ISSN: 2490-3442

UNIVERSITY OF EAST SARAJEVO
FACULTY OF AGRICULTURE

AGROFOR International Journal

AGROFOR International Journal, Vol. 7, Issue No. 2, 2022

agrofor.ues.rs.ba

AGROFOR International Journal

PUBLISHER

University of East Sarajevo, Faculty of Agriculture
Vuka Karadzica 30, 71123 East Sarajevo, Bosnia and Herzegovina
Telephone/fax: +387 57 340 401; +387 57 342 701
Web: agrofor.ues.rs.ba; Email: agroforjournal@gmail.com

Frequency: 3 times per year
Number of copies: 300
ISSN 2490-3434 (Printed)
ISSN 2490-3442 (Online)

CONTENT

Original Scientific paper
10.7251/AGRENG2202005F
UDC 582.998.1:577

EXPLORING AN INVASIVE PLANT *SOLIDAGO CANADENSIS* AS THE POTENTIAL SOURCE OF TRITERPENOIDS

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ABSTRACT

Canadian goldenrod (*Solidago canadensis* L.) is a perennial herb introduced from North America and widely distributed in most European countries, often regarded as an invasive plant threatening the native species, simultaneously being a hard-to-control weed in agriculture. Nevertheless, due to its application in traditional herbal medicine (as *Solidaginis herba*), *S. canadensis* also constitutes a cheap and readily available medicinal raw material. The content of such metabolites as flavonoids, polysaccharides, diterpenes and triterpenoid saponins in this plant is well characterized, whereas the data on the occurrence of triterpenoids and steroids in a free (not conjugated) form are scarce. Thus, the present study was aimed toward the gas chromatography-mass spectrometry (GC-MS) analysis of the extracts obtained from inflorescences (branched panicles) and leaves of *S. canadensis*. Diethyl ether extracts were obtained from dried and powdered samples of *S. canadensis* leaves and inflorescences divided into flowers and stems. Analysis was made by gas chromatography-mass spectrometry method (GC-MS). In both inflorescences and leaves, the significant contents of triterpenoids belonging to lupane-, oleanane- and ursane- groups were found, i.e., lupeol acetate, β-amyrin, and α-amyrin accompanied by its ketone, α-amyrenone. Flowers were the richest source of triterpenoids (approx. 1.2 mg/g of dry weight), whereas the contents in leaves (0.91 mg/g d.w.) and inflorescence stems (0.69 mg/g d.w.) were lower. In all analyzed extracts one compound belonging to plant Δ^7-sterols, spinasterol, was found, present mainly as an ester conjugate.

Keywords: *Canada goldenrod, invasive plants, Solidago canadiensis, steroids, triterpenoids*

INTRODUCTION

Solidago canadensis L. (family Asteraceae, synonyms: Canadian goldenrod, field goldenrod) is an erect rhizomatous perennial plant distributed naturally in North America. In its native range, *S. canadensis* occupies forest edges, roadsides and abandoned fields. Flowering occurs from July to October, the yellow flowers are arranged into small heads on branched pyramidal shaped inflorescences. After its introduction as an ornamental plant, this species (and the closely related *S.*

gigantea) has spread throughout almost all Europe, being among the most successful invasive plants. It is found also in Asia, Africa and Australia. In an invaded area, *S. canadensis* exists in the similar habitats as in the natural range, rapidly colonizing abandoned fields and unmanaged urban sites. As a successive intruder, it is threatening the native species, exerting a negative impact on biodiversity. One of this plant's few benefits is its late blooming period in the second half of summer, when there is a deficiency of bee forage species, therefore, *S. candensis* is valued by beekeepers as nectar- and pollen-providing plant (Guzikowa and Maycock 1986, Weber 1997, Bielecka and Królak 2019).

A developing bioeconomic approach leads to search for new high added-value bioproducts that can be obtained from available local natural resources, including invasive species. In this context of bioeconomy, *S. canadensis* has been suggested for the use in biomass production and biorefinery, however, its traditional applications as a medicinal plant might suggest also other possibilities (Zihare and Blumberga 2017). Historically, the flowers and aerial parts of *S. canadensis* were used in Amerindian medicine to treat urinary tract, tuberculosis, diabetes, hemorrhoids, internal bleeding, asthma, arthritis, ulcers and other skin disorders (Bradette-Hébert *et al*. 2008, Kołodziej *et al*. 2011, Baki *et al*. 2019). In Europe, *Solidago* species were widely used as *Solidaginis herba* to treat urinary tract infections and inflammations, as well as to prevent formation or facilitate elimination of kidney stones and urinary gravel (Kelly *et al*. 2020). Extracts obtained from *S. canadensis* flowers or aerial parts were reported to exhibit several biological activities including antibacterial, antimycotic, antitumor, analgesic, antioxidant, cytotoxic, spasmolytic, sedative and hypotensive properties. Phytochemical characterization of these extracts led to identification of carotenoids, flavonoids, phenolic acids (e.g., hydroxycinnamates), polysaccharides, diterpenes, sesquiterpenes, triterpenoid saponins, tannins, alkaloids, polyacetylenes and essential oils (Baki *et al*. 2019, Shelepova *et al*. 2019, Kelly *et al*. 2020). The aim of the present work was the qualitative and quantitative determination of one of the less characterized group of compounds, i.e., steroids and triterpenoids (occurring in non-glycosidic form) in the aerial parts of *S. canadensis*, i.e., leaves and inflorescences divided into flowers and stems, analyzed separately.

MATERIAL AND METHODS

Aerial parts of randomly chosen plants of *Solidago canadensis* were collected in early September 2021 from the urban habitat in the Pole Mokotowskie park in Warsaw, Poland. Selected not damaged inflorescences and leaves were air-dried at room temperature. Prior to the extraction, inflorescences were separated into flowers and stems. The samples of leaves (3.23 g), flowers (3.20 g) and stems (1.13 g) were homogenized in a laboratory mortar. The ground plant material was placed in thimbles and extracted using a Soxhlet apparatus for 8 h with diethyl ether. The obtained extracts were evaporated to dryness under reduced pressure on a rotary evaporator.

Obtained diethyl ether extracts were fractionated by adsorption preparative thin-layer chromatography (TLC) on 20 cm × 20 cm glass plates coated manually with silica gel 60H (Merck). The solvent system chloroform:methanol 97:3 (v/v) was applied for developing. Three fractions were obtained as described earlier (Dashbaldan *et al*. 2020): free (non-esterified) steroids and triterpenoids, triterpenoid acids and steroid/triterpenoid esters. Fractions were eluted from the gel in diethyl ether. Subsequently, fractions containing free neutral triterpenes and sterols (R_F 0.3-0.9) were directly analyzed by GC-MS, fractions containing triterpene acids (R_F 0.2-0.3) were methylated with diazomethane, whereas fractions containing esters (R_F 0.9-1) were subjected to alkaline hydrolysis. For methylation, nitrosomethylurea (2.06 g) was added to a mixture of 20 mL of diethyl ether and 6 mL of 50% aqueous KOH, and the organic layer was then separated from the aqueous layer. Samples containing triterpenoid acids were dissolved in 2 mL of the obtained solution of diazomethane in diethyl ether, and held at 2 °C for 24 h. For alkaline hydrolysis, the ester fraction was treated with 10% NaOH in 80% MeOH at 80 °C for 3 h. Subsequently, the obtained mixtures were extracted with diethyl ether, and the obtained extracts were fractionated by preparative TLC as described above.

An Agilent Technologies 7890A gas chromatograph (Perlan Technologies, Warszawa, Poland) equipped with a 5975C mass spectrum detector was used for qualitative and quantitative analyses. Samples dissolved in diethyl ether:methanol (5:1, v/v) were applied (in a volume of 1-4 μL) using 1:10 split injection. The column used was a 30 m x 0.25 mm i.d., 0.25-μm, HP-5MS UI (Agilent Technologies, Santa Clara, CA). Helium was used as the carrier gas at a flow rate of 1 mL/min. The separation was made with the following temperature program: initial temperature of 160°C held for 2 min, then increased to 280°C at 5°C/min, and the final temperature of 280°C held for a further 44 min. The other employed parameters were as follows: inlet and FID (flame ionization detector, part of 7890A chromatograph) temperature 290°C; MS transfer line temperature 275°C; quadrupole temperature 150°C; ion source temperature 230°C; EI 70 eV; *m/z* range 33-500; FID gas (H_2) flow 30 $mL \cdot min^{-1}$ (hydrogen generator HydroGen PH300, Peak Scientific, Inchinnan, UK); and air flow 400 $mL \cdot min^{-1}$. Individual compounds were identified by comparing their mass spectra with spectral libraries (Wiley 9th ED. and NIST 2008 Lib. SW Version 2010), previously reported data and the results of earlier experiments, as well as by comparison of their retention times and corresponding mass spectra with those of authentic standards, where available. Quantitation was conducted with a FID detector and performed using an external standard method based on calibration curves determined for authentic standards of ursolic acid methyl ester, α-amyrin and stigmasterol (Rogowska *et al*. 2022).

RESULTS AND DISCUSSION

The diethyl ether extracts were obtained from the dried plant material with yield ranging from 2% to 4%, depending on the plant part (the highest yield, 4%, was obtained from the flowers; 2% from the stems; and 2.4% from the leaves). GC-MS analysis revealed the presence of triterpenoids belonging to three of the most common carbon skeleton groups found in higher plants, i.e., lupane-, oleanane- and ursane-type: lupeol acetate, β-amyrin and oleanolic acid, as well as α-amyrin accompanied by its ketone, α-amyrenone. Both amyrins were also found in the ester fraction analyzed after alkaline hydrolysis. The fraction of steroids occurring in a free form was composed only of one sterol, identified as belonging to a group of Δ^7-sterols, spinasterol (stigmasta-7,22-dien-3-ol) and one steroid ketone, tremulone (stigmasta-3,5-dien-7-one). Spinasterol was also identified in the ester fraction analyzed after hydrolysis.

The quantitative determination of identified compounds is presented in Table 1. The amount of triterpenoids occurring both in free and esterified forms was the highest in the extract of *S. canadensis* flowers (reaching 1.2 mg/g of dry weight), whereas the contents in leaves (0.91 mg/g d.w.) and inflorescence stems (0.69 mg/g d.w.) were lower. The predominating compound in all analyzed extracts was α-amyrin, constituting more than 50% of analyzed triterpenoids (with the highest proportion in the flower extract, reaching almost 60%). Free forms of triterpenoids were more abundant than their ester forms, approx. 8-fold in the flower and leaf extracts, and even more, 12-fold, in the inflorescence stem extract.

The content of a free form of spinasterol was relatively low in all analyzed samples, the highest amount was found in the flower extract (approx. 0.2 mg/g d.w.). Sterols are important constituents of plant cell membranes, regulating their fluidity and permeability (Rogowska and Szakiel 2020), therefore, such low content of a free form of spinasterol could suggest more abundant occurrence of sterol conjugated forms. Indeed, significant amounts of spinasterol released after alkaline hydrolysis of the ester fractions were found in all analyzed extracts. This might suggest the occurrence of sterols also in other forms, mainly as glycosides, however, due to their higher polarity, these forms could not be extracted by diethyl ether and therefore they were not present in extracts obtained in the present study. Regarding the results of other reports (Janson *et al*. 2009), the sterol conjugates seemed to be the prevailing form of these compounds in Solidago species, e.g., *S. altissima*, where the sterol esters and glycosides constituted 85% of the total sterol profile. The present study on *S. canadensis* revealed clearly only the presence of spinasterol, however, it cannot be ruled out that the other related compounds (as 22-dihydrospinasterol or other Δ^5 and Δ^7-sterols, reported for leaf extracts of *S. altissima*) were also present in analyzed samples, however, in very small amounts below the limit of MS identification.

Table 1. The content of free and ester forms of triterpenoids and steroids in extracts obtained from various parts of *S. canadensis* aerial part: leaves, flowers, and the inflorescence stems.

Compound	Plant part		
	leaves	flowers	inflorescence stems
	Content (µg/g d.w.)		
Triterpenoids			
free forms:			
α-amyrin	434.77	640.98	322.86
α-amyrenone	66.40	87.62	7.61
β-amyrin	229.68	240.7	270.14
lupeol acetate	16.41	112.54	29.87
oleanolic acid	66.52	14.91	6.01
Sum of free forms	*813.78*	*1096.75*	*636.49*
ester forms:			
α-amyrin	70.05	105.65	32.94
β-amyrin	32.51	48.78	18.07
Sum of ester forms	*102.56*	*154.43*	*51.01*
Steroids			
free forms			
spinasterol	69.90	214.57	12.91
tremulone	59.20	108.43	35.44
Sum of free forms	*129.10*	*323.0*	*48.35*
ester forms:			
spinasterol	114.64	340.24	42.93

The phytochemical characteristics of many alien invasive species have not yet been fully studied in terms of bioeconomy. The preliminary studies on *S. canadensis* demonstrated that this plant can constitute a valuable raw material for many sectors of the industry, with the possibility of its wider applications in the future as a new source of functional ingredients for food, nutraceuticals, cosmeteuticals, and medicines (Mietlińska *et al*. 2019, Shelepova *et al*. 2019). Other studies pointed to this plant as potentially valuable as a bioaccumulator and phytoremediator of heavy metals (Bielecka and Królak 2019). Any of the potential application can be helpful for the balanced and optimized management proposal, involving not just the trials of mechanical elimination but the simultaneous practical utilization of obtained *S. canadensis* biomass (Zihare and Blumberga 2017, Baranová *et al*. 2022).

CONCLUSIONS

This report supplements the phytochemical characteristics of an invasive plant *S. canadensis* as a potential source of bioactive ingredients, demonstrating the occurrence of significant amounts of triterpenoid alcohols, α- and β-amyrins, particularly in the flower extract.

ACKNOWLEDGEMENTS

The study was performed during the internship of Coralie Fourcade in the Department of Plant Biochemistry, Faculty of Biology, University of Warsaw, Poland, funded by SIGMA-Clermont. Analyses were carried out with the use of CePT infrastructure financed by the European Union—the European Regional Development Fund (Agreement POIG.02.02.00-14-024/08-00).

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Review paper
10.7251/AGRENG2202012T
UDC 338.43 (437.3)

PEASANT AND NATURE AT THE ONSET OF MODERN FORMS OF FARMING IN CZECH LANDS

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ABSTRACT

Traditional farming in the 19th century is often perceived as an era of farmers who live in harmony with nature. The research questions were: What forms of approach to nature can be identified in the period of the onset of modern forms of farming in the Czech lands? how is this approach interpreted? And how was it shaped? Farming textbooks issued between the years 1820 - 1914 were used for the content analysis. The categories were identified: a) Systematization, calculation, rationalization; b) Modern practices as a yield guarantee; c) Fertilizers - necessity for high production; d) An animal like a machine; e) Machinery as a means of perfect work; f) Science as a higher authority; g) Agriculture as the basis for the welfare of the nation; h) Nature as a subject of adjustment. The partial approaches can be summarized into one. This is the perception of nature as a machine. The approach to nature as an environment of production can be interpreted in the form of modern practices, which began to be used to achieve the highest yields. Implementation of such practices required extensive landscaping and regulation of water elements in the landscape. Understanding nature as the environment of production was formed mainly as a result of abandoning traditional values as they were replaced by science and industry.

Keywords: *Traditional farming, content analysis, Czech Republic, peasant, modernity.*

INTRODUCTION

As stated by Almstedt et al. (2014): "Globalisation, increasing competition on the world market, increased environmental awareness, and the orientation towards the service sectors in Western economies has dramatically altered the preconditions for development, not only in urban centres but also in rural areas. Furthermore, despite popular perceptions, the consumption and provision of rural products and services has increased with demand mainly coming from urban areas. A countryside dominated by traditional occupations in agriculture separated from urban life is now regarded as 'a rural myth'". At the same time there is enough evidence of the negative environmental impact of modern industrial agriculture (Kimbrell, 2002;

Horrigan et al., 2002). This discrepancy between the perception of the countryside as an "intact environment" and yet the documented deterioration of the environment leads us to search for its roots.
The work of the peasant in the 19th century is often idealized. He is imagined as a farmer who cultivates the land without the use of modern technologies, by hand and with the help of animals. When a general public thinks about the time, they tend to see farmers who live in harmony with nature and do not significantly affect the environment. As early as the end of the 18th century, however, a modern transformation of agriculture began and resulted in far-reaching environmental consequences. Nowadays in the Czech lands, the attention is mostly paid to the changes in the post-war years (after 1945), when a collectivization in agriculture associated with land consolidation and the expansion of the application of artificial fertilizers. However, these and many other changes have taken place more than 100 years ago and so far they were rather in the shadow of the above-mentioned event.

Industrialization in agriculture is a long-term process, still ongoing, when agrarian society changes into a modern industrial and consumer society. Population predominantly living in rural areas and subsisting on agriculture is disappearing and being replaced by urban population working mainly in industry and services. Industrialization process began in the 18th century in northwestern Europe (Jindra et al. 2015).
According to Šindlářová (1997), consequences of industrializations are as follows:

- Positives of industrialization: increasing labor productivity, improving farmers' skills in working with machinery, elimination of much of the simple manual labor, creating conditions for comparable working and living conditions for farmers, in general, greater openness and the possibility of mobility for technically qualified workers.
- Negatives of industrialization: investment intensity, consequences for the soil, water resources and the whole agricultural landscape and the environment, creation of types of industrial monotonous and tiring work.

From the end of the 1840s, agriculture began to gain momentum rapidly in the Czech lands. This situation was caused by the abolition of feudal wages and forced labor, but above all by introduction of new production processes and dissemination of expertise. However, development did not happen without consequences. Capitalist agriculture had faced economic crises from overproduction (Kubačák, 1994). The great agricultural depression after 1878 was preceded by an industrial, banking and commercial crisis leading to the Vienna stock market crash of 1873 (Lom, 1979). This crisis is considered to be the most serious the economy has ever experienced (Beranová and Kubačák, 2010). The development of long-distance trade had also a great influence on trade with agricultural goods, which was supported by new transport technology (Petráň and Patráňová, 2000).
The greatest development of agricultural production took place in the years 1845-1880, mainly in technology. This was due to rising prices for agricultural products

and especially by the development of agricultural sciences (Lom, 1930). From the end of the 18th century until 1850,
the domain of agriculture was crop production, especially the cultivation of cereals. Livestock, especially cattle, served as a means to the greatest cereal production (Beran, 1978).

All major changes in agriculture (sowing practices, intensive cultivation, fertilization with industrial fertilizers, drainage, new agricultural technology, newly bred crop varieties and animal breeds) first took place on large farms (Beranová and Kubačák, 2010). The economic and technical rise took place unevenly. According to Lom (1979), there were differences *"in soil fertility and the advantage of economic conditions, capital the strength of agricultural holdings, in principle to meet the needs of the family from agricultural production and in skills of farmers"*. At first, farms remained behind, mainly due to small land area and land fragmentation and did not directly participate in total production. It was not until the end of the 19th century that peasants, thanks to agricultural education and cooperative self-help began to balance large farms (Beranová and Kubačák, 2010; Lom, 1979).

MATERIALS AND METHODS

The goals of the research and the research questions were: What forms of approach to nature can be identified in the period of the onset of modern forms of farming in the Czech lands? How is this approach interpreted? And how was it shaped? The research consists of an analysis of selected agricultural texts from the 19th century. The analysis was performed by a qualitative method – a text analysis. The research sample consists of texts with an agricultural theme published between 1820 and 1914. The research sample includes the following documents:

Červený, A. (1871), Horský, F. (1861, 1863, 1872), Jettmar, J. (1887), Prokůpek, J. A. (1899), Richter, J. (1864). Zeithamer, L. M. (1874).

