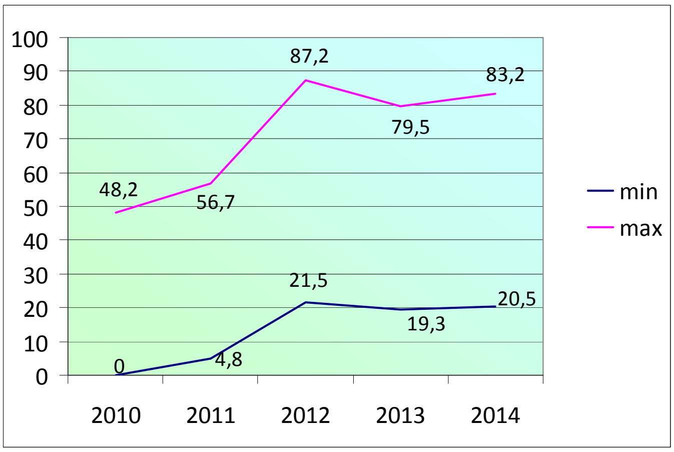

Figure 1. Fruit damage rate by the sea buckthorn fly (%) during the investigation period.

Table 1. Distribution of sea buckthorn varieties on resistance degree to *Rhagoletis batava* Hering

Resistance classes	Fruit damage rate, %	Variety, hybrid
Highly resistant	21.5-25.0	'Baikal', 18/89, 114-2008-02
Medium resistant	26.0-50.0	'Botanicheskaya', 'Dar Kazakovu', 'Mariya', 'Riabinka', 'Syurpriz Baltiki', 'Zheltoplodnaya', 'Zhyoltaya rannyaya', 'Zolotaya kosa', 'Zolotoi klyuchik', 15/88, 03-22-00
Susceptible	51.2-71.3	'Desert maslichnyi', 'Karamelka', 'Dyuimovochka', 'Mendeleevskaya', 'Nivelena', 'Plamennaya' 'Petrovka', 'Podarok sadu', 'Vasilisa', 'Yolochka', 'Zarevo', 7/71, 21/90, 38/90, 11-28-00
Hyper susceptible	81.3-87.2	'Trophimovskaya', 'Finskaya'

Green sea buckthorn aphid and sea buckthorn psylla are widely-spread pests as well (Figure 2).

Caterpillars of leaf-eating lepidopterans (*Archips rosan* L. and *Zeuzera pyrina* L.) are spread sporadically in sea buckthorn plantings. Number of this group of pests did not exceed 0.6 caterpillars per 2 m of branches and 0.1 % of damaged shoots during the research years.

The most widely-spread disease in sea buckthorn plantings is the wilt (drying), caused by fungi from genus *Verticillium* Nees.; *Fusarium* Link. (Shamanskaya 2009); *Corineum elaeagni* Jacz. (Khovalyg 2005); *Stigmina* Sacc. (Kauppinen 2013). Non-infectious types of sea buckthorn wilt were diagnosed as well with damage of plants up to 100 % (Kondrashov 1996). According to

E.M.Drozdovski and I.A.Eremenko (1983) young plants die of root rot and adult plants die of *Verticillium* wilt which is complexed with some other factors. Fungi from genus *Fusarium* Link. – *F. Culmorum* (W.G.Sm.) Sacc., *F. Sumbucinium* Fack., *F.* Oxysporum, *F. gibbosum* (Garanovich *et al.*, 2009) and *Verticillium* Nees. and bacteria *Pseudomonas syringae* (Shalkevich, 2001) were revealed in plant samples with wilt symptoms during the investigations carried out in Belarus. The studied varieties and hybrids had various wilt resistance (Table 2).

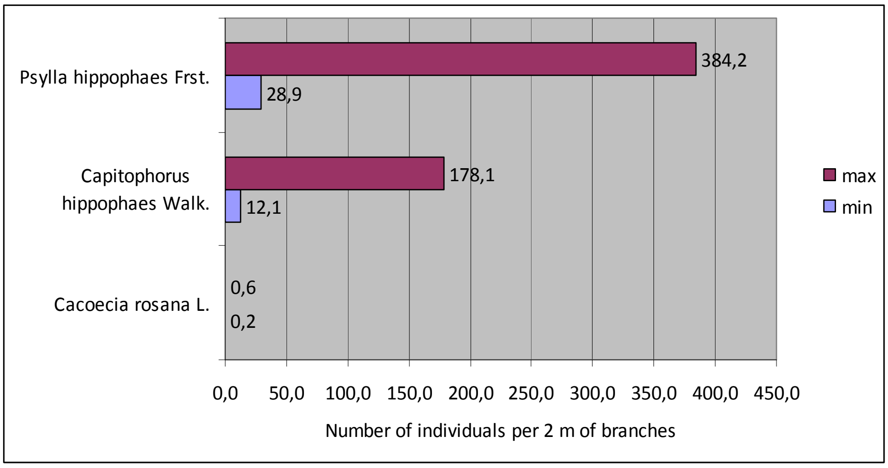

Figure 2. Number of sea buckthorn pests during the investigation period.

Table 2. Distribution of sea buckthorn varieties on resistance degree to wilt

Resistance classes	Disease development, %	Variety
Relatively resistant	0-8,3	‘Desert maslichnyi’, ‘Yolochka’, ‘Mendeleevskaya’, ‘Syurpriz Baltiki’, ‘Zolotoi klyuchik’, 11-28-00
Weakly susceptible	12,5-25,0	‘Baikal’, ‘Botanicheskaya’, ‘Dyuimovochka’, ‘Finskaya’, ‘Gaspadar’, ‘Karamelka’, ‘Mariya’, ‘Nivelena’, ‘Plamennaya’, ‘Riabinka’, ‘Trophimovskaya’, ‘Vasilisa’, ‘Zarevo’, ‘Zheltoplodnaya’, 20/88, 10/86, 15/88, 21/90, 4/87
Medium susceptible	31,7-50,0	‘Dar Kazakovu’, ‘Priokskaya’, ‘Otradnaya’ ‘Zhyoltaya Rannyaya’, 23-34, 38/90
Highly susceptible	53,3-87,5	‘Botanicheskaya luchistaya’, ‘Inya’, ‘Krasnoplodnaya’, ‘Kudrina’, ‘Lomonosovskaya’, ‘Petrovka’, ‘Podarok Sadu’, ‘Vorob’yovskaya’, ‘Zolotaya kosa’, 18/89, 7/71

In certain years “endomycosis” (decoloration and softening) of fruits does a significant damage. The disease is caused by saprophyte micro-flora, represented by the following species: *Aureobasidium pullulang* (D.B.) Ar. El., *Penicillium cyneotuhum* B. Rap. Thorns, *Penicillium rubrum* Stadt. Rap. Thorns., *Aspergillus niger* V. Fieg. Rap. Fen., *Trichoderma viride* Pors, Ritai., *Alternaria alternata* (Fr.) Keissler (Shamanskaya, 2009). In our investigations “endomycosis” of fruits was observed annually. The fruit damage degree was 7-75%. The majority of the studied varieties showed relative resistance (disease expansion was not more than 10%). The most susceptible varieties were ‘Botanicheskaya’, ‘Mendeleevskaya’ and ‘Yolochka’.

Thus, the most harmful pest was defined to be sea buckthorn fly and the most harmful disease the wilt. The varieties most resistant to the pest (‘Baikal’, 18/89, 114-2008-02) and to the disease (‘Desert maslichnyi’, ‘Yolochka’, ‘Mendeleevskaya’, ‘Syurpriz Baltiki’, ‘Zolotoi klyuchik’, 11-28-00) were revealed. The further researches directed on perfection of methods for diagnosis and selection of resistant varieties as well as for biological techniques of plant protection are highly required.

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Sea buckthorn leaves and shoots – a promising source of active compounds

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Abstract

All parts of the sea buckthorn (*Hippophae rhamnoides* L.) plant are considered to contain remarkable amount of compounds that are believed to have beneficial health effects. Till now different parts of sea buckthorn (SBT) plant were used for the treatment of diseases in traditional medicine in various countries. For example, extracts of leaves and bark were used to treat colitis and enterocolitis, leaves also are reported to exhibit anti-oxidant, anti-bacterial, anti-tumour and anti-inflammatory activity, meanwhile having no cytotoxicity and adverse effects after oral usage. Therefore SBT parts would be a good raw material not only for medicinal properties but also for food products with functional properties.

The aim of the research was to study the content of phenolic compounds and antioxidant activity of sea buckthorn parts.

All experiments were carried out at Latvia State Institute of Fruit-Growing. The research object was various parts of female and male sea buckthorn bushes. Total phenols, flavonoids and tannins in extracts of leaves and shoots were analyzed.

Study showed the significant differences ($p < 0.05$) in the content of total phenols, total flavonoids and condensed tannins between parts of sea buckthorn plant. The leaves of female and male plants were the best sources of total phenols (165.76 mg/g DW), and 123.78 mg/g DW, respectively). The total flavonoids content differed reaching the highest level in male flowers (81.72 mg/g DW) and the lowest in male leaves (36.47 mg/g DW). The shoots of female sea buckthorn plant contained the highest amount of condensed tannins reaching 22.47 mg/g DW what is about 20 times higher than in both gender leaves. Similar to phenolic compounds, also the results of both antioxidant activity assays proved significant differences ($p < 0.05$) between sea buckthorn leaves and shoots, furthermore activity detected by FRAP method showed higher values compared to the ones measured by DPPH radical assay. The highest antioxidant activity by both methods was reached in leaves of female plant (220.97 mg/g DW for FRAP and 43.76 mg/g DW for DPPH assay), followed by male leaves, female and male shoots.

Pearson correlation coefficients were positive, strong and significant between total phenols and FRAP ($r = 0.945$), and total phenols and DPPH ($r = 0.883$). Further research of particular phenols and other constituents is needed to explore the potential of sea buckthorn plant in aim to obtain extracts with high biological activity.