RESULTS AND DISCUSSION

Based on the content analysis, the following main categories were identified: a) Systematization, calculation, rationalization; b) Modern practices as a yield guarantee; c) Fertilizers - necessity for high production; d) An animal like a machine; e) Machinery as a means of perfect work; f) Science as a higher authority; g) Agriculture as the basis for the welfare of the nation; h) Nature as a subject of adjustment.

Systematization, calculation, rationalization - in the analysed texts, emphasis is placed on the organization of the economy associated with the introduction of modern practices. According to the authors of the texts, this is the only way to achieve required yield. The belief in the need for the system is given in a way that leaves no room for possible doubts. According to Horský (1863), the farmer becomes a farmer only by introducing a system: *"I believe that only a system makes the peasant a farmer"*. It is a system that gives the impression of uniformity if everyone manages in the same way, texts encourage their readers. The monotony is manifested not only in the very activity of the peasants, but also in nature. This is mainly due to the introduction of new technologies that turn away users from creative activity and allow control over nature. A transformation of peasant into a farmer evokes a transition from the traditional concept of farming into the modern one. Traditionally, agriculture has provided a livelihood for a small circle of people. With the advent of modernity, emphasis began to be placed on the greatest possible production and economic effectivity.

Modern practices as a yield guarantee - a fundamental change towards a modern way of farming was the transition towards the crops ration. This is discussed in all researched texts and it is considered the basis for progress in agriculture: "*introduction of crop rotation, together with a proper farm management result in progress*" (Horský, 1872). Thus, the crop rotation can be considered as a stepping stone in the direction to the perfect and intensive use of land that farmers seek. Of course, it leads to high returns and securing a steady income. Therefore, nature is seen clearly as an environment that provides production, profit and money. From the contemporary environmental view, it is interesting how introducing crop rotations led to the conversion of pastures into fields, thus gaining another space for growing crops and therefore additional yield. The disappearance of cattle grazing also has another dimension, which was mentioned only in one of the texts, but it seems important to mention it and it is also connected with others emerging trends. Namely, because children cannot go to school due to cattle grazing: "*It is cattle grazing, which prevents many children from attending schools, feeding cows in stables is possible due to crop rotations* "(Horský, 1861: 33). As a result, farming will allow children to go to school, thus breaking down an obstacle to the education, which in turn is essential for agricultural progress and higher production.

Fertilizers - necessity for high production – a farmer would not consider artificial fertilizers necessary. He only got along well with the application manure. On the contrary, buying fertilizers meant additional costs for him. But in researched texts there is a constant idea that with the use of fertilisers it is possible to raise to the highest possible degree of profitability. Until the full potential of soil is reached, it is possible to increase the benefit. Man-made fertilizers make it possible to exceed soil fertility limits and nature thus again finds itself in the role of an artificial device, which is to ensure only and only production. According to Prokůpek (1899), manure was sufficient only when a fallow farming was practiced: "*Barn manure was enough at a time when 1/3 of the land was left fallow every third year lying and for two years the field was just sown…* ". Here again it may be seen how individual changes in agriculture are related. Introduction of crop rotation required more intensive fertilization, which only manure could not provide. Use of fertilizers thus go hand in hand with the expansion of crop rotations.

An animal like a machine - in the texts, cattle breeding is mainly reduced to the issue of feeding in which they prevail certain paradoxes. On the one hand, the animal is placed in a position of importance and care is taken about his natural demands. On the other hand, it sounds like it is considered inferior and serves as a "*money factory".* It is often stated, that a peasant held more cattle to bring the desired benefit, but at the same time did not provide the animal sufficient supply of new energy. The cattle suffered over the winter, the peasant did not take care of it and often he did not notice animals until the spring, when they were needed to work. Cattle was treated like a tool that puts off after work is completed. A typical modern approach, in this case

however, it seems to be based on the needs of the farmer himself and not from needs of society (for the market). This approach is often criticised in the researched texts, many improvements are presented in animal nutrition, housing, veterinary care. An example – "*In winter, let's often put pork cold wash with water, then bathe in the summer, which is good for him*". Such approach does not comply with modernity.

Machinery as a means of perfect work - agricultural machines and implements are presented in texts as means for a perfect job. This is essential for emerging intensive agriculture. Every farm should be equipped with the technology to ensure the highest possible yield. The peasant turns into a robot by working with the machine, and nature is subdued by perfection. "*Time is money and money is the nerve of the state organism*" (Horský, 1861) - the quote says "time is money", this is a typical modern approach. It cares about speed and maximum performance and does not look at impacts. The comparison of money to the nerve and the state to the organism is also of interest. Money, like the state, is an institution created by humans and spoken of as living. While it is nature that really is living, in researched texts is understood as a machine, an artificial device. In pursuit of profit, traditional values have completely reversed and nature is definitely not doing well.

Science as a higher authority - The modern transformation of agriculture would not be possible without scientific knowledge. The peasant is encouraged to follow science and use it to his advantage: *"from a Man of Progress it is required that such a man must be in charge of science and experience for his own profit to a reasonable extent…*". There is even a peasant here marked as a man of progress. Its role thus goes beyond agriculture. On him as if all development depended. The peasant is the one who is to make progress. As if he was expected to be a role model for others and to give them proper results.

Agriculture as the basis for the welfare of the nation - the task of a peasant is to provide food for all mankind, and therefore the economy is necessary amended to achieve this. As stated above by Horský text (1863), a farmer draws crops from the ground. It is therefore necessary to feed humanity. Of course, the peasant with his work he procures that food. But no matter how noble his work is, he will still not get without land nothing. Rather, the nobility should be geared towards land management practices. Although new technologies in agriculture have guaranteed higher production, they are environmentally friendly far. Attention is focused on people's satisfaction, the good of nature is in the background of interest. Traditionally, the peasant took care of supplying his own large family, or exchanged his own products with locals. Along with the advent of modernity, however, came the demand to produce for market and sell for money. There is a need to provide food for a growing number of residents. Agriculture thus became the basis for the welfare of the nation. The peasantry guarantees security of the state. Inexhaustibility is even considered as resulting from agriculture: "*Agricultural empires are opening up everywhere, our gold mines are barely exhaustible*" (Komers, 1861). Nature, however it is definitely not inexhaustible. And since it is central to production, it cannot be dispensed with. But it is treated as if it were. When the soil no longer provides the expected output, measures are taken to return it to form and harvest. Modern forms of farming leave far-reaching consequences for nature, but they allow the people to live better and this is considered essential.

Nature as a subject of adjustment - Land reclamation, land consolidation and others are a relatively large topic in the texts. Drainage or irrigation have become one of the main tasks to increase yields: "*Ingenious use, distribution and management of both the superior and groundwater for irrigating dry and drying wet lands matters much…"* (Zeithamer, 1874). In addition to land reclamation and land consolidation, meadow management and regulation of watercourses occur as text topics. It is advised that adjustments of meadows and streams were made as easy as possible and possible damage was prevented. In the meadows no inequality or barrier is tolerated, otherwise the return from them will not be satisfactory.

Let's now look at how nature as an environment for production is approached and how it is interpreted. Generally speaking, it is portrayed through newly introduced practices in agriculture. These modern forms are most evident in contemporary texts farming: crop rotations, deep ploughing, application of fertilizers, stable animal husbandry associated with the extinction of pastures, the use of agricultural machinery and land reclamation. All these methods are interconnected and cause other necessary changes. These include land consolidation, meadow landscaping and watercourse regulation, and conversion of pastures and other crops in the field. For the farmer, the intensification of agriculture will make work easier, it will reduce costs, help to achieve perfect management, but above all it will increase production. This is stated in the texts as the main goal to which every peasant should aim. Modern production technologies in agriculture are to meet the ever-increasing demands population and thus improve their lives. We are thus touching the theory of progress, when it was believed that development will bring benefits. The researched texts also present the belief that the new management system guarantees satisfaction and well-being for all. Progress linked to modernity however, guarantees instead a rather ruined environment, as the analysis has shown and how Wuketits (2006), Loewenstein (2006) or Pepper (1996) speak for example. The last point of the research question is how the approach to nature as an environment for production was formed. At the very beginning, one can see a departure from tradition. It happened especially due to the development of science and industry linked to market production. Traditional wisdom of the elders was replaced by science, which with its knowledge provided the basis for intensification of agriculture. In the texts, there is a direct appeal to abandon old (traditional) practices with which higher production cannot be achieved. With science the education is interconnected that every peasant should attain to a sufficient degree to be able to rationalize its management and thus increase revenues. Rationalization with science and subsequent growth are signs of modernity. It is not left for tradition space, as well as not for nature.

CONCLUSION

The expansion of the industry in the 19th century required new raw materials provided by agriculture and increasing cultivation of industrial crops. Peasants in this sector saw secured sales, and so they followed these demands. Changes took place throughout the whole economy. Not only the peasant but also the whole nation benefited from the production. The industry, for which sufficient raw materials were secured, could then earn more profit. According to researched texts, the well-being of the whole nation is based on a peasant effort, he has to secure it through sufficient income. The basis was to produce as much as possible for as many people as possible. Peasant newly began to be driven by market demand, not household or community needs. Traditional self-sufficiency has given way to a modern focus on performance in an effort to be competitive. Following the thesis of classical economists of the 18th century, this situation could be understood as a transition to the desire to satisfy everyone's needs as much as possible. Regardless the consequences. From the analysis of contemporary texts and its confrontation with theoretical concepts of modernity, tradition and progress, it may be concluded that the relationship of the peasant to nature at the time of onset of modern forms of farming has been determined primarily by an emphasis on utility and potential production. Emancipation from traditional beliefs has also caused disconnection from nature as living entity. Within the established modern methods in agriculture, nature is understood as a machine that offers endless possibilities of use.

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Original Scientific paper
10.7251/AGRENG2202020M
UDC 633:631.67(510)

ASSESSMENT OF REGIONAL IRRIGATION WATER REQUIREMENTS AND ACTUAL SUPPLY IN BAODING, CHINA

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ABSTRACT

Nowadays, water resources scarcity has become a severe issue that interferes with economic, social, and ecological development. As the most significant use of water resources in agriculture, irrigation plays a crucial role in ensuring agricultural production when rainfall is insufficient to maintain the growth of crops. In this paper, estimation of water resources in irrigation practices was carried out in Baoding since the area suffers from water scarcity, and the situation there is worsening, especially after the founding of Xiongan New District. This study aimed to evaluate the supply and demand balance of irrigation at the municipal and county levels through the climate information and crop pattern summarization in Baoding. Data provided by Agricultural Machinery Bureau was integrated with CLIMWAT 2.0 to compute crop water requirements, irrigation requirements, and irrigation schemes in CROPWAT 8.0. Results show that an estimated sum of 1881.3 mm annual crop water requirements comprising the vast majority of crops in agricultural production led to 1493.5 million m^3 irrigation requirements, creating an irrigation deficit of 119.2 million m^3. The greater cutdown on annual water supply compared to the irrigation requirements led to a consecutive exacerbation of annual irrigation deficits. Suggestions, including physical and political measures, were provided to improve the current situation in Baoding.

Keywords: *Crop water requirements, Irrigation requirements, CROPWAT 8.0, Baoding.*

INTRODUCTION

Water is a precious natural resource for ecology and the environment, and it plays an irreplaceable role in economic and social development. Due to climate change, it aggravates existing pressures on water-stressed regions and intensifies the competition between the ecosystem and human beings. As the targeted area, Baoding in Hebei Province has experienced rapid social and economic developments under the Beijing-Tianjin-Hebei synergetic strategy, which aims to alleviate government functions unrelated to the capital city Beijing and enhance adaptabilities among socio-economic development, population resources, and environment (Fang et al., 2018; Chu et al., 2018). Moreover, Xiongan New District formed inside Baoding is conspicuous and has boosted ecological development since 2017 (Xiongan, 2022). Meanwhile, agricultural production is crucial in Baoding since it plays an essential role in maintaining the local grain supply and the supply of areas under the synergetic strategy. Baoding has recently experienced industrial transformation and improvement by shifting its singular agricultural production pattern to the coordinated development between agriculture and industry. The newly established Xiongan District also brings the demand for ecosystem sustainability (Liu et al., 2020). Hence, it requires Baoding to rationally allocate water resources with a diminishing total annual supply. As a principal means, irrigation ensures sufficient water is supplied to crops, which is the primary source of water resource consumption in agricultural production. Further, water scarcity and unfavourable climate conditions intensify the pressure on the irrigation system. An inefficient management system will cause issues in irrigation, negatively influencing the sustainable development of water resources.

Studies were carried out to assess the crop water requirements (CWR), irrigation performance, and management to improve the current situation in Baoding. Yang et al. confirmed that the piedmont region has serious water resource sustainability problems as agricultural production intensifies and groundwater tables in Hebei Province decline (Yang et al., 2014). Feng et al. estimated that CWR for maize was 445.3mm in Hebei Province, and two to three times irrigation should be scheduled for maize cultivation (Feng et al., 2007). Wang et al. analyzed ETc (Crop Evapotranspiration) of wheat and the impact of water consumption under the shrunken wheat cultivated areas (Wang et al., 2014). Yang et al. found that the plain region of Baoding has the highest irrigation requirements, where wheat, maize, and cotton altogether account for 64% of total irrigation requirements in Hebei Province (Yang et al., 2010).

In this study, besides all previous research on Hebei province, the assessment was provided by adopting CROPWAT 8.0 to evaluate the sum of CWR based on the crop pattern on a regional scale, instead of focusing on a particular crop in Baoding. Estimation of annual overall irrigation requirements and irrigation requirements of different counties were carried out to evaluate the supply and demand balance to develop suggestions for improvement of the current irrigation performance and management.

MATERIALS AND METHODS

Baoding is located in the temperate continental monsoon climate zone. It consists of 43% piedmont and 57% plain areas, covering a total area of 22,000 km^2 (BMPGO, 2022). The surface runoff mainly situated in the piedmont region makes the water supply scarcer and more competitive in the plain region (Yang et al., 2010; Yang et al., 2014). The inbound water diversion project plays an essential role in maintaining the ecosystem and sustainability of surface water resources, which took over 61% of the total surface water supply in 2019 from 43% in 2010 (BWCB, 2022; BWRER, 2006). In Hebei province, agricultural irrigation primarily depends on groundwater extraction, and the groundwater consumption in Baoding exceeds the provincial and national averages, which are 84.8% and 30.1%, respectively (GMIA, 2013). Despite the fact that the government has emphasized the severity of groundwater overexploitation and implemented strict regulations to supervise the groundwater resources, groundwater still has been persistently overexploited, resulting in a consecutively deteriorated average buried depth of 26.41m (BWRER, 2006).

The crop pattern refers to the spatial distribution of various crops during a year, and agricultural production is generally categorized as grains and cash crops in Baoding (BMPGO, 2022). Grains, including maize and wheat, take up the majority of total agricultural production, followed by vegetables, oil plants, and fruits. The rest cultivars(others) consisting of a fraction of millets from grains and cotton from cash crops are considered within the estimation of CWR.

The total cultivated area and the area equipped for irrigation have simultaneously shrunk due to urbanization and industrial development over the past decades (BWCB, 2022; BWRER, 2006). It is expected to decrease further when the local authority continues promoting the "Returning Farmland to Forests" strategy and sustainable ecological development. Moreover, the available water resources supplied to irrigation were gradually reduced mainly due to the water competition between different industries. As a result, the corresponding consequence was reflected in the proportion of the area actually irrigated reduced from 95.4% to 85.6% in Baoding, which was close to the national average of 85.2% in 2020 (GMIA, 2013).

CROPWAT 8.0 is a computer-based program developed by FAO, which calculates CWR, develops irrigation schedules for individual conditions, and optimizes water supply schemes according to various crop patterns. Moreover, irrigation practices and crop performance can be evaluated through the program (FAO, 2022). This study adopted the data collected from the Agricultural Machinery Bureau and climate data used in CLIMWAT 2.0 to analyze the climate condition in Baoding. Both sources captured a series of monthly data with more than 15 years of observation. The reference evapotranspiration and effective rainfall used for estimations were obtained from Agricultural Machinery Bureau. It consists of a series of data provided by 17 meteorological and hydrological stations distributed over the entire Baoding. The reference evapotranspiration was collected through the φ20 type pan, which was used at most meteorological and hydrological stations in China over the last century (Yu et al., 2017). Subsequently, it converted to the values of E-601 type pan with a coefficient of 0.65 for computations in CROPWAT 8.0 (BWRER, 2006). Values of the E-601 type pan show a relatively stable and acceptable systematic deviation from the Penman-monteith method estimate, compared to the φ20 type pan (Chen et al., 2005).

The precipitation distributes unevenly during a year from the perspective of spatial-temporal distribution. Its piedmont region has higher precipitation than the plain region, which creates a significant uneven spatial distribution of precipitation (BWCB, 2022). The precipitation intensifies between June and September (flood season) with a monthly average of 97mm, and lessens from December to March of the following year with an average slight amount of 5 mm monthly. Effective rainfall is primarily affected by the climate, soil texture, structure, and root zone depth (Brouwer et al., 1986). The FAO/AGLW formula below (1) and (2) was used to determine the effective rainfall:

$$Peff = 0.6 * P - 10 \quad \text{for } Pmonth <= 70 \text{ mm} \qquad (1)$$

$$Peff = 0.8 * P - 24 \quad \text{for } Pmonth > 70 \text{ mm} \qquad (2)$$

In Baoding, agricultural production, especially irrigation projects, mainly occurs in the plain region. The plain region is combined with alluvial fan plains and flood plains, where the brown earth and the fluvo-aquic soil are formed (DARA, 2022). The soil texture in most areas is classified as loam, consisting of very deep, well-drained, and moderately permeable soils on alluvial fans. Loam accounts for 51% of the entire plain region in Hebei Plain (Zhang et al., 2017). CROPWAT 8.0 soil file has stored default values on heavy (clay), light (sand) and medium (loam) soil in the model, and medium (loam) soil was applied to this study.

Crop factors (Kc) values provided by FAO were adopted for CWR estimation. When dealing with comprehensive irrigation planning and management on a city-level scale, a constant and averaged coefficient can be applied, which is more flexible than capturing Kc daily by selecting various crops and soil coefficients. When summarizing various crops in specific fields for a period of time, a combination of transpiration and evaporation coefficients should be considered (Allen, Richard G. et al., 2000). The crop factor mainly depends on the crop type, the growth stage of the crop, and the climate. It may vary during different growth stages of a single crop (Brisson, Nadine, et al., 1998). The total growing period covers the days from sowing to the last day of harvesting. In other words, the growing period primarily depends on local circumstances (Ko, Jonghan, et al., 2009). The total growing period can be divided into four growth stages: the initial stage, the crop development stage, the mid-season stage, and the late-season stage. In general, crops require less water during the initial and late stages. The water demand will continuously increase during the crop development stage until it reaches its peak point, which roughly uses three times the water than the initial stage (Cakir, 2004; Allen, Richard G., et al., 2000). Overall, if effective rainfall is inadequate to cover the water demand of the crops during any growth stage, irrigation is inevitable under such circumstances.

RESULT AND DISCUSSION

CWR, irrigation requirements, and irrigation schemes are computed according to climate, soil, and crop data on a monthly basis from 2015 to 2019 in Baoding. CWR of each crop was calculated based on four growth stages of crops. The CWR was obtained by subtracting the effective rainfall amount from ETc.

Table 1. Estimated Annual CWR, ETc, Effective Rainfall (ER) Based on the Crop Pattern in Baoding

		Oct	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Total**
Wheat	Stage	Int	Dev	Dev	Dev	Dev	Mid	Lat	Lat	Lat				
	ETc	45	31.2	24.7	30	50	108.4	163	117.1	6.4				575.8
	ER	3.7	0.1	0	0	0	0.1	5	9.8	2.5				21.2
	CWR	41.7	31	24.7	30	50	108.2	157.9	107.3	3.2				554
Maize	Stage									It&Dv	Mid	Lat	Lat	
	ETc									27.8	161.4	124.8	48.5	362.5
	ER									21.4	103.5	59.2	22.5	206.6
	CWR									7.1	58.1	65.6	24.3	155.1
Others	Stage								Int	Dv&Md	Mid	Mid	Lat	
	ETc								50	136.2	154.6	127.3	77.7	559
	ER								9.8	30.8	103.5	59.2	24.1	230.9
	CWR								40.2	105.3	51.3	68.1	53.4	328.2
Vegetables	Stage					Int	Dev	Mid	Lat					
	ETc					31.4	89.9	145.6	29.9					296.8
	ER					0	0.1	5	1.3					6.4
	CWR					31.4	89.8	140.6	28.8					290.6
Oil Plants	Stage								Int	Dev	Mid	Md&Lt	Lat	
	ETc								65.6	145.8	159	118.3	15.6	504.3
	ER								9.8	30.8	103.5	59.2	8	211.3
	CWR								55.8	114.9	55.5	59.1	4.2	289.5
Fruits	Stage								Int	Dev	Mid	Lat		
	ETc								80.7	142.3	145.8	98.4		467.2
	ER								9.8	30.8	103.5	55		199.1
	CWR								70.9	111.4	42.3	39.2		263.8

The results in table 1 showed that the highest CWR was estimated for growing wheat, followed by others (small grains), vegetables and oil plants, and growing maize has the least irrigation requirement. However, the total amount of irrigation applied to crops primarily depends on the estimated cultivated areas of individual crops. From the perspective of monthly CWR, the highest requirement occurred during the intensified crop production period from April to June. There were no production activities from November to January except for wheat production in Baoding.

The irrigation scheme can be calculated after importing data of the crop pattern into CROPWAT 8.0, as shown in Table 2.

Table 2. Estimation of Irrigation Scheme and Total Irrigation Requirements Based on the Crop Pattern and Irrigated Areas in Baoding

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Precipitation Deficit												
Wheat	30	50	108.3	157.9	107.3	3.2	0	0	0	41.7	31.1	24.7
Maize	0	0	0	0	0	7.1	58	65.6	24.3	0	0	0
Others	0	0	0	0	40.2	105.3	51.2	68.1	53.4	9.9	0	0
Vegetables	0	31.4	89.8	140.6	28.8	0	0	0	0	0	0	0
Oil Plants	0	0	0	0	55.8	115	55.5	59.1	4.2	0	0	0
Fruits	0	0	0	0	70.9	111.4	42.3	39.2	0	0	0	0

Total	30	81.4	198.1	298.5	303	342	207	232	81.9	51.6	31.1	24.7
Net scheme irr. req. in												
mm/day	0.3	0.7	1.4	2.1	1.3	0.5	1.1	1.2	0.4	0.4	0.3	0.2
mm/month	9.3	18.6	42.5	63	41	14.7	33.8	38.1	13.5	13.1	9.6	7.6
l/s/h	0.03	0.08	0.16	0.24	0.15	0.06	0.13	0.14	0.05	0.05	0.04	0.03
Irrigated area (% of total area)	31	41	41	41	50	90	59	59	58	33	31	31
Irr.req. for actual area (l/s/h)	0.11	0.19	0.39	0.59	0.31	0.06	0.21	0.24	0.09	0.15	0.12	0.09
million m^3	44.09	100.72	206.74	312.76	200.41	69.82	160.20	183.08	67.49	64.00	48.10	36.07
Total												1493.48
Average Annual Actual Supply												1374.33
Irrigation Deficit in million^3												-119.15

Table 2 indicates that the irrigation requirements reached the highest amount in March, April. and May. In June, the total irrigated area reached the maximum during a year, which covered 90% of the total area equipped for irrigation in Baoding. The total irrigation requirements can be obtained by multiplying the irrigation requirements with the irrigated area of each month. The result showed that the maximum amount of irrigation was required in April, while the minimum amount of irrigation was required in December. Given the fact that the average annual actual water supply was 1374.33 million m^3, which resulted in an annual irrigation average deficit of 119.15 million m^3 in Baoding.

Similarly, estimations of irrigation requirements were repeated based on a regional scale, including all counties and districts in Baoding from 2015 to 2019 (Table 3).

Table 3. Supply and Demand Balance in All Counties and Districts in Baoding

	2015			**2016**			**2017**			**2018**			**2019**		
	Irr. Req.	**Actual Supply**	+/-	**Irr. Req.**	**Actual Supply**	+/-	**Irr. Req.**	**Actual Supply**	+/-	**Irr. Req.**	**Actual Supply**	+/-	**Irr. Req.**	**Actual Supply**	+/-
Urban	367	391	24	371	371	0	380	345	35	380	304	76	373	273	100
Laishui	28	73	46	26	68	43	25	68	42	24	43	19	23	34	11
Fuping	25	15	10	26	15	11	24	15	9	24	24	0	24	21	3
Dingxing	154	162	9	154	154	0	154	143	10	154	126	28	154	90	64
Tang	65	57	8	65	52	13	58	38	19	60	92	33	60	59	1
Gaoyang	89	64	25	89	64	25	91	64	27	81	56	25	81	43	38
Laiyuan	10	9	1	10	9	2	9	8	1	9	7	2	9	11	1
Wangdu	79	82	3	81	78	3	81	73	8	80	64	16	80	64	16
Yi	67	54	13	63	48	15	64	47	17	61	42	20	61	57	5
Quyang	51	73	23	51	69	19	49	64	16	47	57	10	48	56	8
Li	118	72	46	118	68	50	103	63	40	113	56	57	114	56	58
Shunping	64	107	44	63	102	38	66	95	28	66	83	17	65	67	1
Boye	76	54	22	76	55	21	78	55	23	78	41	37	78	37	41
Zhuozhou	97	137	40	100	130	31	101	121	20	101	107	6	99	90	9
Anguo	112	119	7	113	113	0	112	105	7	112	92	20	112	82	30
Gaobeidian	95	113	18	97	104	6	99	86	13	99	83	16	100	81	19
Total	1496	1583	87	1503	1501	2	1493	1391	103	1489	1277	212	1481	1120	361

The result in Table 3 shows a decreasing trend in overall irrigation requirements, mainly due to the shrinkage of irrigated areas and the variation of the crop pattern in Baoding. Meanwhile, the intensive competition between agriculture, domestic needs, and industry, as well as the ecological restoration urgently needed in Xiongan New District, significantly reduced the annual actual water supply. The greater cutdown on annual water supply than irrigation requirements resulted in annual irrigation deficits since 2016, and the deficit rapidly increased to 361 million m^3 in 2019. The continuous increase in irrigation deficits led to 75% of the counties suffering from irrigation deficits in Baoding. In 2015, there were nine over-irrigated counties, resulting in a total amount of 87.22 million m^3 irrigation surplus, which covered more than 50% of administrative regions in BC. The irrigation requirements and actual annual supply tended to balance with only 2 million m^3 irrigation deficits in 2016. The continuous increase in irrigation deficits led to 75% of the counties suffering from irrigation deficits except for Laishui, Laiyuan, Quyang, and Shunping in 2019.

Under the pressure of water resources scarcity, the actual irrigation supply was insufficient to maintain the overall irrigation requirements. CWR and irrigation requirements maintain intense while conditions remain unchanged within crop pattern, irrigation performance and water resource management. The source of water supply predominantly relies on water diversion projects, while the inbound water resources are far insufficient for local development. Urbanization and industrial transformation have intensified the water competition when an increasing number of counties have become irrigation deficient since 2015. As the role player in the grain supply within the synergetic strategy, the irrigation deficit cannot be solved by simply reducing the total cultivation areas and shrinking the production in agriculture. The CWR differences among each month during cultivation and uneven supply distribution between each county have emphasized the importance of integrality and comprehensiveness in water resources management. Meanwhile, the situation will worsen because 1) groundwater resources covered nearly 89.7% of the total irrigation supply, and the number needs to be cut down to at least 30.1% in the future for the purpose of ecological restoration; 2) the reduced amount of water can not be obtained from surface water resources since it is already insufficient for agricultural production; 3) urbanization and industrial development will intensify the water resources competition. Therefore, the situation of irrigation must be urgently improved to solve water scarcity.

The improvement can be implemented through physical and political measures. From physical perspectives, 1) the inbound water diversion project, as the most reliable inbound surface runoff, has to ensure a sufficient amount of water resources continuously flow into Baoding; 2) the scale of the area equipped for irrigation can be reduced, which a limited amount of irrigation water can be rationally distributed to maintain the irrigation requirements for a reduced area, which will increase the production per ha; 3) the water-saving irrigation technology has to be extensively adopted to enhance the irrigation efficiency. From political perspectives, 1) the crop pattern should be adjusted at the municipal or county level to reduce the irrigation requirements; 2) a sound dispatching regime for irrigation water resources coordination among each county should be established to balance the supply and demand; 3) a critical standard that aims for water-saving in Baoding should be established for irrigation management; 4) awareness of saving water has to be improved on both management and operational levels.

CONCLUSIONS

Under the water scarcity conditions in Baoding, this study aims to obtain the irrigation requirements on both municipal and county levels via CROPWAT 8.0. An average annual irrigation deficit of 119.15 million m^3 was estimated. The greater cutdown on annual water supply compared to irrigation requirements led to a consecutive exacerbation of the annual irrigation deficit since 2016. Despite counties including Laishui, Laiyuan, Quyang, and Shunping showing a surplus in irrigation supply, the rest of the counties had a shortage of irrigation supply in 2019. To improve the current situation, securing inbound water diversion projects, reducing the area equipped for irrigation corresponded to the limited water resources, and promoting water-saving technology should be urgently implemented in the short term. In the meantime, adjusting the crop pattern, improving the dispatching regime among individual counties, creating critical standards on management, and strengthening the awareness of saving water should also be considered in the long term.

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Original Scientific paper
10.7251/AGRENG2202029K
UDC 633.63

THE PHYSIOLOGICAL AND BIOCHEMICAL ESTIMATION OF THE ADAPTIVE ABILITY OF SUGAR BEET (*BETA VULGARIS* L.) TO SHADING AND PLANT DENSITY

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ABSTRACT

The study of adaptive resistance to adverse environmental effects, which is connected with mechanisms of ontogenetic adaptation investigation, is an important direction of crop breeding. The adaptive resistance is identified on the phenotype level within the information stored and expressed by the genome. Ukrainian and Swedish lines, hybrids, and hybrid parent components of sugar beet under different shading conditions (30 and 60% of natural light) were investigated. Genotype-specific general physiological and biochemical features of the adaptive changes in leaves and productivity components in the course of metabolism are revealed. A significant decrease in the photosynthesis intensity and photochemical activity of chloroplasts occurred during the sugar beet plant adaptation to shading. The adaptive level of different sugar beet genotypes to low light was also expressed through the significant changes in the water-soluble carbohydrate pool of leaves themselves and leaf petioles. An important physiological parameter of sugar beet adaptive reaction is a response to shading in specific leaf weight (SLW), which is used in plant breeding as the trait of increased photosynthesis intensity. As a result of the study, it was found that the shading impacted significantly on the distribution and ratio of sucrose in the ring zones of vascular bundles and adjacent zones of the root storage parenchyma. The ontogenetic adaptation to photosynthetic active radiation light regime of photosynthetically active radiation (PAR) of sugar beet lines, hybrids, and their parent components of different origins under shading and different plant density in the field was shown. It was found that the stress intensity is a key characteristic of changes in physiological, biochemical, anatomical, and morphological traits of the leaf, which maintain plant homeostasis and ensure maximum efficiency of photosynthesis and productivity of different sugar beet genotypes under these conditions.

Keywords: *ontogenetic adaptation, sugar beet, physiological and biochemical features, adaptation.*

INTRODUCTION

Management of physiological processes, such as photosynthesis at different levels of its organization, optimization of source-sink relations (Kyrizii, 2015), light (Zhu, 2010) and dark (Golovko, 1998) respiration is the modern strategy of plant breeding (Stasik *et al*., 2016). One particularly important environmental cue with economic consequences, that is, early allocation versus later growth trade-offs, is shade. In dense plant canopies such as weedy crop fields, the plant environment is enriched with far-red (FR) light due to reflected FR by green vegetation. For example, the ratio of reflected red (R) to FR was 0.06 when sugar beet was surrounded by common lambsquarters (*Chenopodium album* L.) compared to 0.7 when surrounded by bare soil. With the aid of a family of photoreceptors, particularly cryptochromes and phytochromes, plants can sense and respond to changes in R:FR and blue-violet light in their surroundings. Therefore, a search for informative physiological and biochemical indicators associated with plant productivity in a wide range of growing conditions, which may be used as physiological and biochemical markers to increase the efficiency of the breeding process, is important. The new sugar beet hybrids contain sugar in the amount of 75-76% of dry matter, and a further increase in sugar content is an extremely labor-consuming process. It was found that the anatomical structure of the storage organ is the basis of the pattern of slowing down the rate of sucrose accumulation (Elliot *et al.*, 1996; Fasahat *et al*., 2018). Thus, to improve the existing breeding methodology for obtaining high-sugary breeding genotypes, it is necessary to study the relationship between the anatomical structure and the sugar accumulating capacity of the roots. An important direction in crop breeding is the study of the adaptive resistance of plants to the adverse impact of the environment, which is associated with the study of the mechanisms of ontogenetic adaptation, which is found at the phenotypic level within the information stored and implemented by the genome (Kyrychenko, 2002; Lv *et al.*, 2019; Ghaffari *et al*., 2021). These changes are considered a manifestation of the forms of reliability of biological systems (Grodzinskiy, 1983) from the standpoint of implementing their adaptive potential (Zhuchenko, 2008; Li *et al*., 2019) at the autologous, physiological, biochemical, genetic, and molecular levels. Studies on the influence of seasonal weather variability on sugar beet development recognized that amongst the different environmental variables, the amount of available light for the crop is a predominant factor driving the biomass accumulation after crop canopy closure (Artru *et al.*, 2018).

This study aimed to identify the most informative physiological and biochemical indicators in the formation of high productivity of sugar beet in connection with their resistance to shading and thickening.

MATERIALS AND METHODS

A diploid hybrid ‘Lgovsko-Verkhniatskyi ChS21’ (‘LV ChS21’) and its parent components, CMS line ‘Hill 13’ (Sweden) and simple interlinear sugar beet hybrids ‘SKF5050’, ‘SKF5084’, and ‘SKF4973’ were used. Plants were grown in soil culture in Wagner's vessels with a capacity of 14 kg of soil at 60% of full soil moisture. A part of 15-day-old plants was shaded with screens of gauze (2-3 layers) and white cloth for the entire growing season (140 days). Biological repetition was 20 plants. The decrease in natural illumination under the screens was 60% (treatment I) and 30% (treatment II). Plants that were grown in natural light served as control. Field two-factor experiments on shading and thickening were carried out at the Yaltushkiv Research Station of the National Academy of Sciences of Ukraine. The intensity of photosynthesis was recorded under controlled conditions using an installation mounted based on an optical-acoustic infrared gas analyzer GIAM-5M, set according to a differential scheme. The contents of chlorophylls *a*, *b*, and carotenoids were determined spectrophotometrically and calculated according to the formula (Musienko *et al.*, 2001). The photochemical activity (PCA) of chloroplasts was assessed spectrophotometrically by the reduction of potassium ferricyanide. The total content of albumins and globulins, protein nitrogen, and water-soluble carbohydrates was determined in accordance with Yermakov (1987), the content of sucrose by the method of cold digestion (Pochynok, 1976), the distribution of sucrose in the zones of the interring parenchyma and vascular bundles on the cross-section of roots in accordance with Okanenko (1968). All experiments were carried out at the experimental plots and laboratories of the National University of Life and Environmental Sciences of Ukraine (Kyiv, Ukraine) in the years 2015-2018. Statistical processing of the obtained experimental data was performed with the Excel Data Analysis package.

RESULTS AND DISCUSSION

As a result of studies of the sugar beet lines, hybrids, and their parent components of Ukrainian and Swedish origin under different shading conditions (30 and 60% of natural light) we found general physiological and biochemical features of adaptive changes in the leaf apparatus and productivity components as well as genotype-specific differences in the metabolic processes of plants. As the data in Tables 1 and 2 show, under shading conditions, there is a clear tendency of increasing the content of chlorophylls *a* and *b* compared to the control per specific leaf weight (SLW) unit by 10-50% in treatment I and 25-60% in treatment II). These parameters reproduce various aspects of the genetic determination of the optical system of a leaf (Lutkov, 1986; Zou *et al*., 2019). At the same time, shading affected the content of chlorophyll *b* more than chlorophyll a, which led to a decrease in the ratio of chlorophyll a/*b*, which is one of the most distinctive characteristics of the adaptation of plant photosynthetic apparatus to the light factor (Mokronosov, 1981). This is a sign of the acquisition of shadow endurance by the photosynthetic apparatus (Dymova, 1998; Holovko, 1998). A similar pattern of adaptation to shading has been described in the literature for sugar beet (Kyriziy, 2004; Artru *et al*., 2018; Zeng *et al*., 2022) and other crops (Guliyev, 1990).
In the process of adapting sugar beet plants to shading, there was a significant decrease in the intensity of photosynthesis and PCA of chloroplasts compared to the control. An important physiological parameter of adaptive reactions of sugar

beets to shading is the specific leaf weight (SLW) which is used in breeding as a sign of the increased intensity of photosynthesis (Criswell and Shibles,1971), the value of which naturally decreased in plants of all the studied genotypes along with the increasing stress factor (Tables 1, 2). In the whole plant system, SLW is associated with the level of the pool of assimilates and is an indicator of their utilization for the growth of leaves to form the assimilation surface (Tooming, 1984). From the data given in Tables 1, 2, it can be seen that the decrease in SLW in shading conditions is accompanied by an increase in the leaf area.

Table 1. Physiological and biochemical indices of leaf apparatus of plants of different genotypes of sugar beets in shading.

Hybrid, component	Treatment	Chlorophyll content (mg/g)		Ratio of chlorophylls a/b	PCA (µM [Fe (CN)6]3- /mg chl.·year)	SLW, g/dm^2	Water-soluble carbohydrates (% of dry weight)				Total albumins and globulins (% of dry weight)
							leaf blades		petioles		
		a	b				mono-sugar	total sugars	mono-sugar	total sugars	
75 days											
‘LV ChS21’	C** I II	0.98 1.11 1.14*	0.34 0.45 0.49	2.85 2.45 2.31*	53.4 50.3 47.5*	0.56 0.52 0.49	4.25 3.21 1.99*	9.29 7.54 5.62*	30.90 28.87 28.10*	37.84 35.15 34.40*	11.7 10.85 9.94*
CMS	C I II	1.09 1.25 1.36*	0.45 0.54 0.60	2.62 2.33 2.25*	42.8 40.3 37.9*	0.63 0.58 0.53	3.84 3.27 2.89*	7.13 5.96 4.39*	33.40 32.17 30.31	39.74 36.81* 33.65*	14.5 12.1 11.35
Multigerm	C I II	0.87 1.12 1.16	0.32 0.43 0.46	2.69 2.61 2.52	31.9 29.7 27.6*	0.69 0.63 0.59*	3.49 2.92 2.54*	6.69 5.84 4.65*	24.10 23.09 21.87	30.79 29.66 26.59*	14.09 12.50 11.39*
LSD05		0.09	0.06		1.70	0.02	0.52	0.95	1.01	1.15	0.75
140 days											
‘LV ChS21’	C I II	1,45 1.71 1.76*	0.46 0.66 0.71	3.17 2.60 2.43*	64.9 58.7 53.0*	0.63 0.54 0.51	11.56 9.47 5.32	16.68 13.18 9.34*	36.10 34.04 30 .49*	42.79 37.40 35.91*	10.1 8.7* 7.6*
CMS	C I II	1.56 1.87 2.10*	0.49 0.77 0.88	3.21 2.39 2.31*	52.6 47.5* 42.9*	0.67 0.58 0.47	9.15 8.06 7.25	13.58 11.25 8.11*	38.89 36.44 32.71*	45.23 39.36 33.05*	13.6 8.8* 7.3*
Multigerm	C I II	1.15 1.65 1.73*	0.34 0.53 0.60	3.43 3.10 2.80*	46.4 41.9* 37.8*	0.73 0.62 0.53	8.61 7.46 6.71	12.22 10.53 8.15*	33.40 32.39 31.44*	40.34 38.09 31.75*	13.3 9.3* 7.9*
LSD05		0.10	0.07		2.1	0.04	1.11	1.80	1.51	2.11	1.5

*The difference is significant at $p<0.05$ relative to the control.