Key words

leaves, shoots, gender, flavonoids, phenols, tannins, antioxidant activity.

Review of sea buckthorn pests in Latvia

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Abstract

Since introduction of common sea buckthorn (*Hippophae rhamnoides*) into the territory of Latvia it was used mainly as ornamental plant. Only in 1984 the first sea buckthorn plantation was planted as fruit crop orchard. Nowadays the sea buckthorn is one of the commercially important fruit crops in Latvia. Although the growing of sea buckthorns rapidly increased during the last 20 years, no attention was dedicated to their pests until the first records of *Gelechia hippophaella* in Latvia. That actualized the need for wider studies on sea buckthorn pest diversity and their importance in commercial plantations.

Preliminary research results, including the pests listed in the literature, show more than 30 invertebrate pests associated with sea buckthorn that are recognized in Latvia. The majority of pests encountered are polyphagous insects that in most cases do not have commercial importance. Monophagous insects associated with sea buckthorn are *Cacopsylla* sp., *Gelechia hippophaella, Capitophorus elaeagni, Capitophorus hippophaes, Capitophorus similis*, and *Rhagoletis batava*. More widely recognized polyphagous pests are some moths, especially *Archips rosana,* and *Phyllopertha horticola*. Previously was known that unidentified monophagous *Cacopsylla* sp. can cause serious damage in some local sea buckthorn plantations, but during the last two years this psyllid species in plantations was observed rarely or was absent. Today only *Gelechia hippophaella,* and *Rhagoletis batava* can be considered as main and potentially important invertebrate pests of sea buckthorn in Latvia, but additional research is needed.

Other group of important pests of sea buckthorn are some members of vertebrata – birds (*Corvus corax, Corvus cornix,* and *Sturnus vulgaris*) and mammals (*Capreolus capreolus*, and *Sus scrofa*). Birds can cause significant damage to fruit yield and are commercially most important sea buckthorn pests in commercial plantations in Latvia and almost no possibilities to reduce the damage to the yield. Importance of wild boars (*Sus scrofa*) is observed only locally, where animals destroy sea buckthorn roots. Almost in the all observed parts of the country sea buckthorn was damaged by European roe deer (*Capreolus capreolus*). Only fences builded around the sea buckthorn plantations can limit damage made by mammals.

Effect of different preconditioning treatments on textural and physical properties of sea buckthorn seeds

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Abstract

Information on textural and physical properties of sea buckthorn seeds is necessary for the construction of a dehulling system and the mechanical compression of oil. Therefore, the present study was done to determine the textural and physical properties of sea buckthorn seeds and the effect of different preconditioning treatments.

Two different cultivars were conditioned to five levels of moisture content from 6.26 to 24.26% for *Leikora* and 5.48 to 30.10% for *Hergo*.

The textural characteristics of these cultivars were measured in terms of hardness and fracturability. In addition, the attended force at different test times of compression was determined. The average dimensions area, outer diameter max and min, aspect ratio and circumference were measured at the different moisture contents of the cultivars. Additionally, a thousand seed mass and a bulk density were determined.

For both cultivars the hardness decreased with increasing moisture content. A trend of decreasing attended force was found at higher moisture content levels. The seeds of *Leikora* and *Hergo* didn´t show a significant change in fracturability due to change in moisture content.

The area and the outer diameter max and min of the *Hergo* seeds increased with increasing moisture content. *Leikora* seeds didn´t show any significant changes in area and outer diameter max and min due to change in moisture content. The aspect ratios and the circumference values of *Leikora* and *Hergo* seeds were not linear related with moisture content. *Leikora* seeds had higher values of aspect ratio and circumference values than the seeds of *Hergo*. The thousand seed mass increased with the increase in moisture content for both cultivars. A linear relationship and a negative correlation for the bulk density of both cultivars with the moisture content were found. *Leikora* seeds had higher values of thousand seed mass and bulk density than the seeds of *Hergo* at all moisture content levels.

Key words

Sea buckthorn seeds, textural properties, physical properties

Introduction

Sea buckthorn (*Hippophaë rhamnoides*) is a nitrogen-fixing *actinomycetes* plant species native to Europe and Asia. The fruits are yellow-orange berries that can be processed to oil, juice and food additives mainly used for production of confectionery and consumer care products. (Beveridge et al. 1999, Mörsel & Singh 2009)

Two different types of oil can be obtained from the fruit: oil from the seed and oil from the pulp and skin of the fruit. The amount of oil depends mainly on the origin of the fruit and other factors (Yang & Kallio 2002, Tittinen, Hakala, & Kallio 2005) The quality of edible oils depends not only on the production method, but also from other factors e.g. the raw material and its characteristics at object time (Sharma, Sogi & Saxena 2009)

The common mechanical compression is mainly done with the whole sea buckthorn seeds. According to other studies, dehulling of seeds could improve the quality of the oil and the de-oiled by-product. Evangelista et al. (2010) reported that hexane-extracted oil from the dehulled cuphea seed contained 70% less chlorophyll compared with similarly extracted whole seed oil. Chlorophyll leads to a loss of quality in taste and color of oil. Subramanian et al. (1990) describes an improved color, a lower wax content of the dehulled extracted sunflower oil as well as lower fiber and higher protein content of the de-oiled press residue. In addition, a reduction of physical damage of oil expression unit was reached due to absence of hull.