**C – Control

Table 2. Physiological and biochemical characteristics of leaf apparatus of sugar beet lines (130 days).

Line	Treatment	Chlorophyll (mg/g)			Carotenoids (mg/g)	Carotenoids (mg/dm^2)	Intensity of photosynthesis (mg CO_2·dm^{-2}·h	PCA (μM $[Fe(CN)_6]^{3-}$/mg chl.·year)	SLW (mg/dm^2)	Albumins + globulins (% of dry matter)	Leaf area (dm^2)
		a	b	a/b							
'SKF 5084'	c**	0.382	0.260*	1.46	0.228	0.68	15.4	34.68	668.2	14.71	25.43
	s***	0.621	0.573	1.08	0.682*	0.69	6.95*	15.61*	476.3*	13.85	53.88*
'SKF 5050'	c	0.340	0.211	1.61	0.215	0.61	16.1	29.40	696.5	15.91	33.39
	s	0.789	0.561*	1.40	0.283*	0.67	7.24*	12.94*	479.6*	14.83	60.42*
'SKF 4973'	c	0.456	0.348	1.31	0.170	0.52	11.85	37.68	691.2	11.54	41.77
	s	0.683*	0.537*	1.27	0.200	0.56	5.45*	16.96*	527.5	12.18	48.92*
'Hill 13'	c	0.736	0.463	1.58	0.456	1.36	11.08	30.60	747.9	13.60	37.97
	s	1.076	0.879*	1.22	0.335*	0.87	4.76*	12.85*	508.8*	7.6*	50.16*
LSD_{05}		0.120	0.040	0.23	0.01	0.12	0.70	0.80	14.10	1.40	1.12

*The difference is significant at $p<0.05$ relative to the control.
**C - Control
***S - shading

Shading was also accompanied by a decrease in the total content of albumins and globulins in the leaves depending on the genotype, especially at the end of the growing season. The decrease ranged from 13.9 to 35% in treatment I and from 24.8 to 46.4% in treatment II (Table 1, 2). The degree of adaptability of various sugar beet genotypes to reduced illumination was also manifested due to significant changes in the pool of water-soluble carbohydrates in leaf blades and petioles, the content of which decreased in the studied genotypes by 11.9-25% (treatment I) and by 20.5-54% (treatment II) compared to the control (Table 3, 1).

Table 3. Water-soluble carbohydrate content in leaves and sugar content of roots in sugar beet lines.

Line	Treatment	Leaf blades		Petioles		Root
		monosaccharides	disaccharides	monosaccharides	disaccharides	sucrose content (%)
		(% of dry matter)				
'SKF 5084'	control	14.18	18.29	30.81	46.54	15.95
	shading	12.8	16.75	27.2	40.9	14.75
'SKF 5050'	control	10.91	16.15	34.69	47.15	15.17
	shading	6.07*	8.77*	30.5	44.65	13.55
'SKF 4973'	control	19.15	22.88	41.87	51.44	16.83
	shading	7.5*	12.81*	34.6*	46.71	14.55
CMS line	control	9.1	13.58	32.71	45.23	18.84
	shading	8.06	12.52	26.44*	33.0*	15.9
LSD_{05}		1.20	1.22	1.30	1.25	0.90

*The difference is significant at $p<0.05$ relative to the control

Studies have shown that the adaptation of sugar beet to shading also occurred as a result of a change in source-sink relations, the original program of which is embedded in the plant genome (Mokronosov, 1981). In the conditions of shading, photoassimilates were mainly directed to the growth of the leaf apparatus of plants, the leaf area of which in all studied genotypes increased 1.5 to 2 times compared to the control plants (Table 1, 4), which was also noted by other authors (Kiriziy, 2002). The rate of aging and withering of leaves, which lifespan is under the influence of phytohormonal status (Sytnik *et al*., 1978), was significantly slowed down, especially in CMS lines (Table 5), hybrid, and its components (Table 4).

Under the conditions of shading, the weight of raw and dry matter of roots in the hybrid and its components decreased from 21.3 to 35.4% (treatment I) and from 40.1 to 53.2% (treatment II), which was accompanied by an increase in the ratio of the tops/root, a decrease in the ratio of the weight of root to the weight of the whole plant, and an increase in the ratio of the dry matter of petioles to the dry matter of tops (Table 4). In the studied sugar beet lines, the decrease in the accumulation of dry matter of roots compared to the control was 30% in multigerm sugar beet lines and 16% in CMS lines (Table 3). Shading negatively affected the process of sugar accumulation due to the primary biosynthesis of sucrose in the leaf and its entry into the storage compartment of the root (Kuznecov *et al*., 1990; Khozaei *et al*., 2020). Among the studied sugar beet genotypes, the greatest decrease in the sugar content of roots was 1.8-4.6% in treatments I and II in 'LV ChS21', and the least decrease, from 0.9 to 1.2% was observed in the 'Hill 13 (Table 4). In the sugar beet lines, sugar content decreased in the range from 1.24 to 2.8% (Table 3).

The revealed peculiarities of the formation of sucrose content in various sugar beet genotypes are generally consistent with the peculiarities of the growth and development of the leaf apparatus and the functional photosynthetic characteristics of leaves in the donor-acceptor system. In shaded plants in the second half of vegetation, the period of leaf apparatus formation was prolonged, and the competitive relations between young growing leaves and roots in the aspect of photoassimilation were aggravated. These circumstances limit the inflow of sucrose from the above-ground part into the roots and lead to inhibition of the growth processes and accumulation of sucrose in the roots (Pavlinova, 1981). In addition, in shaded sugar beet plants, the intensity of photosynthesis decreases, which in turn leads to a decrease in the pool of newly formed plastic substances.

Studies have shown that shading significantly influenced the distribution and ratio of sucrose in the zones of vascular bund rings and adjacent zones of the stocking root parenchyma. At harvest, these adaptive changes are more clearly expressed in the inner part of roots (1^{st} to 3^{rd} rings), which is supplied with the assimilates by phloem vascular bundles of mature leaves (Kliachenko and Shevchenko, 2007; Kliachenko, 2007), than in the middle (4^{th} to 5^{th} ring) and peripheral part (Table 6). At the same time, the greatest stability of the concentration of sucrose in the transporting and storing root tissues was demonstrated by the hybrid component 'Hill 13'. The results of field experiments on the influence of different plant densities on the production process of sugar beet showed that the studied CMS hybrids and their parent components demonstrated different adaptive resistance to the thickening of sowings. At the plant densities of 100 and 120 plants/ha, the most resistant appeared the component of 'Hill 13' and CMS component of 'LV ChS21', which yield increased by 4.6-4.4 and 4.2-4.1 t/ha, sugar yield by 1.1-0.4 and 1.09-0.5 t/ha, respectively (Table 7). Thus, as a result of the studies of ontogenetic adaptation of sugar beet lines, hybrids, and their parent components to the light regime of PAR under shading and different plant density, it was found that the stress factor intensity is a key characteristic of changes in the physiological, biochemical, anatomical, and morphological parameters of the leaf apparatus, which provide plant homeostasis and ensure the maximum efficiency of photosynthesis and productivity of different sugar beet genotypes under these conditions.

Table 4. Indicators of plant productivity of sugar beet genotypes under shading.

Hybrid, component	Treatment	Dry matter (g)					Leaf area (dm^2)	Root weight (g)	Sucrose (% of wet weight)
		leaf blades	petioles	root	whole plant	dead leaf			
75 days									
'LV ChS21'	Control	39.3	16.9	71.9	128.1	7.5	60.5	323	12.64
	I	40.5	20.1	51.0*	111.6*	1.3*	63,6	288	12.19
	II	43.9*	22.8*	39.9*	106.6*	1.2*	67.1*	275*	11.72
CMS	Control	45.6	21.3	58.9	125.8	6.8	76.5	283	13.04
	I	46.9	25,2	45.3*	117.7*	1.2*	79.6	258	12.61
	II	48.4*	27.5*	34.7*	110.6*	.07*	83.7*	235*	12.58
Multigerm	Control	31.5	14.1	53,9	99,5	5.1	59.9	291	12.98
	I	32.9	15.8	35.6*	84.3*	0.7*	61.5	248*	12.56
	II	34.1*	17.2*	30.7*	82.0*	0.5*	62.9*	223*	12.43
LSD_{05}		1.2	1.7	5.6		0.04	1.21	6.58	0.11
140 days									
'LV ChS21'	Control	43.1	22,7	273.8	339.6	65.4	45.9	1126	17.55
	I	45.7	29.2	194.6*	269.5*	12.3*	52.1	885*	15.75*
	II	52.4*	40.4*	156.7*	249.5*	10.8*	59.2*	793*	12.95*
CMS	Control	54.3	22.3	260.7	337.3	66.1	31.4	1031	18.20
	I	56.3	30.1	202.6*	289.0*	11.8*	51.7	850*	16.50*
	II	59.8*	34.5*	153.7*	248.0*	6 7*	60.5*	675*	15.90*
Multigerm	Control	42.8	23.6	269.1	335.5	80.5	46.2	1097	17.90
	I	45.5	27.1	177.5*	250.1*	10.7*	49.3	770*	16.22
	II	47.9*	30.1*	128.2*	206.2*	9.5*	51.4*	600*	15.15*
LSD_{05}		1.9	1.1	10.3		1.92	1.5	15.4	0.35

*The difference is significant at p<0.05 relative to the control.

Table 5. Accumulation of dry matter in plants of sugar beet lines.

Line	Root (g)		Leaf blades (g)		Petioles (g)		Whole plant weight (g)		Weight of withered leaves (g)		Above-ground mass root	
	c**	s***	c	s	c	s	c	s	c	s	c	s
'SKF 5084'	116.15	47.04*	37.04	39.48	16.73	36.58*	169.92	123.11*	21.92	6.72*	0.46	1.61*
'SKF 5050'	84.11*	40.*65	53.42 *	57.89*	20.34	43.64*	157.87	142.18*	22.11	13:30	0,87	2.49*
'SKF 4973'	142.87	53.85*	52.81	40.38*	40.19	47.88*	235.87	182.49*	15:57	7.17*	0.65	1.63*
CMS line	260.20	152.68*	56.16	66.39*	22.65	34.57*	339.01	253.58*	65.55	6.74*	0.30	0.66*
LSD_{05}	10.11	1.15	1.10	1.21	1.13	1.07	11.12	10.12	1.09	0.07		

*The difference is significant at $p<0.05$ relative to the control
**C - Control
***S - shading

Table. 6. Distribution of sucrose (% to wet weight) in roots of different sugar beet genotypes under shading (140 days).

Hybrid, component	Treatment	1st ring		2nd ring		3rd ring		4th ring		5th ring		Periphery
		vascular bundles	parenchyma	vascular bundles	parenchyma	vascular bundles	parenchyma	vascular bundles	parenchyma	vascular bundles	parenchyma	
'LV ChS21'	C** I II	17.1 15.3 13.8*	16.4 14.7 13.3*	17.5 15.6 15.3*	16.6 15.4 13.6*	17.7 16.8 15.6	16.9 16.3 14.8*	18.1 17.8 16.9	18.0 17.6 16.8	18.4 18.0 17.6	18.6 18.2 18.0	18.3 18.1 17.8
CMS	C I II	15.7 15.1 14.8	15.0 13/6. 13.3	16.0 15.9 15.6	15.6 15.0 14.7	16.4 15.9 15.7	15.9 15.1 15.0	17.0 15.7 15.4	16.8 15.6 15.4	17.4 16.0 15.8	17.8 16.8 16.6	17.6 16.9 16.7
Multigerm	C I II	19.8 17.8 16.2*	15.8 15.2 1.7*	20.2 17.8 16.7*	15.6 15.2 14.9	20,6 18.8 17.3	17.4 16.4* 15.7*	21.0 19.3 18.0	18.6 18.0 17.8*	21.2 20,6 19.0	22.6 21.0 20.2*	20.8 20.4 19.8
LSD_{05}		0.30	0.25	0.35	0.20	0.35	0.25	0.35	0.35	0.35	0.30	0.20

*The difference is significant at $p<0.05$ relative to the control
**C - Control

Table 7. Yield performance of sugar beet under different plant densities.

Hybrid, component	Plant density (1000 plants/ha)	Yield (t/ha)	Root weight (g)	Sucrose content (% of wet weight)	Sugar yield (t/ha)
'LV ChS21'	80	40.0	501.0	17.1	6.8
	100	43.4	434.4	17.4	7.4
	120	42.9	357.8	15.9	6.9
CMS	80	38.8	485.5	17.6	6.8
	100	43.0	437	17.9	7.7
	120	42.9	357.5	16.9	7.3
Multigerm	80	39.9	498.5	17.3	6.9
	100	42.9	429.0	17.8	7.6
	12	42.5	354.1	16.9	7.2
LSD_{05}		2.1	19	0.40	0.60

CONCLUSIONS

Since a certain type of reaction or the norm of reaction to environmental conditions (i.e. the ability to optimally change the organization in response to changes in internal and external factors) is inherited, in the process of obtaining thickening-resistant breeding genotypes of sugar beet, their comprehensive assessment and selection should be carried out by the integral physiological and biochemical parameters, with selecting progeny of plants that are less responsive to a decrease in illumination.

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Review paper
10.7251/AGRENG2202040E
UDC 631:574.1(6-15)

BIODIVERSITY – FOOD SYSTEMS NEXUS: UNPACKING LINKAGES BETWEEN BIODIVERSITY, DIETS AND NUTRITION IN BURKINA FASO AND NIGER

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ABSTRACT

A growing body of evidence shows the strong linkages between biodiversity and food and nutrition security. However, there is a gap in knowledge on such linkages in developing countries, which are more affected by food insecurity and malnutrition. Moreover, it is not clear what are the implications of the ongoing biodiversity loss for food security and nutrition. In this context, the present review analyses the state of research on the nexus between biodiversity, diets and nutrition security in Burkina Faso and Niger. It draws upon a search of scholarly literature indexed in the Web of Science. The review suggests that there is a huge gap in research on this topic in both countries. In general, the literature shows a positive association between biodiversity and dietary diversity, nutrition and food security. Indeed, dietary diversity is affected by the variety of farm crops and animals, as well as foraged wild plants and trees. The pathways linking biodiversity and diets are context-specific. The effects of cropland expansion on dietary diversity are mixed and depend on farming systems diversity. The contribution of agrobiodiversity to nutrition is affected by the nutritional quality of products. Moreover, traditional knowledge plays a central role as an interface between biodiversity and dietary/food diversity. This review suggests that biodiversity is necessary for dietary diversity and nutrition security. Although still small, the existing body of evidence supports investments in agrobiodiversity to address food insecurity and malnutrition in Burkina Faso and Niger.

Keywords: *agrobiodiversity, crop diversity, animal diversity, dietary diversity, food security, nutrition, West Africa, Sahel.*

INTRODUCTION

According to the Convention on Biological Diversity (UNEP 1992), biodiversity (i.e. biological diversity) refers to "*the variability among living organisms from all* *sources, including, inter alia, terrestrial, marine, and other aquatic ecosystems, and the ecological complexes of which they are part: this includes diversity within species, between species and of ecosystems*" (p. 27). Biodiversity refers to diversity at different scales of biological organization (genes, populations, species, and ecosystems) and multiple geographic scales (local, regional, or global) (Millennium Ecosystem Assessment 2005). Biodiversity is the foundation of ecosystems, which provide different services that are fundamental for human well-being. These include provisioning services (e.g. food, fibre, fuel, water), regulating services (e.g. climate, diseases, water quality), supporting services (e.g. nutrient cycling, soil formation, photosynthesis) and cultural services (e.g. aesthetic enjoyment, recreation, spiritual fulfilment) (Millennium Ecosystem Assessment 2005). The Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES) introduced recently a new concept, viz. Nature's Contributions to People (NCP) (Díaz et al. 2018), which is closely related to the ecosystem services concept. Food provisioning is one of the most important services delivered by ecosystems. Therefore, biodiversity loss can have high economic and social costs and increase poverty and hunger (Millennium Ecosystem Assessment 2005). In this context, agricultural biodiversity has always played a crucial role in sustaining and strengthening food security, nutrition, and livelihood security (Toledo and Burlingame 2006). However, the genetic diversity of food crops and animal breeds is diminishing rapidly (Millstone and Lang 2008).

Biodiversity loss is one of the most pressing challenges facing humanity. This explains why biodiversity conservation is addressed in many Sustainable Development Goals (SDGs) such as SDG 2 "Zero Hunger" and SDG 15 "Life on Land" (United Nations 2015). Humans rely on an increasingly limited number of plant and animal species for their nutrition. Indeed, only 30 crops provide 90% of dietary needs, with only three crops (viz. wheat, rice and corn) providing about half of the food consumed worldwide (FAO 2010). Likewise, fewer than 14 species (e.g. cattle, goats, sheep, chicken) account for about 90% of the global trade in animal products (Secretariat of the Convention on Biological Diversity 2008). The most important drivers of biodiversity erosion and loss are overexploitation (e.g. over-hunting, over-fishing), habitat change (e.g. land-use changes), climate change, pollution (nitrogen, phosphorus), and invasive alien species (Millennium Ecosystem Assessment 2005).

A growing body of literature links biodiversity to food and nutrition security (Burlingame et al. 2009; Fanzo et al. 2013; Foresight 2011; Toledo and Burlingame 2006). Indeed, agricultural biodiversity results instrumental to diversify diets and improve nutrition and health (Fanzo et al. 2013). This is particularly important to address the issue of micronutrient malnutrition (i.e. hidden hunger), which affects approximately 2 billion people worldwide (FAO et al. 2017). Diets simplification and the erosion of agro-biodiversity are responsible for nutrient deficiencies in many parts of the world (Johns and Eyzaguirre 2006). The strong linkage between biodiversity and nutrition is also acknowledged in the definition of sustainable diets (FAO 2011). However, it seems that the relationship between biodiversity and dietary diversity, i.e. number of food items or food groups consumed over a given period of time (Ruel 2003), is not straightforward. For instance, El Bilali et al. (2017), in their analysis of the relationship between biodiversity and dietary diversity in the Mediterranean, found that despite a general decline in agricultural biodiversity, dietary diversity increased over recent decades, possibly reflecting greater affluence and the effects of globalization and agri-food trade.

However, it is assumed that the relationship between domestic biodiversity and diversity of diets changes from one country to another. Moreover, it can be posited that it is stronger in developing countries, especially landlocked countries that are less exposed to international agri-food trade. This makes the examples of Burkina Faso and Niger particularly interesting.

Burkina Faso and Niger are two landlocked West African and Sahelian countries. They have low human development (UNDP 2019), are affected by multiple forms of malnutrition (FAO et al. 2018; USAID 2018a, 2018b), and have a gap in terms of sustainable development (Sachs et al. 2017). Agriculture is a leading sector for the economies of Niger and Burkina Faso, with a significant contribution to the gross domestic product (GDP) and employment (World Bank 2019a). However, agriculture is extensive, poorly mechanized and almost entirely reliant on the variable summer rainfall, making it vulnerable to climate change. Staple dryland crops include cereals (e.g. sorghum, millet, maize) and legumes (e.g. cowpea), while major cash crops are cotton and groundnut (USAID 2017). Natural resources are deteriorating in a context of changing climate and recurrent droughts (USAID 2017). Climate change represents a challenge for agriculture (Mainardi 2011; Sultan et al. 2005; USAID 2017) and is also an important driver of poverty and livelihoods vulnerability. The prevalence of undernourishment is high (FAO et al. 2018), while deficiencies of micronutrients (e.g. iron, vitamin A, iodine) continue to affect the health and well-being of a wide share of the population in both countries (Institut National de la Statistique and ICF International 2013; USAID 2018a, 2018b).

In this context, the present review analyses the state of research on the nexus between biodiversity, diets and nutrition security in Burkina Faso and Niger.

METHODS

The present systematic review follows the PRISMA guidelines (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) (Moher et al. 2009). It draws upon a search of all documents indexed in the Web of Science (WoS) carried out in May 2021 using the following Title-Abs-Key search query: *(biodiversity OR “species diversity” OR “genetic diversity”) AND (diet OR consumption OR consumer OR nutrition OR intake OR nutrient) AND (Burkina OR Niger OR “West* Africa” OR Sahel).* The search on WoS yielded 147 documents. The selection of the documents to include in the systematic review was informed by the methodology suggested by El Bilali (2021; 2020). In particular, three eligibility criteria were considered: geographical coverage (viz. the document deals with Burkina Faso and/or Niger); thematic focus (viz. the document deals with *both* biodiversity and diets); and document type (viz. only research articles, chapters or conference papers were selected; letters to editors, commentaries, notes as well as reviews were discarded). Only the documents that meet all the eligibility criteria were included in the systematic review. Table 1 describes the selection steps and process.

Table 1. Articles selection process.

Selection step	Number of selected documents	Number of documents excluded and exclusion reasons
Search on WoS	147	--
Screening of documents based on titles	147	45 documents excluded because they deal with other countries than Burkina Faso and Niger viz. Argentina, Benin, Botswana, Brazil, Cape Verde, Chile, China, Congo, Cote d'Ivoire, Ethiopia, Germany, Ghana, Guinea, Guinea-Bissau, India, Madagascar, Mali, Mauritania, Nigeria, Sao Tome, Saudi Arabia, Senegal, Switzerland, Togo, and UK.
Screening of documents based on abstracts	81	81 documents excluded: 35 documents do not deal with Burkina Faso and/or Niger; 5 documents do not address biodiversity; 41 documents do not deal with diets or nutrition.
Scrutiny of full-texts	22	6 documents excluded: 3 documents not addressing Burkina Faso and/or Niger; 1 document not addressing diets or nutrition; 2 reviews.
Inclusion in the systematic review	15	--

First, 45 documents were excluded following the screening of titles, as they do not refer to Burkina Faso and/or Niger; documents covering wider geographical areas (e.g. West Africa, Sahel, Sub-Saharan Africa,) or those where the geographical scope is not reported in the title were kept for further scrutiny. Second, 81 documents were excluded following the scrutiny of abstracts as they do not meet the eligibility criteria. Finally, 6 documents were discarded following the analysis of full-texts, including 2 reviews. Consequently, only 15 documents were included in the systematic review (Table 2).

Table 2. List of the eligible documents included in the systematic review.

Year	References
2021	Lourme-Ruiz et al. (2021)
2020	Morgan and Moseley (2020)
2019	Baudron et al. (2019)
2018	Félix et al. (2018); Sandwidi et al. (2018)
2017	Sunderland et al. (2017); Gaisberger et al. (2017)
2016	Poole et al. (2016); Agúndez Leal et al. (2016)
2015	Taylor et al. (2015)

Year	References
2021	Lourme-Ruiz et al. (2021)
2020	Morgan and Moseley (2020)
2019	Baudron et al. (2019)
2013	Stadlmayr et al. (2013); Barnaud et al. (2013)
2010	Garrity et al. (2010)
2009	Belem et al. (2009)
2003	Wahlqvist (2003)

RESULTS AND DISCUSSION

The low number of the selected documents suggests a huge gap in research on the relations between biodiversity, diets and nutrition in Burkina Faso, Niger and West Africa at large.

Many articles analyse the relationship between biodiversity in general, and agrobiodiversity in particular, and dietary diversity (Baudron et al. 2019; Lourme-Ruiz et al. 2021; Morgan and Moseley 2020). Indeed, the literature shows that the diversity of diets is affected by the diversity of farm crops and animals, as well as wild plants, forest trees and wild animals that households have access to and can use. Lourme-Ruiz et al. (2021) found that WDDS-10 (a women's dietary diversity score based on ten food groups) is positively associated with production diversity score (PDS) and the number of agroforestry trees species in farms of the Hauts-Bassins (western Burkina Faso). Therefore, the dietary diversity of women in farming households depends on the diversity of on-farm production (Lourme-Ruiz et al. 2021). Local farm management and crop associations provide opportunities to collect diverse nutrition-rich species year-round thus sustaining household nutrition in semiarid West Africa (Félix et al. 2018). Baudron et al. (2019) highlight that three pathways may link forests to diet diversity viz. direct pathway (forest products consumption), income pathway (forest-related income used to purchase food), and agro-ecological pathway (forests and farm trees sustaining on-farm food production). They found that the agro-ecological pathway was more plausible in Burkina Faso as greater forest cover in the landscape improved crop and livestock production. They conclude that the different pathways are not straightforward and context-specific (Baudron et al. 2019). Likewise, as highlighted by Lourme-Ruiz et al. (2021), the results of the assessment of the relationships between dietary diversity and agro-biodiversity depend on how agricultural biodiversity is evaluated i.e. indicators and metrics used.

In this context, particular attention is paid to foraging as a source of nutrient-rich wild plants (Belem et al. 2009; Morgan and Moseley 2020). Foraging can concern a wide range of wild plants such as herbaceous plants (Belem et al. 2009) and woody species (Agúndez Leal et al. 2016; Belem et al. 2009; Gaisberger et al. 2017). Belem et al. (2009) found that 43 tree, shrub, and annual herbaceous plant species are foraged in the community of Seguenega, northern Burkina Faso. In their analysis of the use of biodiversity by smallholder farmers in semi-arid West

Africa, Félix et al. (2018) put that "*Wild plants (i.e. grasses, shrubs, vines, and trees) collected from surrounding landscape play an important role in sustaining micro-nutrient accessibility at the household level*" (p. 83). Morgan and Moseley (2020) found that foraging represents an important source of micronutrients and dietary diversity for female farmers in Burkina Faso. However, foraging may lead to the overexploitation of food plants and trees thus their genetic erosion and loss (Belem et al. 2009; Gaisberger et al. 2017). Belem et al. (2009) point out that leafy wild food species are threatened by over-harvesting, animal browsing, cutting, and bushfires in Northern Burkina Faso. The use of the meat of wild animals (i.e. bushmeat) (Taylor et al. 2015) results particularly problematic; it certainly contributes to the food security of riparian communities, but represents a threat to wildlife.

The literature also addresses the effects of cropland expansion, especially mono-cropping, on agro-biodiversity and consequently dietary diversity. Morgan and Moseley (2020) suggest that rice New Green Revolution projects do not affect foraging in Burkina Faso. However, Sunderland et al. (2017) argue that the transition from a forested landscape to a more agrarian system might have unintended consequences such as the loss of dietary diversity and ecosystem services/forest products. Gaisberger et al. (2017) found that overexploitation and cotton production represent the most serious short-term threats to food tree species in Burkina Faso. Lourme-Ruiz et al. (2021) show that the relationship between cotton production and dietary diversity of women depends on the type of management; it was positively associated with WDDS-10 when managed by women but not when men managed cotton production (Lourme-Ruiz et al. 2021). However, changes in land use can also have positive impacts on biodiversity and food security. For instance, some land management approaches such as Evergreen Agriculture (Garrity et al. 2010) have allowed restoring exhausted and degraded soils, thus increasing food crop yields and improving household food security, in several Sub-Saharan Africa countries such as Burkina Faso (cf. pit technology, zai, along with farmer-managed regeneration of trees) and Niger (cf. *Faidherbia albida* agroforests in sorghum and millet fields).

The contribution of agro-biodiversity to nutrition is affected by the quality of agri-food products, especially their nutritional quality. In this context, food composition tables become fundamental to have precise data about the nutrient content of foods, including orphan crops and underutilized cultivars/varieties (Stadlmayr et al. 2013). Lourme-Ruiz et al. (2021) call for paying more attention to the nutritional quality of agri-food products in agriculture policy in Burkina Faso by providing support to the production of nutrient-dense crops (e.g. vegetables, fruits, beans), instead of focusing only on mass production of cotton and maize.

Some documents analyse the role of biodiversity in food security (Belem et al. 2009; Garrity et al. 2010; Poole et al. 2016). This relates to food availability and utilisation as well as food access. In fact, the use of biodiversity plays a crucial role in the livelihoods of many rural communities, especially those living close to forests and other biodiverse ecosystems. Agúndez Leal et al. (2016) suggest that

woody food species complement the diets of rural populations in Niger in times of food scarcity. Scholars also analyse different approaches that are supposed to reconcile the divergent objectives of food security, climate change mitigation/adaptation and biodiversity conservation such as Evergreen Agriculture (Garrity et al. 2010), an agroforestry system consisting in the integration of tree species into annual food crop systems. However, Baudron et al. (2019) warn that the positive contribution of forest ecosystems to rural livelihoods and food access/security cannot be generalized and change from one context to another.
Further documents highlight the central role of traditional knowledge (cf. culture, traditions) as an interface between biodiversity and dietary/food diversity (Sandwidi et al. 2018; Wahlqvist 2003). In fact, traditional, local knowledge and culture determine the way local biodiversity is used, especially in local cuisine (Wahlqvist 2003). This, in turn, determines the level of resource use sustainability. Women play an important role in the conservation and dissemination of knowledge on local agrobiodiversity and its use, especially that relating to neglected and underutilised species such as baobab (*Adansonia digitata*), nere (*Parkia biglobosa*) and shea (*Vitellaria paradoxa*) in Burkina Faso (Poole et al. 2016).
Other articles address the implications of the linkages between biodiversity and nutrition for breeding of different relevant crops in West Africa such as fonio (Barnaud et al. 2013). In particular, participatory breeding processes ensure that nutritional traits valued by local communities are taken into consideration by breeders (Sandwidi et al. 2018). The participation of local communities is also paramount for the conservation of local diversity of cultivated and wild food species (Belem et al. 2009; Sandwidi et al. 2018).

CONCLUSIONS

This review provides a comprehensive analysis of how the nexus between biodiversity, diets and nutrition in Burkina Faso and Niger is addressed in the scholarly literature. The low number of documents addressing this topic suggests a huge gap in the research field and the need for more attention and investments. In general, the literature shows that dietary diversity and nutrition are affected by the diversity of farm crops and animals, as well as wild plants, forest trees and wild animals to which households have access through foraging. However, there are different, complementary pathways linking biodiversity and diets, and they are context-specific. These include a direct pathway, involving consumption of foraged products, or an income pathway, implying selling them to buy food. However, foraging may lead to the overexploitation of food plants and trees. The relationship between dietary diversity and agro-biodiversity depends on how they are evaluated. Moreover, the effects of cropland expansion on dietary diversity are mixed and depend on the diversity of farming systems. The contribution of agro-biodiversity to nutrition is affected by the quality of agri-food products, especially their nutritional quality. Biodiversity is also associated with food security; wild food products complement the diets of rural communities in times of food deficit. Traditional knowledge (cf. culture, traditions) plays a central role as an interface between biodiversity and dietary/food diversity. In this context, the participation of local communities in breeding and conservation endeavours becomes paramount for their success.

Biodiversity, diets, and food and nutrition security are strongly interlinked. Enhancing agro-biodiversity could be an effective strategy to address food insecurity and malnutrition in Burkina Faso and Niger. Research is highly needed to strengthen the body of evidence on the interlinkages between biodiversity and nutrition, point out the potential solutions to optimise synergies between biodiversity and nutrition while minimising trade-offs, inform policy to adopt appropriate approaches by the governments and other stakeholders to address present and future challenges in Burkina Faso and Niger. Research is also needed to examine further the relationships between (agro)biodiversity and nutrition security from different angles, at different levels and in different contexts, also concerning increasing socio-economic affluence and regional agri-food trade. Further research should also explore the associations between biodiversity, dietary diversity, and health and nutritional well-being outcomes.

ACKNOWLEDGMENTS

This work was carried out within the project SUSTLIVES (*SUSTaining and improving local crop patrimony in Burkina Faso and Niger for better LIVes and EcoSystems* - https://www.sustlives.eu), of the DeSIRA initiative (Development Smart Innovation through Research in Agriculture), financed by the European Union (contribution agreement FOOD/2021/422-681).

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Review paper
10.7251/AGRENG2202051E
UDC 631.95(6-15)

AGROECOLOGY IN BURKINA FASO AND NIGER

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ABSTRACT

Agroecology is considered a science, a practice and a social movement, which shows the centrality of research in agroecology development. Interest in agroecology has been shown in many developing countries such as Burkina Faso and Niger. Therefore, this paper analyses the state of research on agroecology in Burkina Faso and Niger, by drawing upon a search of scholarly publications performed in June 2021 on the Web of Science. The analysis of the scholarly literature suggests that the scientific component of agroecology is underdeveloped in both countries. Despite the recurring discourse on agroecology in West Africa, quality research is far below expectation and this might hamper the development of the agroecological movement as well as the documentation and dissemination of agroecological practices. Agroecology is presented as an instrument to address several environmental (e.g. biodiversity loss, land degradation), social (e.g. food insecurity) and economic (e.g. unemployment, poverty) challenges. Indeed, agroecology could contribute to food security, biodiversity conservation and rural livelihoods. The literature also highlights that agroecological management is knowledge-intensive so farmers' capacities need to be strengthened to increase the adoption of agroecological practices. Agroecology is also labour intensive, which can increase its contribution to local economies and livelihoods (cf. employment) but could also hamper its adoption where there is limited labour availability. Further research is needed to foster agroecological transition in Burkina Faso and Niger, which is fundamental to move towards sustainable agriculture and food systems that ensure food and nutrition security without undermining the fragile natural resource base.

Keywords: *agroecological transition, biodiversity, food security, livelihoods, West Africa.*

INTRODUCTION

Agroecology is gaining ground, both in developed and developing countries, as one of the most prominent and promising pathways for the transition towards sustainable agriculture and food systems (El Bilali, 2019; FAO, 2018; HLPE, 2019; Ollivier et al., 2018; Wezel et al., 2016). The transformative potential of agroecology is nowadays widely recognised not only by many scholars and organic agriculture movements (Herren et al., 2015) but also by several international organisations (FAO, 2015; HLPE, 2019) and expert panels (IPES-Food, 2016). Agroecology is an approach that dates back to the beginning of the 20th century and links together science, practice and movements focused on social change (Wezel et al., 2011). It utilizes ecological principles to design and manage productive, resilient and sustainable farming and food systems (Gliessman 2015; IPES-Food 2016). Recently, Wezel et al. (2020) defined 13 consolidated agroecological principles viz. recycling, biodiversity, land and natural resource governance, input reduction, soil health, animal health, connectivity, synergy, fairness, participation, co-creation of knowledge, social values and diets, and economic diversification. The integration of the three practical forms of agroecology (viz. scientific discipline, agricultural practice, social movement) and linkage with other food movements (e.g. food sovereignty) provided a collective action to contest the dominant agro-food regime and create agro-food alternatives (Levidow et al., 2014). The agroecological philosophy and message have also profoundly influenced and shaped other alternative agro-food movements and communities such as organic agriculture, permaculture and conservation agriculture (El Bilali, 2019; HLPE, 2019). Agroecological practices embrace soil fertility management, pest control, biodiversity conservation and agroecosystem integrity (Lampkin et al. 2017; Wezel et al. 2014) and contribute to food security and livelihoods (HLPE, 2019). However, despite the well-documented positive impacts of agroecology, the agroecological transition is hampered by many context-specific (technical, political, social, cultural, economic) obstacles of different nature requiring solutions from different fields of competence (Beudou et al., 2017; El Bilali, 2019). West Africa and Sahel regions face the challenge of feeding a growing population in a context of accelerated degradation of natural resources and climate change. Therefore, agroecological interventions have been promoted by a network of stakeholders to increase food production while conserving the natural resource base (Tapsoba et al., 2020). Agroecology has a longstanding history in Burkina Faso and Niger. For instance, in Burkina Faso, the agroecological alternative is rooted in the revolutionary period of president Sankara in the 1980s who was strongly concerned about environmental issues linked to agricultural development and supported endogenous development principles. The agroecological movement was reactivated in the country in the 21st century, among others, through small projects supported by different NGOs such as the French association *Terre et Humanisme* (Gross & Jaubert, 2019). However, it is widely recognised that research is fundamental for the development of agroecology and there are still many knowledge gaps to be addressed (HLPE, 2019). In this context, it is not clear whether the agroecological movement is supported by evidence from science in Burkina Faso and Niger. Therefore, the present review paper analyses the state of research on agroecology in Burkina Faso and Niger.

METHODS

The paper is based on a systematic review of all documents about agroecology in Burkina Faso and Niger indexed in Clarivate Analytics - Web of Science (WoS), following the PRISMA guidelines (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) (Moher et al., 2009). It draws upon a search performed on June 19th, 2021, on WoS using the following search string: *(agroecology OR "agro-ecology" OR "agro-ecologic" OR agroecologic) AND (Burkina OR Niger OR "West* Africa" OR Sahel)*. The initial search yielded 44 documents published between 1990 and 2021. Three inclusion criteria were considered for the selection of documents to be included in the systematic review: geographical coverage (viz. document deals with Burkina Faso and/or Niger); thematic focus (viz. main topic is agroecology); and document type (viz. only research articles, book chapters or conference papers were selected; letters to editors, commentaries and/or notes as well as reviews were excluded). The screening of titles allowed excluding 8 ineligible documents that do not refer to Burkina Faso, Niger or the wider West Africa/Sahel region; the excluded documents deal with Benin, Brazil, Cameroon, Ghana, Nigeria, and Sierra Leone. Additional 22 documents were excluded following the scrutiny of abstracts as they do not deal with Burkina Faso and/or Niger (9 documents) or agroecology (12 documents) as well as one editorial material without abstract. Furthermore, 3 reviews (El Bilali, 2021; Kanlindogbe et al., 2020; Tapsoba et al., 2020) were discarded after the analysis of full-texts. Therefore, only 11 documents were included in the systematic review: Dowd-Uribe (2014); Gross and Jaubert (2019); Guébré et al. (2020); Lappé (2013); Masse et al. (2013); Nana et al. (2015); Osbahr and Allan (2003); Ouédraogo et al. (2019); Ratnadass et al. (2011); Saqalli et al. (2010); and Vidal et al. (2020).

RESULTS AND DISCUSSION

Agroecology has several impacts on the environment and natural capital. Some studies show that agroecological practices have positive effects not only on soil fertility but also on its biological activity. For instance, Guébré et al. (2020) found that plant residue amendments from stems and leaves of agroforestry shrubs increased nitrogen availability and triggered the soil macro-faunal activity (cf. termites, earthworms) with a consequent improvement in crop performance in Burkina Faso. Ouédraogo et al. (2019) found that in gardening farms in Bobo-Dioulasso (Burkina Faso), there is a widespread practice of crop rotations and associations, and organic fertilization. However, different factors affect soil fertility; according to Osbahr and Allan (2003), drawing upon local ethno-pedological knowledge in Fandou Beri village (Niger), "*Local soil fertility management depends on individuals' capabilities, perceptions of constraints and* *opportunities, and their ability to mediate access to different types of resources*" (p. 457). Indeed, villagers in Fandou Beri were able to define soils based on location, production potential and interaction with the wider ecological environment.