Physical and mechanical characteristics of seeds, kernels and fruits were reported by many studies e.g. for corn (Seifi et al. 2010), for pomegranate seeds (Kingsly et al. 2006), for glory lily beans (Suganya et al. 2013), for sunflowers (Subramanian et al. 1990), for neem nuts (Viswanathan et al. 1996), for apricot pit and kernel (Fathollahzadeh et al. 2008), for blueberries (Chiabrando et al 2009) and for sea buckthorn berries (Khazaei et al. 2004).

Information on textural and physical properties of sea buckthorn seeds is necessary for the construction of a dehulling system and the mechanical compression of oil. However, only a few literatures are available regarding the textural and physical attributes for sea buckthorn seeds. Therefore, the present study was done to determine the textural and physical properties of sea buckthorn seeds and the effect of different preconditioning treatments.

Materials and methods

Sample conditioning

Dried sea buckthorn kernels *Hippophae rhamnoides* 'Leikora' and seeds of *Hippophae rhamnoides* 'Hergo' (standard market quality) with the initial moisture content of 6.26% (w/w) for 'Leikora'and 5.48% (w/w) for 'Hergo'were used in the experiments. Additionally, fresh seeds were taken from pomace with moisture content of 24.26% (w/w) for 'Leikora'and 30.10% (w/w) for 'Hergo'. The samples of medium range moisture content were affected by adding a defined amount of distilled water to the dried sea buckthorn seeds to obtain moisture levels as 11.48, 13.60 and 18.68% (w/w) for 'Hergo'and 11.12, 15.11 and 21.30% (w/w) for 'Leikora'. (Viswanathan et al. 1996)

Dimensions and size

To determine the dimensions area (calculated by the amount of pixel multiplied by calibration factors of the directions X and Y) in mm^2, aspect ratio (ratio of the sample height to its width), circumference in mm, outer Diameter max and min in mm were determined by the computer software Image analysis-Software analySIS 5 (Co. SIS, Germany).

Thousand seed mass

Randomly selected 100 seeds were weighed in an electronic balance with 0,001g graduations. This weight was converted into 1000 seed mass (Bundessortenamt Germany, 2000).

Bulk density

The bulk density in kg/hl was calculated from the mass of seeds divided by the volume containing mass (DIN ISO 7971-3:2009).

Texture measurements

The textural properties of the sea buckthorn seeds were measured using the texture analyzer Zwicki 2.5KW (Co. Zwick/Roell, Germany) and the related computer software testXpert II (V3.5). Textural properties were deduced from the force-time curves. To interpret the textural parameters from the graph, the initial peak position was considered as fracturability (N) (Fi_1; first peak of compression) and the maximum peak was considered as hardness (N) (Fi_3; maximum peak of compression) (Bourne 2nd Edition 2002, Kahyaoglu & Kaya 2006).

Statistical analysis

The analysis of significance (p-value) was carried out using Systat Software (Systat Software GmbH, Germany). The coefficient of determination (R^2) and standard derivation was calculated by MS Excel (Co. Microsoft Corp., Redmond, WA, USA).

Results and discussion

The mean values for the area, 1000 seed mass and bulk density of seeds in dependence of moisture content and variety are given in Figure 1.

The area of the 'Hergo'seeds increased significantly ($p<0.05$) with increased moisture content due to swelling. The coefficient of determination as a function of moisture content and area of seeds of 'Hergo'was $R^2=0.98$. 'Leikora'seeds didn´t show any significant change in area due to change in moisture content ($R^2=0.02$, $p>0.05$).

The 1000 seed mass of 'Leikora' was significantly higher than the 1000 seed mass of 'Hergo'at all determined moisture contents ($p<0.05$). The 1000 seed mass increased linear with increase in moisture content for both cultivars ($R^2=0.99$ for 'Hergo', $R^2=0.92$ for 'Leikora'). The equal trend has been observed for pomegranate seeds (Kingsly, Singh, Mankantan, & Jain 2006), neem nuts (Viswanathan 1996) and lily beans (Suganya & Kailappan 2013).

The bulk density decreased linearly in the range of 74.68 to 69.45kg/m^3 with increasing moisture content of 5.48 to 30.10% (w/w) for 'Hergo' ($R^2=0.89$). For 'Leikora', the bulk density decreased linearly from 72.08 to 68.28kg/m^3 as the moisture content decreased from 6.26 to 24.26% (w/w) ($R^2=0.89$).

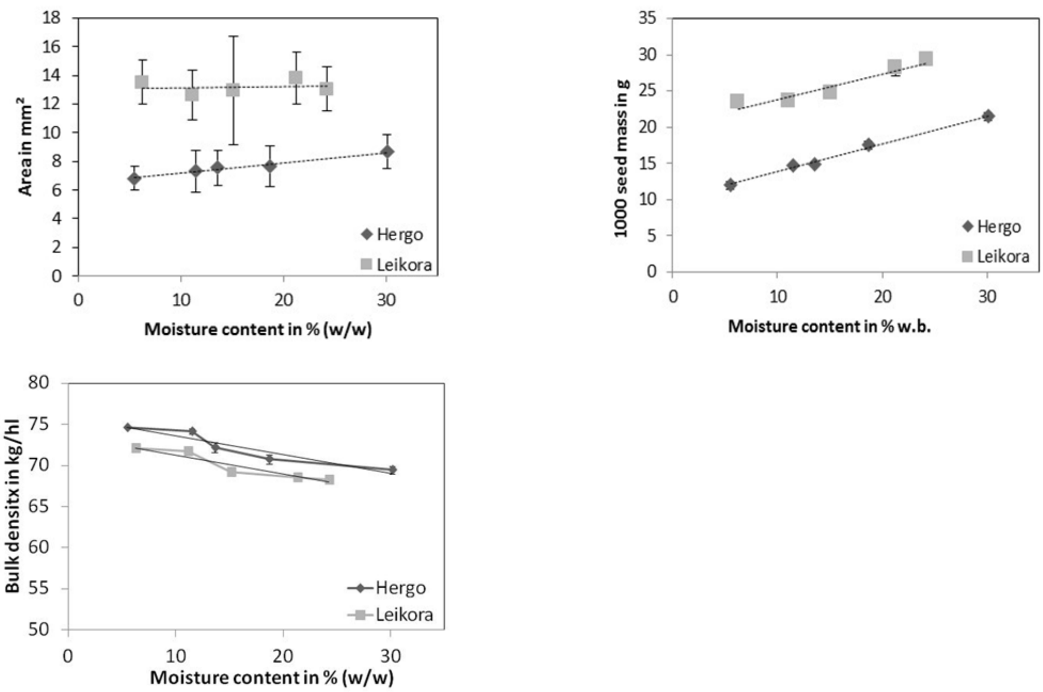

Figure 1. Effect of moisture content and cultivars on area, 1000 seed mass and bulk density of sea buckthorn seeds (Shown from left to right)

The results of linear connection and negative correlation of bulk density and moisture content are similar to that found for other seeds and kernels (Viswanathan 1996, Seifi & Alimardani 2010). Further seed dimensions and the moisture content of the sea buckthorn seed varieties are given in Table 1.

Table 1. Effect of moisture content and variety on dimensions of sea buckthorn seeds. Figures in parentheses are the standard derivations.

Variety	Moisture content in % w. b.	Aspect ratio	Circumference in mm	Outer Diameter max in mm	Outer Diameter min in mm
Hergo	5.48 (0.38)	1.93 (0.15)	11.20 (2.17)	4.23 (0.32)	2.08 (0.17)
	11.48 (0.40)	1.93 (0.13)	10.73 (1.33)	4.35 (0.57)	2.14 (0.32)
	13.60 (0.80)	1.93 (0.15)	11.06 (1.64)	4.44 (0.38)	2.18 (0.21)
	18.68 (0.89)	1.92 (0.15)	10.84 (1.07)	4.44 (0.47)	2.20 (0.22)
	30.10 (0.26)	1.92 (0.14)	11.54 (0.90)	4.72 (0.39)	2.35 (0.19)
Leikora	6.26 (0.09)	2.00 (0.12)	14.48 (0.91)	5.96 (0.40)	2.89 (0.19)
	11.12 (0.19)	2.01 (0.23)	14.88 (2.83)	5.79 (0.44)	2.78 (0.20)
	15.11 (0.40)	2.12 (0.23)	14.52 (2.73)	6.00 (0.94)	2.68 (0.44)
	21.30 (0.60)	2.05 (0.15)	14.86 (1.12)	6.10 (0.48)	2.88 (0.21)
	24.26 (0.22)	2.00 (0.17)	14.27 (0.79)	5.82 (0.34)	2.80 (0.24)

'Leikora'seeds had significant higher values of outer diameter min and max than 'Hergo'seeds ($p<0.05$).

The outer diameter max and min of 'Hergo' increased linearly with moisture content (R^2=0.96 and R^2=0.98). The seeds of 'Leikora'didn´t follow similar pattern. The outer diameter max and min didn´t increased with increasing moisture content (For both R^2=0.01). The aspect ratios of 'Leikora'and 'Hergo'were not polynomial related with moisture content. 'Leikora'seeds had significant higher values of aspect ratio than the seeds of 'Hergo' ($p<0.05$). There were also no significant differences in the circumference values of 'Leikora'and 'Hergo'seeds with increasing moisture content.
The attended force at different test times (values of 4, 6 and 9 seconds) and the effect of different moisture contents of sea buckthorn seeds of 'Leikora'and 'Hergo'is presented in Figure 2.
It was observed that the hardness for both cultivars decreased linearly with the increase in moisture level (Figure 3).