There is a dual relationship between agroecology and biodiversity. On the one hand, agro-ecological practices contribute to enhancing biodiversity in farming systems. On the other hand, biodiversity is essential for providing some ecosystem services (e.g. pest regulation) that are crucial for successful agroecological management. For instance, Ratnadass et al. (2011) refer to a 'strategic' approach based on the exploitation of preventive, agroecological methods for the control of various pests and diseases in horticulture. These include the use of trap plants for reducing the infestation and damage of *Helicoverpa armigera*, tomato fruit worm, on okra in Niger.

Food insecurity is still a challenge in Sub-Saharan African countries. Therefore, Sub-Saharan African agriculture is called to increase agricultural production to achieve food security while reducing its footprints and impacts on the environment. For that, innovative practices, that consider the complexity of the social and biophysical systems of agricultural production and accommodate the ongoing environmental and socio-economic changes, are needed. In this context, Masse et al. (2013) highlight the benefit of this 'ecological engineering approach' and put that "*Innovative agricultural practices will be based on an intensification of ecological processes that determine the functioning of the soil plant system, farmers' fields and agro-ecosystems*" (p. 289). Also Lappé (2013), drawing upon case studies and success stories from Andhra Pradesh (India) and Niger, suggests that agroecology is transforming food systems in ways to address hunger and food insecurity. Indeed, agroecology allows to "*strengthen human relationships, enabling farmers to gain a greater voice in food production and fairer access to the food produced*" (p. 219). Therefore, agroecology supports food sovereignty and the control of local communities over their farming and food systems.

Many studies suggest that agroecology is labour intensive (Nana et al., 2015; Ouédraogo et al., 2019), since agroecological practices reduce input costs but increase labour requirements. This means that agroecology can contribute to local economies and livelihoods by creating job opportunities, but it can also be a barrier to the adoption of agroecology. For instance, Ouédraogo et al. (2019) found that the labour intensiveness of agroecological practices hamper their adoption by irrigated vegetable producers in Réo area (central-western Burkina Faso) since the majority of families cannot or are not willing to allocate more labour to their farming activities. Nana et al. (2015) show that conservation agriculture has positive effects on income.

A network of stakeholders is developing promising initiatives for scaling up agroecological practices and achieving agroecological transition in Burkina Faso, Niger and West Africa at large. However, there are different understandings and conceptualisations of agroecological transitions. Some scholars consider agroecology as similar to organic agriculture and, consequently, conceptualise agroecological transition as conversion to organic agriculture (Vidal et al., 2020).

However, Ouédraogo et al. (2019) found that no gardener in Bobo-Dioulasso (Burkina Faso) practices exclusively organic or agro-ecological production methods. Also Gross and Jaubert (2019) underline that the large diversity of situations and livelihood strategies makes it evident that agroecological gardening can only be adopted by a very small number of family farms in Réo area (central-western Burkina Faso).

Agroecological transitions can follow different pathways. Vidal et al. (2020) identified four trajectories of transformation of agro-pastoral dairy farms in Burkina Faso and France that correspond to different stages of conversion to organic farming and adoption of organic practices at the farm level i.e. organic farms with the use of pastoral resources and integrated animal health, organic farms, farms under conversion to organic agriculture, and conventional farms. Agroecological transition brings about changes in agro-pastoral practices regarding animal health and welfare, reproduction, milking (duration, period), fodder and forage management (harvesting, storage), and rangeland and pasture use. Similarly, Ouédraogo et al. (2019) identify four types of gardening farms in Bobo-Dioulasso (Burkina Faso) based on their location, size and intensity of use of pesticides and fertilizers viz. very intensive, intensive, moderately intensive and lowly intensive farms.

It is widely recognised that agroecological transition processes are context-specific as well as the importance of the direct involvement of the concerned stakeholders, especially farmers, in their framing. In this regard, Vidal et al. (2020) put that "*Agroecological transition would benefit from being co-constructed, by taking into account the diversity of local contexts through research, in partnership with farmers, technical supervision, NGOs and policy makers*". Osbahr and Allan (2003) call for paying more attention to farmers' physical, biological and agroecological knowledge and mechanisms through which it is used to make management decisions at the farm level. Meanwhile, Gross and Jaubert (2019) call on development organisations and public institutions to consider the diversity of family farms in Burkina Faso, as well as diverse farming families' needs and capacities, in supporting their transition to agroecology.

Different factors can enable or hamper the development and dissemination of agroecology. One of the most important constraints relates to the lack of reliable data on the economic, demographic and agro-ecological environments in Sahelian villages and households (Saqalli et al., 2010). The study of Dowd-Uribe (2014) suggests that both social and agro-ecological factors (viz. credit, governance, seed price and pest dynamics) affect not only the processes but also the outcomes of the adoption of new production systems, such as agroecology. Vidal et al. (2020) point to the market as one of the most important drivers of the agroecological transition of agro-pastoral dairy farms in Burkina Faso and France. Further drivers include resource management schemes. One problem faced by agro-pastoralists in West Africa in their move towards reducing reliance on purchased feed is the difficult access to pastures and rangelands for grazing (Vidal et al., 2020). This shows that land-use strategies at the territorial level have implications for the management choices and decisions of single farmers and pastoralists. Ouédraogo et al. (2019) suggest that the main challenges faced by farms Bobo-Dioulasso (Burkina Faso) relate to pesticide use and sustainable fertilization for an agro-ecological transition. Furthermore, gardeners have a weak and imprecise knowledge of the health and environmental impacts of their practices. Moreover, agroecological farming relies on organic inputs (manure, plant biomass) that are scarce during the dry season. Last but not least, the systemic agroecological approach is complex to master for most farmers in Burkina Faso (Ouédraogo et al., 2019). This shows the importance of building the capacities of the involved actors, especially farmers. In this respect, Nana et al. (2015) point out the need to develop collective organizational innovations, especially for the management of crop residues.

By reading the papers in chronological order, it is possible to formulate a reflection on the evolution and maturity of the concept of agroecology in Burkina Faso and Niger. It can be noticed that since 2003, there has been a change in the focus of the topics and interests revolving around the concept of agroecology. At first, the greatest interest was of technical nature; it concerned the possibility of addressing some urgent issues related to the yield gap or of great interest for production purposes by applying agroecological concepts and practices. For instance, Osbahr and Allan (2003) focus on the possibility of managing soil fertility by applying agroecological concepts and practices. Subsequently, in line with international reflections on issues relating to biodiversity and to the provision of ecosystem services able to support farm production, attention in the papers shifted to aspects related to functional biodiversity and to the ability of the agroecological approach to improve the management of agroecosystems, namely ecosystem services regarding pest and disease regulation (Ratnadass et al., 2011). Then, in a later moment, the effectiveness of the agroecological approach in addressing food insecurity was widely verified (Masse et al., 2013) and the interest of scholars was on how agroecology can have a positive impact on the whole food system (Lappé, 2013). The reflection on agroecology, therefore, moved on to how far it is possible to push its adoption among farmers and on what are the factors that limit the adoption of agroecological practices (Dowd-Uribe, 2014; Saqalli et al., 2010). From these analyses arose how important is to consider the level of knowledge of farmers and the possibility of accessing ecological knowledge; a possible solution is identified in developing collective experiences that promote the adoption of organizational innovations through the exchange of knowledge and good practices (Nana et al., 2015). As the reflection on agroecology in Niger and Burkina Faso proceeded, the discourse moved on the need to facilitate the agroecological transition of local production contexts and it is enriched with a series of proposals and solutions by scholars dealing with socio-economic aspects. Gross and Jaubert (2019) and Ouédraogo et al. (2019) underline that the greatest difficulty in adopting agroecological practices lies in the need for a deep knowledge of individual environmental and socio-economic contexts. Other scholars highlight the need to activate research and experimentation activities to provide specific answers for each context and case (Guébré et al., 2020; Ouédraogo et al., 2019;

Vidal et al., 2020). Reading such papers provides not only a picture of the research needs on agroecology in both countries, but above all confirms how a joint reflection by local researchers and experts can lead to identifying political solutions that would allow making decisions and allocate funds for specific research priorities. This approach would allow overcoming the problems of adopting agroecological practices and identifying research priorities that provide technical indications suitable for the different production contexts.

CONCLUSIONS

Agroecology is widely recognised as a promising pathway of transition to sustainable agriculture and food systems. Therefore, interest in agroecology has been growing in many developing countries such as Burkina Faso and Niger not only from governments but also international organisations and NGOs. However, it is not clear whether such an increase in interest determined higher attention to agroecology by the research systems in these countries. Therefore, this paper analysed the state of research on agroecology in both countries. The analysis of the scholarly literature confirms the positive impacts of agroecology in terms of food security, biodiversity conservation and rural livelihoods. Agroecology represents a valid instrument to address several environmental (e.g. biodiversity loss, land degradation), social (e.g. food insecurity) and economic (e.g. unemployment, poverty) challenges. However, the literature review also suggests that, despite the recurring discourse on agroecology, there is a research gap. Indeed, there is a low number of articles addressing agroecology in both countries. This implies that the scientific component of agroecology (which along practices and movement constitute the pillars of agroecology) is underdeveloped. This gap in research might hamper the development of the agroecological movement as well as the documentation and dissemination of agroecological practices, thus slowing down the whole agroecological transition process in the region. Indeed, the literature also highlights that agroecology is knowledge-intensive and the need to strengthen the capacities of all actors involved in the agricultural knowledge and innovation system (AKIS), especially farmers. Further investments in research are needed to bridge the existing knowledge gap and unlock the potential of agroecological transition in Burkina and Niger, which is fundamental to move towards sustainable agriculture and food systems that ensure food and nutrition security without undermining the fragile natural resource base. The development of agroecology can contribute to the achievement of the Sustainable Development Goals (SDGs) in Burkina Faso, Niger and Sahel at large.

ACKNOWLEDGMENTS

This work was carried out within the project SUSTLIVES (*SUSTaining and improving local crop patrimony in Burkina Faso and Niger for better LIVes and EcoSystems* - https://www.sustlives.eu), of the DeSIRA initiative (Development Smart Innovation through Research in Agriculture), financed by the European Union (contribution agreement FOOD/2021/422-681).

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Original Scientific paper
10.7251/AGRENG2202061Q
UDC 630:631(469)

PRESENT AND FUTURE AGROFORESTRY LAND SUITABILITY ANALYSIS IN CENTRAL PORTUGAL USING MULTICRITERIA DECISION ANALYSIS

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ABSTRACT

The expansion of monoculture tree plantations typically made up of pine or eucalyptus trees, is a main problem in Portugal. In recent years, monoculture plantations have spread across the country, driven by the pulp and paper industry. These are generally more susceptible to the spread of fire than mixed forests or agroforestry systems, which among other advantages, hold more water and reduces soil erosion. A diverse landscape with a mosaic of different uses and vegetation cover types provides a greater bulwark or natural barrier against large-scale and uncontrollable forest fires. The objective of this study is to determine the suitability for species cultivated in an agroforestry mode in the *Pinhal Interior Sul* region, as an alternative agricultural system, based on the analysis of the soil and climate limiting factors. For this purpose, the biophysical criteria determining the cultivation of common oak (*Quercus robur* L.), cork oak (*Quercus suber* L.), strawberry tree (*Arbutus unedo* L.), and sweet chestnut (*Castanea sativa* Mill.) were processed using a Geographic Information System for the present time and in the face of two future emission scenarios. The analysis was performed by the Analytical Hierarchy Process (AHP). After dividing the problem into hierarchical levels of decision-making, a pairwise comparison of criteria was performed to evaluate the weights of these criteria. The process was completed by validating the consistency of these operations. The AHP was adequate in the evaluation of the tree species' suitability allowing the integration of different criteria. It is therefore essential to be aware of the suitability and resilience of agroforestry systems to meet the need to adapt to climate change.

Keywords: *Agroforestry, Land suitability, Analytic Hierarchy Process, GIS.*

INTRODUCTION

Large-scale rural fires are a worldwide problem that has been studied in the most fire-prone regions of the world (Tedim, Leone & McGee, 2019; Vigna et al., 2021). Portugal is the most affected country by rural fires in Europe in the period between 2011 and 2020, with an average of 17,712 rural fires per year, corresponding to 131,118 thousand hectares of burned area per year, with 63,828 hectares (49%) of forest stands and 58,343 hectares (44%) of bush and natural pastures, and still affecting 8,947 hectares (7%) of agricultural area (APA, 2022). The increased presence of rural fires can be attributed to the transformation of the Portuguese landscape in the last decades, with the forest cover progressively changing to extensive monoculture landscapes of even-aged stands of fast-growing species, dominated by *Pinus pinaster* Aiton. and *Eucalyptus globulus* Labill. (DGT, 2020). Socioecological causes also act together to create the conditions for a greater incidence of burnt areas in certain regions of Portugal, resulting from the demographic changes (the population exodus from rural areas, and the aging population) that led to the expansion of unmanaged or abandoned land. These events eventually prompted the beginning of large fires in Portugal, particularly since the 80s of the XX century (Nunes, Lourenço & Meira, 2016).

In this context, a rural fire prevention strategy through an integrated and ecologically based planning approach needs to be addressed. This approach should include the implementation of sustainable land uses and well-defined management measures to achieve a fire-resilient and sustainable landscape. Those fire-resistant and resilient land uses include agroforestry systems and woodlands mainly composed of native oak species and other native broadleaved species (Magalhães et al., 2021).

Facing the trends of climate change is urgent to address land-use adaptation more coherently and promote the suitability of plant, weather, and soil conditions. Hence, that investigation identifies the suitable areas for agroforestry using a model based on multicriteria spatial analysis AHP. The suitable areas are determined by an evaluation of the climate, soil, and topographical factors and the understanding of local biophysical restraints. In this kind of situation, many variables are involved and each one should be weighted according to their relative importance in the optimal growth conditions for agroforestry systems through multicriteria evaluation and Geographic Information Systems.

Multicriteria decision analysis (MCDA) has been widely applied in various studies in different fields, many of which are published and have been cited by many authors as processes of relevant decision-making. Various techniques of MCDA have been used extensively in rural land-use suitability analysis (Alkimim, Sparovek & Clarke, 2015; Dedeoğlu & Dengiz, 2019; Mighty, 2015; Wotlolan et al., 2021; Zhang et al., 2015, Quinta-Nova & Ferreira, 2020).

In this study, the Analytic Hierarchy Process (AHP) was used as an MCDA technique with a GIS to identify the areas suitable for promoting agroforestry uses in a study area located in the Center region of Portugal, by the integration of several criteria. AHP is an inductive, multicriteria method, effective in the estimation of the likelihood of unique events, and in the face of multiplicity, uncertainty, and the limitation of information. The suitability analysis was examined for the present time and in the face of two future emission scenarios (RCP 4.5 and 8.5) because it is essential to be aware of the suitability and resilience of agroforestry systems to meet the need to adapt to climate change.

MATERIAL AND METHODS

The study-case area is located in the Central Region of mainland Portugal (Figure 1). It has an area of 2079 km^2 and includes seven municipalities: Castanheira de Pêra, Pedrógão Grande, Figueiró dos Vinhos, Sertã, Pampilhosa da Serra, Oleiros and Proença-a-Nova with a very high risk of rural fire (ICNF, 2022). During the period between 2015 and 2020, the burnt area in the case-study area reached 1145 km^2, corresponding to 55% of the study-case area. This subregion is mainly occupied by forest stands of pine trees and eucalyptus (69.4%) and shrubland (15.0%), with only 11.5% corresponding to woodlands, agroforestry, and agriculture.

The Regional Forest Management Plan for the Coastal Center Region of Portugal recommended a set of wooden species suitable for the study area (ICNF, 2019). For this study, we select the following deciduous species of interest for exploration under agroforestry systems: common oak (*Quercus robur* L.), strawberry tree (*Arbutus unedo* L.), cork oak (*Quercus suber* L.), and sweet chestnut (*Castanea sativa* Mill.).

Data under representative concentration pathway (RCP) 4.5 for 2041-2060 was used for future climate analyses, which is obtained from the WorldClim database (Fick & Hijmans, 2017). In the future conditions, we have considered two representative concentration pathways (RCPs) scenarios (RCP 4.5 and RCP 8.5) fitted for 2070.

Emissions in RCP 4.5 (Intermediate scenario) peak around 2040, then declines, resulting in a global temperature rise between 1.1 to 2.6 ºC by 2081-2100 (relative to 1986-2005). In RCP 8.5 (Worst-case climate change scenario) emissions continue to rise throughout the 21st century, resulting in global temperature rise between 2.6 to 4.8 ºC by 2081-2100 (relative to 1986-2005).

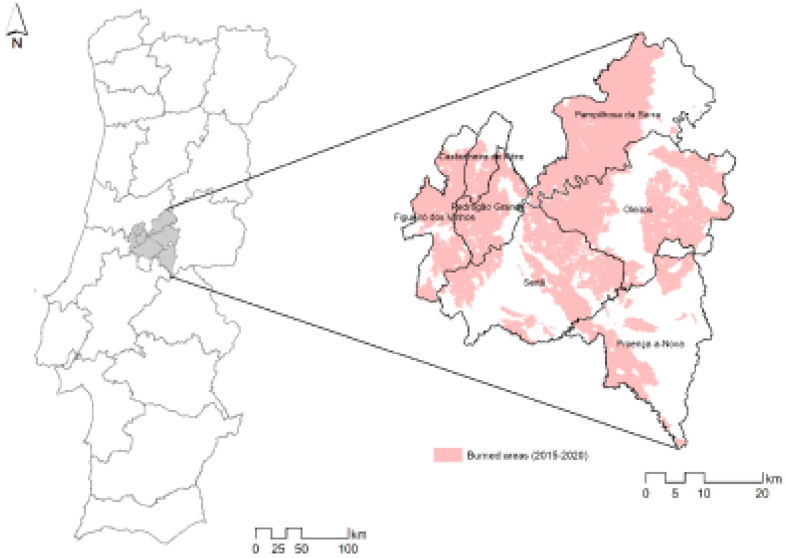


Figure 1. Study area location

The classification of the plant species' suitability resulted from the integration of a set of biophysical criteria based on ecological requirements. Geoprocessing and spatial analysis were performed to geographic data, namely climate (annual mean temperature and annual precipitation), soils (texture, depth, and pH), and altitude. Different references were consulted on the ecological requirements of the species (Ribeiro et al., 2012; Correia & Oliveira, 1999; Correia & Oliveira, 2003).

The different layers corresponding to each criteria were classified in two suitability levels: low to medium suitability (1) and high suitability (2). After creating layers resulting from the reclassification in suitability levels, the general suitability for each species was performed using a multicriteria decision analysis - the Analytic Hierarchy Process - AHP (Saaty, 1987).

The AHP consists of four essential phases: criteria generating and spatial analysis, standardization, and suitability assessment. First, a spatial database was created to include all vector and raster layers and data models. All spatial layers were prepared, and the consistency of coordinates was maintained in ArcGIS 10.8 software. All criteria included in the analysis had to be standardized. Standardization makes all spatial layers constant and in the same measurement units' format (Saaty, 1987). Hence, all vector layers were converted into raster format and the reclassify tool in ArcGIS was used to standardize and assign values for each criterion.