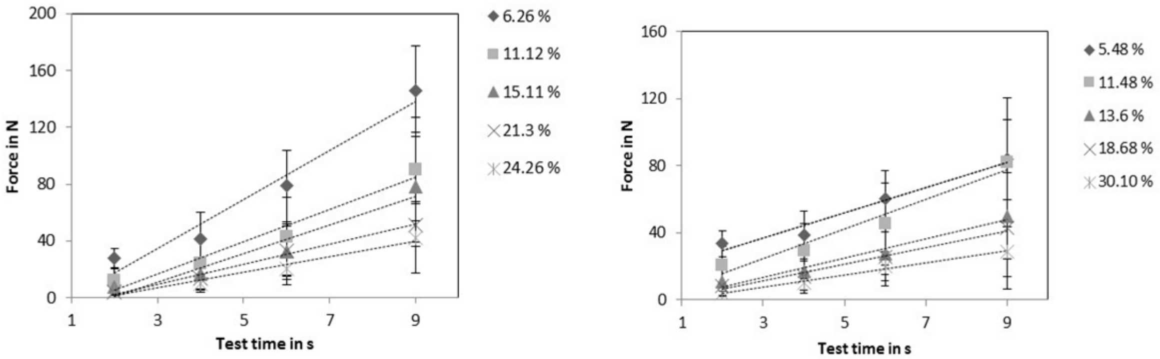

Figure 2. Effect of moisture content on deformation force at different test times of sea buckthorn seeds of Leikora (left) and Hergo (right)

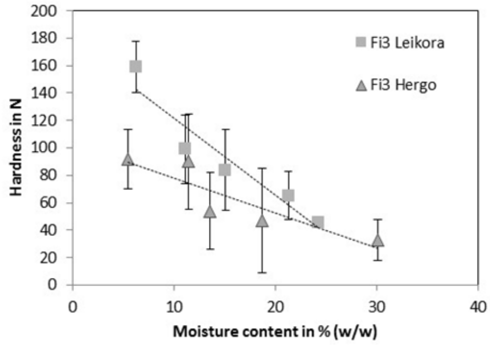

Figure 3. Effect of moisture content and cultivar on hardness of sea buckthorn seeds

The force required for the maximum peak of compression was always observed higher for the 'Leikora'seeds as compared to 'Hergo'seeds ($p<0.05$). The equations show that the decrease in hardness for 'Leikora' (R^2=0.91) is more linear than for 'Hergo' (R^2=0.79).

The results show the same trend in decreasing of hardness due to increasing of moisture content to those reported by Kingsly et al. (2006) for pomegranate seeds and Viswanathan et al. (1996) for neem nut.

The knowledge of the physical and textural properties of sea buckthorn seeds is essential to facilitate the design and development of equipment for dehulling seeds and pressing seed oil. The kernel hardness is an indicator of breaking susceptibility. The fracturability and the required force could be related to dehulling properties of seeds, whereas the hardness and the required force could be associated with the oil extraction. The conditioning of sea buckthorn seeds to higher moisture can reduce the force and energy required to break the hull. Bulk density and 1000 seed mass can be valuable in sizing seed hoppers and storage facilities. The determined dimensions can be used to determine the angle of oil mills and to achieve consistent flow of materials through the chute.

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The Hardiness of Sea Buckthorn Cultivars in Estonian Climatic Conditions

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Abstract

Research on sea buckthorn cultivars in Estonia started in 1973. In 40 years, 52 cultivars have been introduced from Russia (mainly from Barnaul, Moscow and Nizhni-Novgorod), Belarus, Germany and Finland. At present, the list of cultivars recommended for growing in Estonia includes the cultivars `Avgustinka`, `Botanitcheskaya`, `Botanitcheskaya lyubitelskaya`, `Otradnaya` and `Trophimovskaya`. There are 856 ha of sea buckthorn plantations in Estonia.

Estonian winters are characterized by periods of very cold weather followed by days with above-freezing temperatures. This fluctuation leads to winter damage in sea buckthorn. Sea buckthorn buds and bark on the trunk and larger branches are most exposed to winter damage. Harvesting sea buckthorn berries by cutting of branches weakens shrubs and is conducive to winter damage. The state of the shrubs was evaluated in mid-July on a scale from 1 to 9.

Key words

Sea buckthorn, cultivars, adaptation

Introduction

In Estonia, the first trials with sea buckthorn (*Hippophaë rhamnoides* L.) were carried out in the middle of the 20th century with the goal of revegetation of alvars and sandy areas. Research on sea buckthorn cultivars was started in 1973. In 40 years, 52 cultivars from Russia (Barnaul, Moscow, Nizhni-Novgorod), Belarus, Finland and Germany were introduced in Estonia. Many sea buckthorn cultivars do not adapt to the climatic conditions in Estonia, where the vegetative period lasts 150 to 180 days and winters are characterized by intermittent frosts and thaws, which is conducive to winter damage on sea buckthorn shrubs. Larger orchards in Estonia have adopted a harvesting technology whereby fruit-bearing branches are cut off and frozen at -20 ºC to separate the berries. Using this harvesting technology removes a large share of foliage, which interferes with the ability of shrubs to prepare for the winter. Buds are the parts of sea buckthorn plants that are the most sensitive to winter damage, followed by the bark on the trunk and larger branches.

The aim of this paper is to evaluate winter hardiness of different sea buckthorn cultivars based on the state of the shrubs and to ascertain cultivars best suited for commercial orchards.

Materials and methods

Sea buckthorn cultivar comparison trials were started at Rõhu Experimental Station by planting 14 cultivars with 21 shrubs each in 1988 and seven German and Russian cultivars with 15 shrubs each in 2005.

At 26 years of age, the surviving of shrubs was counted; the state of the shrubs was evaluated on a 9-point scale and the circumference of the trunks of the larger shrubs was measured.

In Polli, a collection of sea buckthorn cultivars was planted in 2005, with 2 to 5 shrubs per cultivar. In the winter of 2012, winter damage to the shrubs was evaluated on a 9-point scale, where 1 stood for an undamaged shrub and 9 for a dried shrub. In the fall of 2014, when the shrubs in the orchard were 10 years old, the state of the shrubs was evaluated again on a 9-point scale; in an older, 15-year-old orchard (planted in 2000), the number of surviving shrubs was counted and the state of the surviving shrubs was evaluated. The research team also visited six sea buckthorn growers in

Estonia, of which five were in Southern and one in Northern Estonia. The area of the plantations ranged mostly from 0.1 to 3 hectares, with the largest being 20 hectares.

The state of shrubs and the extent of damage were evaluated on a 9-point scale as follows:
1 – the shrub has dried;
2 – the crown has dried, a few shoots on the trunk or thicker branches growing from dormant buds;
3 – bigger branches have a limited number of one-year shoots;
4 – half of the crown has dried, the shrub has many one-year shoots;
5 – the state of the shrub is satisfactory; 1- to 3-year-old branches that have dried are being replaced by young shoots;
6 – a large part of one-year shoots have dried; young shoots are short; yield is low;
7 – the state of the shrub is good; young shoots are 20 cm long; yield is satisfactory;
8 – the state of the shrub is good; young shoots are 20 to 30 cm long; yield is satisfactory;
9 – the state of the tree is very good; young shoots are over 30 cm long; yield is good.

The results were converted for dispersion analysis using √X+½ and the results were evaluated using the Duncan test.

Results and discussion
Sea buckthorn is an introduced species in Estonia. Sea buckthorn has a characteristically short rest period. In Central Russia (Moscow), buds finish their rest period already in November, and in favorable temperatures the water starts to be absorbed into cell proteins. Long thaw periods in mid-winter reduce the winter hardiness of the generative parts of buds. The generative buds of male plants are particularly sensitive.

In the collection of sea buckthorn cultivars in Polli, winter damage occurred on 8-year-old shrubs in spring 2012. The extent of damage was varied by cultivar. Cultivars 'Tarmo', 'Otradnaya', 'Gibrid Perchika', 'Botanicheskaya aromatnaya' suffered extensive damage that destroyed over ½ of the crown. Other cultivars lost a part of 1- to 3-year old branches, which led to crop failure.

In 2014, the state of shrubs was evaluated in different experimental and commercial plantations (Table 1). The state of shrubs depended on the adaptive capacity of cultivars. Cultivars that are not characterized by slowdown in the growth of shoots and keep their leaves on until permanent cold weather in December are not winter resistant in Estonia. On the other hand, the shrubs of cultivars 'Vorobyevskaya', 'Botanicheskaya', 'Botanicheskaya aromatnaya', 'Botanicheskaya lyubitelskaya', 'Avgustinka', and 'Trophimovskaya' were in good condition both in commercial orchards and in the 26-year-old experimental orchard at Rõhu. These cultivars originate from the Botanical Garden of Moscow State University where they were obtained by free pollination between specimens of the Baltic and Siberian races of sea buckthorn.

The condition of the shrubs was worse in the 10-year-old plantation where berries had been harvested by cutting off the fruit-bearing branches but the shrubs of cultivars 'Botanicheskaya lyubitelskaya', 'Botanicheskaya aromatnaya', 'Botanicheskaya', and 'Tytti' had fared somewhat better than others.

In the experimental orchard planted in 2000, which was first used for studying the effect of fertilizing on shrub growth and later for comparing harvesting technologies, 44% of the 98 shrubs of the cultivar 'Botanicheskaya' had survived and the average state was 7.3. The cultivar 'Prozrachnaya' had survived 67% of shrubs of with the average state 8.1. Harvesting technology used in this orchard – removing fruit-bearing branches with a considerable part of foliage – weakened the shrubs and led to losing many shrubs due to winter damage.