The AHP decomposes a problem, question, or decision, in all the variables that constitute it, in a scheme of criteria and sub-criteria and then makes pairwise comparisons between them (Saaty, 1987).
The pairwise comparison matrix was created using a scale of 1-9 in order to determine the relative importance of each criteria. The matrix format in pairwise comparisons defines $A = [c_{ij}]_{nxn}$ as follows:

$$\begin{bmatrix} c_{11} & c_{12} & \cdots & c_{1n} \\ c_{21} & c_{22} & \cdots & c_{2n} \\ c_{1n} & c_{2n} & \cdots & c_{nn} \end{bmatrix}$$

After generating all pairwise comparison matrices, the vector of weights, w $\{w_1, w_2, ..., w_n\}$ is calculated according to Saaty's eigenvector method. This is followed by two steps to calculate weights: First, normalizing the pairwise comparison matrix $A = [c_{ij}]_{nxn}$ based on the following equation:

$$c_{ij} = \frac{c_{ij}}{\sum_{j=1}^{n} c_{ij}}$$

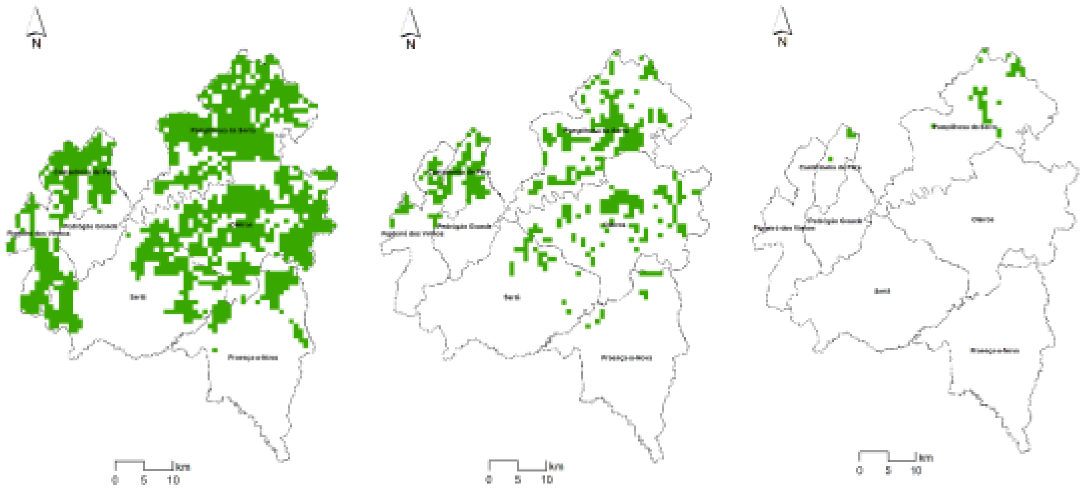
In the pairwise comparison matrix, n refers to the number of elements (Mikhailov, 2003). One of the strengths of the AHP method is measuring the inconsistencies by calculating Consistency Relationship (CR) which is a comparison between the consistency index (CI) and the random consistency (RI) index as follows:

$$CR = \frac{CI}{RI}$$

CR specifies the degree of consistency or inconsistency (Scholl, Manthey, Helm & Steiner, 2005). It denotes the probability that the matrix judgments were made randomly (Saaty, 1987). If the CR value is less than 0.10, the pairwise consistency is fairly acceptable. On the contrary, if the value is higher than 0.10, this indicates inconsistencies in the evaluation and hence the original weights should be recalculated.
In the suitability assessment stage, the weighting linear combination approach is used to produce a composite suitability map. All spatial layers were converted into raster models and the reclassify tool was employed to classify all layers to a standardized measurement suitability scale between 1 and 3, where 1 indicates the least suitable while 3 denotes the most suitable. The weighted overlay technique was performed to combine all weighted spatial layers and produce a suitability map, using the GIS-based AHP extension developed by Marinoni (2004).

RESULTS AND DISCUSSION

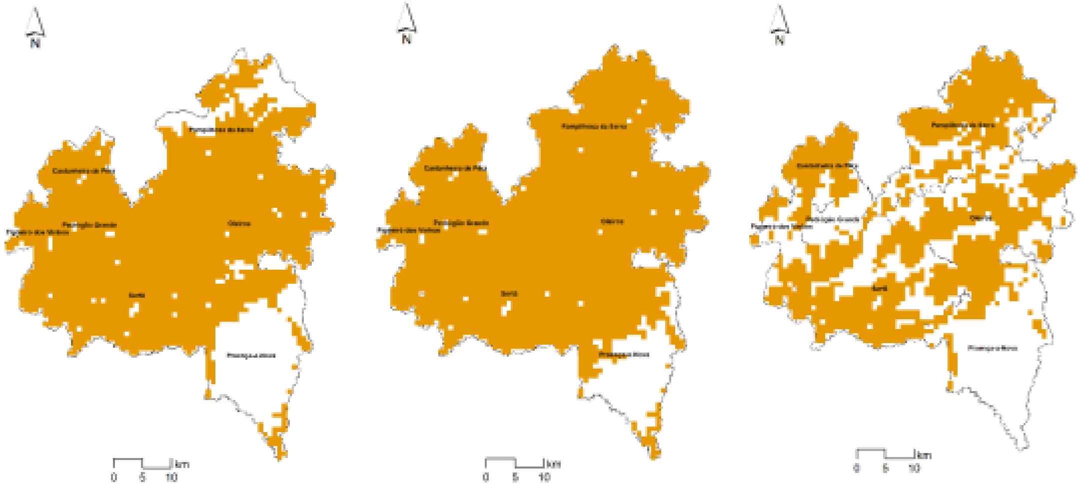
Based on the map analysis (Figure 2), about 861,8 km^2, corresponding to 42.1% of the total area, are classified as highly suitable for common oak in the present situation. For the Intermediate future scenario output (RCP 4.5 - 2070), the total area with high suitability for the common oak decreases to 272.8 km^2 (13.3%) and reduces even more worst-case climate change scenario (RCP 8.5 - 2070) with 24.1 km^2, corresponding to only 1.2% of the total area.



a) b) c)

Figure 2. Common oak highest suitability maps: a) Present; b) RCP 4.5 - 2070; c) RCP 8.5 - 2070.

For the cork oak (Figure 3) in the present the study area presents high suitability in 80.1% of the territory (1639.6 km^2). However, for future scenarios there are notable differences, with an increase of highly suitable area for the intermediate scenario (4.5 - 2070), with a total area of 1812.5 km^2 (88.6%), while for the worst-case scenario the potential distribution of the species drops to 1128.4 km^2 (55.2%) and becomes more fragmented.



a) b) c)

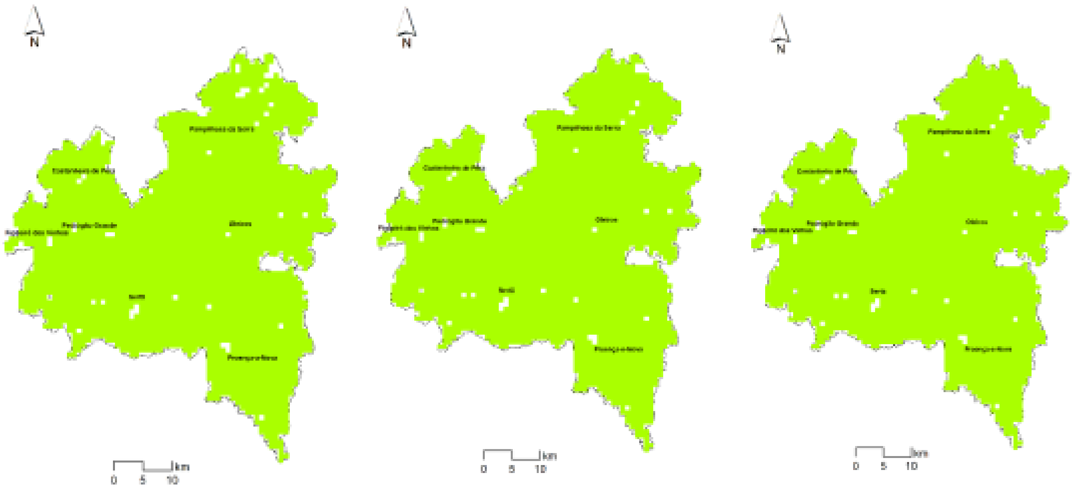
Figure 3. Cork oak highest suitability maps: a) Present; b) RCP 4.5 - 2070; c) RCP 8.5 - 2070.

The high suitability area for the sweet chestnut (Figure 4) in the present corresponds to 1235.2 km^2 (60.4%). For both future climate scenarios the potential high suitability area decreases, with 413.3 km^2 (20.2%) for the intermediate scenario, and 124.0 km^2 (6.0%) for the worst-case scenario.

a) b) c)

Figure 4. Sweet chestnut suitability maps: a) Present; b) RCP 4.5 - 2070; c) RCP 8.5 - 2070.

The high suitability distribution for strawberry tree (Figure 5) occupies practically all the available arable land in the study area (2004 km^2; 97.9%), and will practically not change over time, although there will be a slight increase in both future scenarios, with 2018.5 km^2 (98.7%) for the intermediate scenario, and 2021.9 km^2 (98.8%) for the worst-case scenario



a) b) c)

Figure 5. Strawberry tree suitability maps: a) Present; b) RCP 4.5 - 2070; c) RCP 8.5 - 2070.

CONCLUSIONS

The carrying out of the study on suitable forest wooden species which can be exploited within agroforestry systems in a fire-prone region of Portugal as a way to prevent large-scale rural fires and promote a sustainable land use, using GIS and an MCDA technique, allows the following conclusions to be drawn:

- This methodological approach allowed us to assess the suitability of four forest species in a Central region of Portugal. The AHP was based on a set of criteria contributing to a reflection on the adequacy of those species for the climatic, topographic and soil characteristics of the region.
- The main results obtained indicate that the methodology used could provide a guide map for decision makers to achieve a more sustainable use of the territory facing their ecological limitations and considering future climate scenarios.

- It's important the local authorities that support the farmers create tools to raise awareness and guide them about the global climatic changes and their implications.
- According to the results obtained, that revels that species more adapted to Atlantic conditions like common oak and sweet chestnut will decrease, so it's urgent to promote species more adapted to future climate conditions. On the other hand, the strawberry tree has high suitability in almost all regions in the present and future scenarios.
- For further study is recommend selecting other native species (e.g., holm oak) more adapted to Mediterranean conditions in order to adapt to the bioclimatic shifts towards higher latitudes and/or altitudes.
- It's important to privilege diverse landscapes in the studied region characterized by the mosaic of agrosilvopastoral systems serving as an alternative to the current extensive monoculture landscapes of even-aged stands of fast-growing species.

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Original Scientific paper
10.7251/AGRENG2202071B
UDC 630*44

EFFECT OF FALLOPIA JAPONICA ON SOIL MICROBIAL ACTIVITY DEPENDS ON VARIOUS CLIMATIC AREAS

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ABSTRACT

Biological invasions are one of the main threats to natural ecosystems and the impact of invasive plant species on native species, communities, ecosystems and soil biota has been widely recognized over the last decades. The number of invasive plants and their distribution is increasing in many parts of the world. *Fallopia japonica* (Houtt.) Ronse Decr. (Japanese knotweed) is considered to be one of 100 worst invasive species in the world. The aim of this study was to report that invasive plant species *F. japonica* had an impact on physico-chemical properties and microbial indices in soil ecosystems. The research was carried out in ten research sites in three different climatic areas of Eastern Slovakia. Soil reaction, soil moisture, soil organic carbon, soil basal respiration and soil enzyme activities (FDA, beta-glucosidase, acid and alkaline phosphatases) were determined. Obtained data were compared with uninvaded adjacent sites. Generally, the studied plant invader altered soil parameters, but those changes varied among selected localities. Correlation relationships were found between the individual parameters depending on the location and altitude. The results also suggested that large-scale invasion by *Fallopia* species was, therefore, likely to seriously affect biodiversity and reduce the quality of ecosystems.

Keywords: *Invasive species, Soil indices, Soil enzymes, Climate Areas.*

INTRODUCTION

Soil is a hardly renewable formation that needs to be protected and monitored in changes that would lead to its complete destruction. It is a crucial natural resource, producing food and raw materials, recycles waste, filters and retaining water, ensuring the circulation of substances in nature, maintaining the diversity of animals and plants and shapes the quality of the environment (Forman and Godron, 1986). Soil quality is the ability to maintain and affect the quality of the environment and healthy animal and plant development (Yakovchenko *et al.*, 1996). Soil quality needs to be derived from changes in its parameters - indicators (Wick *et al.*, 2002). As indicators of soil quality, are used microbiological characteristics because they allow an immediate response to different environmental changes (Bobuľská *et al.*, 2021). There are many factors that negatively affect soil quality and one of the factors is human influence (Meena *et al.*, 2020). Currently, biological invasions are critical to human activity. There are many anthropogenic activities that help to spread non-native plant species. Such plant invasions dramatically disrupt native ecosystems, have a massive impact on biodiversity, displace native species and form homogeneous communities and, last but not least, threaten human health. They can have a negative effect on soil properties and thus also affect soil conditions of microorganisms (Ehrenfeld, 2003; Belnap *et al.*, 2005).

Measuring and evaluating soil quality is a relatively big problem. As for indicators are often used biological characteristics that must be sensitive enough to reflect changes in the soil and had a high informative value (Fazekašová and Bobuľská, 2012). The most effective indices are microbiological characteristics, which thanks to their reactivity, distribution, generation time and diversity of the soil microflora allow for immediate response to various changes (Bobuľská *et al.*, 2021). Among the important microbial parameters include soil respiration, soil microbial biomass, soil enzyme activity, fixation of biological N_2, etc. (Fejér and Bobuľská, 2015). The activity and composition of the microbial community are directly affected by the abiotic factors (soil temperature and humidity, nutrient availability), and indirectly affected by biomass and plant diversity (Khan *et al.*, 2018). One of the important components of the soil ecosystem are soil microorganisms. Although microorganisms are the smallest living system, their activity it is very rich and important. They play an important role in the decomposition of plants and animal residues, organic mineralization, humus formation and soil aggregates, maintaining the balance of the soil ecosystem (Wang *et al.*, 2020) and are appropriate and sensitive a tool for predicting changes in the quality of monitored soils (Vinhal-Freitas *et al.*, 2017).

The objective of the study was to assess the impact of invasive plant species on soil properties, with an emphasis on biochemical indices, on soil ecosystems in various climatic areas.

MATERIAL AND METHODS

Site description

The research was carried out on three different climatic areas during May 2021. All research sites have been significantly invaded by *Fallopia japonica* (Japanese knotweed), which greatly affects the characteristics of the soil ecosystem.

Cold climatic area (49°18'06" N, 20°41'19" E) was characterized by annual rain precipitation of 800-900 mm with the average temperature of 6-7°C. The dominant soil type are Cambisols.
Middle climatic area (48°56'09" N, 21°54'24" E) climatic area relatively warm, slightly dry, highland, continental with an average annual rainfall of 700-800 mm with the average temperature of 8-9 °C.
Warm climatic area (48°22'52" N, 20°00'52" E) was characterized as warm, very dry, basin with an average annual rainfall of 600-700 mm with the average temperature 9-10 °C. The dominant soil types for the second and third research localities are skeletal Fluvisols.

Invasive species description

Fallopia japonica (Houtt.) Ronse Decr. (Japanese knotweed) belongs to Polygonaceae family, is considered to be one of 100 worst invasive species in the world. It was introduced into North America and Europe in the 19th century as an ornamental plant and cattle fodder. In its native range of Japan, Taiwan and Korea. This invasive plant is found growing in sunny places on hills, high mountains and along road verges and ditches. Considered an aggressive invader in Europe, the United States, and Canada, *F. japonica* spreads by clonal, rhizomatous growth and can quickly form a monoculture. *F. japonica* can survive very harsh conditions with a pH range of 3.0-8.5, and an ability to survive extreme heavy metal and salt pollution and areas with low available nitrogen (Abgrall *et al.*, 2018; Sołtysiak, 2020).

Soil sampling

At all three research sites (cold, middle and warm climatic areas), 20 localities were identified (10 for invaded and 10 for non-invaded sites) measuring 1m x 1m, which represented a total of 60 bulk of average soil samples. Each sample consisted of three subsamples taken from a depth of 0.1-0.2 m, which were subsequently homogenized. All soil samples were transferred to a laboratory in plastic bags, sieved with a mesh size of 2 mm and stored in a refrigerator before the analyzes. Part of the samples were air dried at the room temperature to determine selected chemical parameters.

Soil physico-chemical and microbial indices

Dry soil samples were completely crushed in a porcelain crucible. These soil samples were used to measure soil reaction, total nitrogen content and organic carbon content. Soil pH was detected in a 1:3 mixture of soil and 0.01 M $CaCl_2$ solution using a digital pH meter. Soil organic carbon (SOC) and total nitrogen content (Ntot) were determined by the Turin's method (Fiala *et al.*, 1999). Gravimetric soil moisture (SM) was calculated on 10 g of fresh subsamples after drying in a 105°C oven for 24 h. Soil basal respiration (SBR) was measured by the CO_2 released from field moist soil in hermetically sealed flasks at 25°C for 48

hours (Alef and Nannipieri, 1995). Enzymatic activity assays, beta-glucosidase (BGL) (Eivazi and Tabatabai, 1988), fluorescein diacetate (FDA) (Green *et al.*, 2006), acid phosphatase (PHOSac) and alkaline phosphatase (PHOSal) (Grejtovský, 1991), were determined using field-moist soil samples using specific substrates of each enzyme. All determinations were performed in duplicate. The corresponding controls were done for each soil and enzyme activity by the same analytical analysis described, but without the addition of the substrate at the moment of initiating the enzyme reaction. Activity of all enzymes was measured in a spectrophotometer crating a reference curve.
Statistical evaluation of the obtained data was performed using Statistica 12 programme. All statistical operations were performed in the PAST 4.03 programme. Data were transformed before log+1 analysis.

RESULTS AND DISCUSSION

Invasive plant species may have the strong impact on several soil properties. The results revealed that soil reaction did not significantly change between invaded (6.9) and non-invaded sites (6.2). The same trend was observed for total nitrogen content, where average values in invaded sites reached 0.39% and in non-invaded sites 0.34%. Statistically, the content of organic carbon and soil moisture showed significant impact of invasiveness. Selected invasive species was able to slightly increase soil moisture (20.7%) compared to the control sites (17.3%). Very important soil chemical parameter organic matter, that affects quality of soil ecosystems was also significantly affected by plant invasion. The content of organic carbon showed higher average values in invaded sites (3.1%) compared to the non-invaded sites (1.84%). Generally, invaded soils are characterized by increased C and N stores and microbial activity (Stefanowicz *et al.*, 2017) which partially corresponds to our results.
The results of soil microbial indices also showed some differences between invaded and non-invaded sites. The study of Čerevková *et al.* (2019) showed a decrease of soil respiration rate affected by *F. japonica*, that has also been demonstrated in our research (Figure 1). The average value of soil respiration was statistically significant lower in invaded soils compared to non-invaded. The study of Koutika *et al.* (2007) showed that invasion of F. japonica reduced the soil C respiration and created soil organic matter which decomposed slowly. Figure 1 represents soil microbial indices in invaded and control sites. The same trend was found for acid phosphatase; it was lower (but not significantly) in the invaded than the non-invaded sites. Alkaline phosphatase was affected by *F. japonica* invasion in different ways in the different climatic areas, but no significant differences were found. Similar trend was also observed with the activity of FDA and beta-glucosidase. Their values were higher under presence of invasive plant species compared to the non-invasive sites. The analysed microbial characteristics in our study revealed that enzymatic activity was slightly higher in all ecosystems invaded by *F. japonica* compared to the uninvaded ones, suggesting that invasion did not significantly affect microbial activity in soil ecosystem. This was in line

with the measured enzyme activities, which remained unaffected by *F. japonica* invasion probably due the unchanged soil acidity, a factor most affecting activity of soil enzymes (Dassonville *et al.*, 2011).