The comparison trial of German and Russian sea buckthorn cultivars established in 2005 revealed a difference in their adaptive capacity in the Estonian climatic conditions. The shrubs that fared relatively well were of cultivars 'Botanicheskaya' (9.0), 'Otradnaya' (8.5), and 'Leikora' (8.0).

The shrubs of cultivars ‘Hergo’ (6.8), ‘Askola’ (6.7), ‘Botanicheskaya lyubitelskaya’ (6.3), ‘Gibrid Perchika’ (6.1), and ‘Podarok sadu’ (5.8) fared satisfactorily. The shrubs of cultivars ‘Sirola’, ‘Frugana’, ‘Habego’, and ‘Hergo’ did not fare well (3.2 to 5.0). The shrubs of cultivars ‘Dar Katuni’, ‘Grodenskaya’, ‘Maria’, and ‘Mendelyevskaya’ were destroyed in the 10-year-old orchard. The shrubs had suffered serious damage; their crowns were destroyed. There were a few shoots on the trunks of some shrubs but some shrubs had been lost in case of cultivars ‘Nivelena’, ‘Pantelyevskaya’, ‘Plamennaya’, ‘Zolotaya rannaya’, and ‘Tarmo’.

Conclusions

Our research indicates that cultivars bred in the Botanical Garden of Moscow State University – ’Vorobyevskaya’, ’Botanicheskaya’, ’Botanicheskaya aromatnaya’, ’Botanicheskaya lyubitelskaya’, ’Avgustinka’, and ’Trophimovskaya’ – are better suited for larger commerical orchards. We conclude that the harvesting technology whereby fruit-bearing branches are cut off from shrubs along with a large share of foliage reduces the winter resistance of shrubs.

Breeding new winter hardy cultivars is important in the Baltic countries, as introducing cultivars from continental Siberia and Western Europe has not been promising.

Table 1. State of sea buckthorn shrubs in 2014 and the extent of winter damage in 2012.

Cultivar	State of shrubs in Polli, winter damage		Rõhu Experimental Station, 2014		Commercial orchards, 2014
	2012 (1 to 9)	2014 (1 to 9)	Surviving shrubs, %	State of shrub (1 to 9)	State of shrub (1 to 9)
Botanicheskaya	2.3 a	4.8 a	28	8.3 a	8,2 a
Botanicheskaya lyubitelskaya	2.0 a	5.5 a	5	8.0 a	8,1 a
Botanicheskaya aromatnaya	5.5 c	5.0 a	19	8.7 a	8,0 a
Avgustinka	4.7 bc	3.5 b	5	7.0 b	8,1 a
Otradnaya	6.5 c	3.5 b	33	8.6 a	8,0 a
Gibrid Perchika	6.5 c	3.0 b	38	8.7 a	5,9 b
Trophimovskaya	3.0 ab	3.0 b	19	8.7 a	8,0 a
Vorobyevskaya	2.0 a	4.0 b	38	8.7 a	8,3 a
Podarok sadu	4.0 bc	4.0 b	48	7.0 b	4,3 b
Terhi	3.7 ab	3.0 b	-	-	-
Tarmo	7.3 c	2.5 c	-	-	-
Tytti	1.7 a	4.5 a	-	-	-
Nivelena	5.0 bc	2.0 c	-	-	-
Zolotaya rannaya	3.7 ab	2.0 c	-	-	-
Plamennaya	2.3 a	2.0 c	-	-	-

Different letters per column indicate a significant difference.

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Triacylglycerol composition of developing sea buckthorn seeds and the related gene expression levels

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Abstract

Sea buckthorn (*Hippophaë rhamnoides* L.) seed oil and pulp oil contain large amounts of unsaturated fatty acids, including the omega-7 fatty acids vaccenic acid (18:1*n*–7) and palmitoleic acid (16:1*n*–7). The oils are largely made up of triacylglycerols (TAG). There is evidence suggesting positive effects of the pulp oil on atopic dermatitis.

The aim of this work is to study the biosynthesis of TAGs in developing seeds of sea buckthorn. We target the problem by studying the expression of genes encoding enzymes in the TAG synthesis pathway in a time series collected during two years from two experiments grown in two locations within Finland (south, Turku: 60° N and north, Kittilä: 68° N). Two cultivars (Tytti and Terhi) are included. The study is conducted using quantitative PCR for the genes of interest.

The experimental part of the work is still ongoing. Our first results show the expression levels of different enzyme genes for a period of 12 weeks from early berry development until ripe berries. The expression of several genes changed significantly during the growing season. The most notable increases during seed development were detected in the expression of the genes for Δ^9-desaturase and diacylglycerol acyltransferase (DGAT) in both of the studied cultivars. These results are being complemented with analysis of fatty acid and TAG composition at the same time points using gas chromatography (GC) and high performance liquid chromatography (HPLC).

Key words

Triacylglycerol biosynthesis, gene expression, seed oil, pulp oil, gas chromatography, high-performance liquid chromatography

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