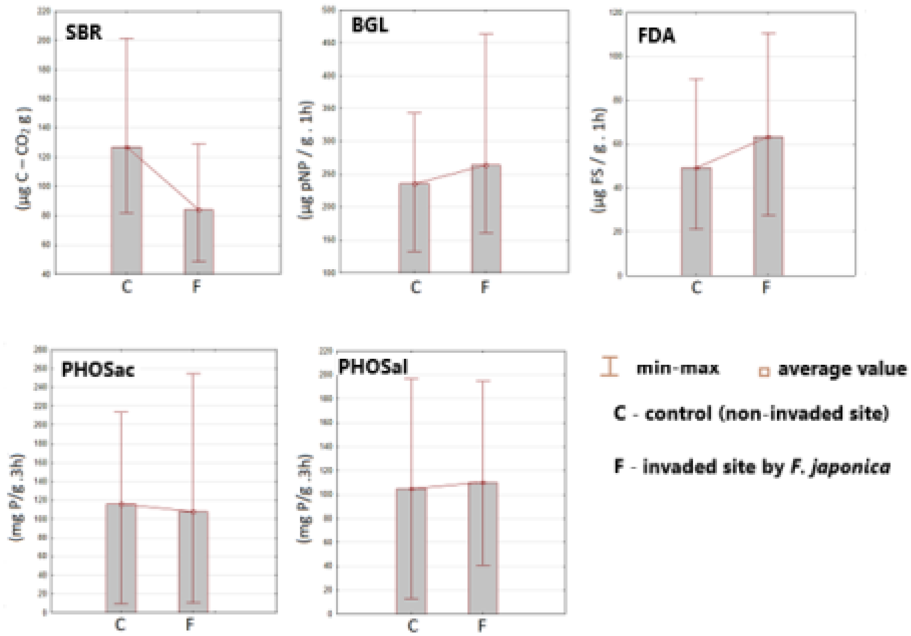

Figure 1. Average values of soil microbial indices in invaded and non-invaded (control) sites.

Native flora, as well as actual weather conditions, soil and ecosystem type, date of soil sampling, etc., are probably responsible for the variable impacts of invasion on soil acidity and moisture among studies. *F. japonica* forms dense stands that prevent other species from growing. The study of Kappes *et al.* (2007) and Čerevková *et al.* (2019) evaluated the impact of this plant exotic species on soil fauna and concluded that *F. japonica* significantly affect the faunal community, more that physical and microbial properties, regardless of selected habitat. Their study also concluded that large-scale invasion by *Fallopia* species is therefore likely to seriously affect biodiversity and reduce the quality of riparian ecosystems for amphibians, reptiles, birds and mammals whose diets are largely composed of arthropods.

Activity of microorganisms is also affected by climate that was also shown in our study. Figure 2 represents average values of microbial parameters according to different climatic regions. Statistically significant differences were shown for all monitored parameters according to the climatic area ($p<0.05$). The study of some authors (Rilling *et al.*, 2019, Zhou *et al.*, 2019) show that climatic factors have big impacts on soil parameters development, especially soil organic carbon. Our study revealed that soil microbial parameters, except activity of BGL, reached higher values in warm conditions compared to the cold region. This result corresponds with the finding of Misiak *et al.* (2021). Interestingly, the enzymatic activity, in which the enzyme activity such as FDA, acidic and alkaline phosphatase was lowest in the coldest climate region compared to others. High precipitation and low temperature may result in reduced activity of microorganisms. Statistically significant differences have been demonstrated in soil reaction, organic carbon content, soil respiration and the FDA in invaded soils compared to non-invaded regardless of climatic area. It follows that biological invasions affect the soil environment and are capable of significantly change soil properties.

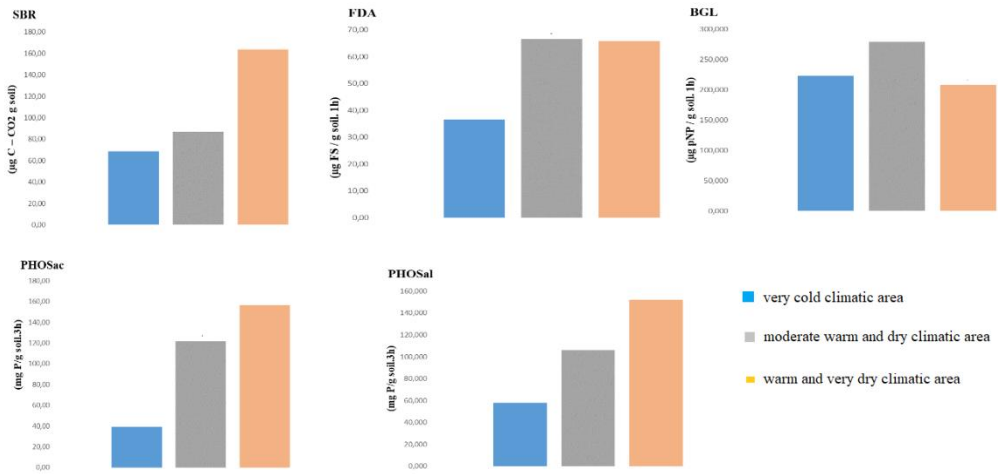

Figure 2 Average values of soil microbial indices in three different climatic regions.

CONCLUSION

The results showed that invasive *Fallopia japonica* have been able to alter the values of biochemical soil properties in soil ecosystem. There have also been recorded specific correlations among single parameters and region, which means that the effect of climatic conditions and different altitudes also impact microbial activity and other soil parameters.

ACKNOWLEDGMENTS

This study was supported by the project of Slovak Scientific Agency VEGA No. 2/0018/20.

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Original Scientific paper
10.7251/AGRENG2202079K
UDC 631.878:631:452

AMARANTHUS CAUDATUS AS A PUTATIVE BIOENERGY PLANT FOR PREPARATION OF BIOCHAR TO ENHANCE BIOMASS PRODUCTION

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ABSTRACT

Conversion of low-density wood into biochar faces many problems and results in ash formation. Development of three-container indirect heating retort can help to combat with this problem and could lead to production of good quality biochar. To validate, the *Amaranthus spp*. biomass has been dried, ground <0.1 mm and put it in a small container which was placed in a bigger container and finally surrounded by a large stainless-steel container which was filled with feedstock and allowed to combust for two hours under limited oxygen condition. Analysis of prepared biochar showed alkaline pH, high water holding capacity and electrical conductivity. Pot scale study was performed on *Amaranthus caudatus* L. var. gibbosus rubra which showed that application of 1% biochar has not only enhanced fresh and dry shoot biomass but also improved biometric growth parameters (length of shoot and number of leaves) as compared to control plants. The study showed that the application of a low dose of biochar from *Amaranthus spp.* improved the growth of *A. caudatus* plants and thus increased the lignocellulose content which can be used for bioenergy production.

Keywords: *Amaranthus, biochar, retort, plant biomass, lignocellulose*.

INTRODUCTION

Continuous use of non-renewable fuels is a serious global concern. The best possible way to minimize the problem is to replace these with sustainable resources. Currently, lignocellulosic crop, which is the most abundant available biofuel feedstock on earth, is being used to reduce this dependency. In one way application of pesticides and fertilizers has resulted in enhanced productivity however, on the other side they contaminated the soil with heavy metals (Tripti et al., 2017). High lignocellulosic biomass plants can help in mitigating such problems and can help in many other ways. Firstly, they can immobilize metals by sequestering them in roots (Limayem and Ricke, 2012). Secondly, the biomass can be used for preparation of biochar and biofuel by enzymatic hydrolysis. Biochar is a carbonaceous material produced by burning in limited oxygen condition (Hussain et al., 2017; Vecstaudza et al., 2017). It has very high surface area, rich in macro- and microelements, with a high ability to absorb metals. It is also a suitable carrier for the immobilization of microorganisms because of its high porosity (Li et al., 2022). Since high density woods produces good quality biochar, low density woods often convert into ashes. Thus, it is very crucial to know at what temperature the biomass should be burnt and for this, a good quality biochar retort is required. There are three types of pyrolysis conventional or fast, slow and ultra-fast or flash which can be direct or indirect. Both direct and indirect heating of biomass in a retort is a popular method of production of biochar however direct method fail sometimes in case of low-density wood as biomass will convert in ashes at higher temperatures. An approach has taken to develop and construct a retort which can produce good quality biochar. To check the efficiency of produced biochar a pot scale plant growth experiment with fixed percentage of biochar as additive is required which will help to evaluate the biometric growth and developmental parameters of plant.

Thus, the aim of the study was to prepare a three-stage container-based biochar retort to produce biochar with minimum ash content. In addition, to evaluate the efficiency of produced biochar the pot scale experiment was performed to see its efficiency on *Amaranthus caudatus* L. growth and development.

MATERIALS AND METHODS

To produce a high-quality biochar from *Amaranthus* biomass, a suitable torrefaction oven is must. A torrefaction oven/pyrolysis retort was prepared using cylindrical stainless-steel containers of different sizes. The retort comprises of 3 containers with lids and a chimney (40 cm height) attached at the top to remove the fumes and gases during operation of retort. The volume of the bigger, medium and small container was 1.27×10^5 cm^3, 0.62×10^5 cm^3 and 0.01×10^5 cm^3, respectively. The dry wood of tree was collected and dried to use it as feedstock (fuel) for the retort.

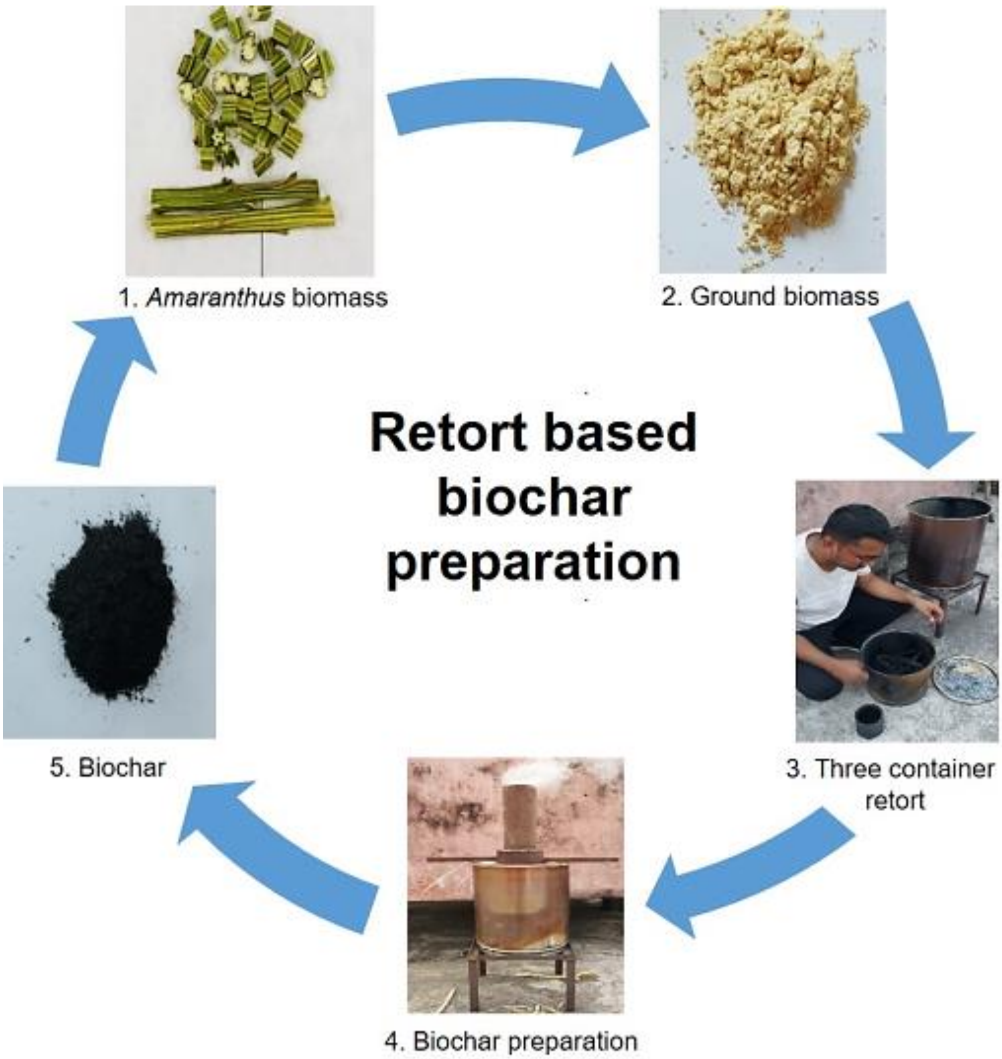

Figure. Schematic diagram showing conversion of biochar from biomass using a retort.

A total of 8 small holes were made at the bottom of the big and medium container to allow a limited amount of oxygen to pass through the bottom to the upper side to burn the feedstock and biomass (*Amaranthus* plants were collected from the Botanical garden of the Ural Federal University, Ekaterinburg) filled in medium container for a longer duration of time to produce a better-quality biochar. This process is called as indirect heating of biomass were biomass burns slowly for a longer duration of time. Since the ground raw biomass can convert into ash if burned directly, they were placed in small container to prepare a biochar with low ash content and minimum loss in ignition and pyrolysis was performed during autumn season of 2021 in the pyrolysis unit of the University campus A schematic representation of the biochar production process is presented in Figure.

Amaranthus caudatus L. var. gibbosus rubra a plant rich in lignocellulose content and can produce big biomass in a short period of time. The seeds were collected from the Botanical garden of University. The healthy seeds were selected and soaked overnight in distilled water. Next morning, a 25-well seedling tray, each well filled with 20 g of soil was sown with one seed. A total of 3-replicates of such seedling trays were prepared. The plants were watered every evening and allowed to grow for next 20 days under natural day:night regime. Once the seedlings reached a height of 7–10 cm, they were transferred in big pots of 10 L soil capacity. A total of 12 pots were prepared and divided into two sections to prepare two treatments, i.e., garden soil (as a control) and another with 1% biochar. The plants were watered regularly every evening and allowed to grow for a period of another 40 days. At harvest, shoot fresh and dry biomass, length, and number of leaves were determined (Kumar et al., 2021).
The seeds from plants were collected, and then harvested to collect the biomass for further analysis. The stem of plants was carefully separated from root and washed several times to remove any adhered soil particles. The samples were dried to obtain moisture-less biomass and cut into small pieces of about 1 cm using an electric cutter and further ground below < 0.1 cm using a 30000× high speed grinder. The prepared biochar was characterized for its physicochemical parameters such as water holding capacity, pH, and electrical conductivity (EC) (Kumar et al., 2017).

RESULTS AND DISCUSSION

The stainless-steel constructed biochar retort was able to withstand the high temperature ranged between 200–500 °C. It was observed that the feedstock was completely burned in the first hour of operation whereas the energy transferred into second container remain ignited for next one and half hour. The feedstock was mainly converted into ash whereas the ash content in the second container was lower than feedstock. The ground biomass kept in the small container converted into high carbon rich (>60%) biochar. The produced biochar showed an alkaline pH of 9.75 ± 0.25, with water holding capacity 52.00 ± 4.40 and ES of 10.05 mS/cm which showed its suitability in agroecosystem especially for acidic soil.
The pot scale study was conducted using *A. caudatus* plant species and shoot fresh and dry biomass and length was recorded in the presence and absence of biochar (Table). It was observed that application of biochar (1%) has significantly improved the biomass of the plant. Both dry weight and fresh weight has improved by 21 and 33%, respectively. It also helped in elongation of shoot length which was increased by 24%. Tripti et al. (2017) and Kumar et al. (2021) had also reported that application of biochar enhanced the plant growth and development.

Table 1. Shoot fresh and dry biomass, length and number of leaves in the control and biochar treated soil (Mean ± SD, n = 6) of *A. caudatus*.

Treatment	Fresh biomass, g	Dry biomass, g	Length, cm	Number of leaves, n
Control soil	35.0 ± 8.0	11.5 ± 1.5	18.5 ± 2.0	5.0 ± 1.0
Soil + 1% biochar	52.5 ± 4.5*	14.0 ± 1.2*	25.5 ± 1.5*	8.0 ± 1.0

**Significantly different according to the independent sample t-test at p < 0.05.*

Altogether, the detailed methodology for production of biochar and its use in plant growth experiment reported will give a clear idea for early researchers, scientists, and stakeholders to produce biochar in most efficient manner which can be used in agriculture to reduce dependency on chemical fertilizers and exhaustible fuels.

CONCLUSIONS

The study showed a detailed pictorial method for preparation of retort for the generation of biochar using plant biomass. The three-container biochar retort showed a significant result in production of biochar by minimizing the ash content. Moreover, the biochar produced showed a high water holding capacity with alkaline pH and high electrical conductivity which showed ability for its use in acidic environment. A pot scale experiment using biochar prepared from *Amaranthus spp*. showed enhanced shoot fresh and dry biomass as well as length depicting its role in plant growth improvement which could help to increase the lignocellulose content in *Amaranthus caudatus* plant for bioenergy production.

ACKNOWLEDGMENT

The research was supported by the Russian Science Foundation grant No. 21-76-00011, https://rscf.ru/project/21-76-00011. We also acknowledge Prof Maiti S.K., Department of Environmental Science and Engineering, IIT-ISM, Dhanbad for his support and suggestions.

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Original Scientific paper
10.7251/AGRENG2202085M
UDC 630*17:582.632.2(439)

LONG-TERM DYNAMICS OF SUBCANOPY LAYER AS NEW LAYER IN AN OAK FOREST OF HUNGARY

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ABSTRACT

Structural dynamics of the shrub layer were analysed in a Hungarian oak forest after the serious oak decline pandemics. Vertical foliage distribution changed in the understory and a new subcanopy layer appeared below the oak canopy in the last decades. This paper focuses on the following questions: (1) how have the new foliage layer developed after oak decline? (2) Which woody species are the most frequent in this layer? (3) How have the mean sizes of these species changed? The forest association in the monitoring site is *Quercetum petraeae-cerridis* with *Quercus petraea* Matt. L. (sessile oak) and *Quercus cerris* L. (Turkey oak). The site was subdivided into 144 permanent subplots. Woody individuals were classified as subcanopy trees between 8.0–13.0 m in height. Measured structural parameters were carried out in the period 1982-2017. Three woody species, *Acer campestre* L. (field maple), *Acer tataricum* L. (Tatar maple) and *Cornus mas* L. (European cornel) played a key role in the new layer and their height was between 8.0–13.0 m or higher than 13.0 m. The density of species in this layer increased considerably between 1982 and 2002. The most frequent woody species was *A. campestre*. The mean height, diameter and mean cover of the dominant woody species increased significantly after the decreasing oak density. Our results suggest that the mixed oak forest responded to oak decline with significant structural rearrangement in the shrubs and three woody species compensated for the remarkable foliage loss in the canopy. These species formed a new subcanopy layer.

Keywords: *Shrub community, Oak decline, New foliage layer, Acer campestre, Dominant woody species.*

INTRODUCTION

Oak decline has been described as a widespread and complex phenomenon worldwide in many countries (Tomiczek, 1993; Sonesson and Drobyshev, 2010). Various abiotic (air pollution, nitrogen eutrophication, soil chemical stress, climatic extreme events, site conditions) and biotic factors (insect defoliation, borer attack, infection by pathogenic fungi, microorganisms) have been related to the serious oak decline in the world (Thomas *et al.*, 2002). An increase in the death of oak trees has been observed in many regions of Hungary since 1978 (Igmándy *et al*., 1986). In the Síkfőkút forest stand species composition of the canopy was stable until 1979 and the healthy *Quercus petraea* Matt. L. (sessile oak) and *Quercus cerris* L. (Turkey oak) also remained constant. Oak decline was first reported in 1979–80 and by 2017, 62.9% of the oak trees had died in the mixed-species oak forest stand (*Quercetum petraeae-cerris* Soó 1963). This means that the oak trees density decreased from 816 living trunks to 651 trunks in the first decade. The oak decay continued at a variable rate in the following decades, from 651 - through 408 trees in 1988 and 372 trees five years later - to 303 healthy trunks by 2017. Tree decline resulted in an opening of the canopy. Many papers have reported that oak mortality is key factor influencing the structure and dynamics of forest community (Moraal and Hilszczanski, 2000; Woodall *et al*., 2005).

Relatively few studies deal with shrub layer dynamics after oak death and the possible relation between trees and shrubs (Légaré *et al.*, 2002). Understory and overstory relationships are complex but are dominated by the canopy layer condition and structure (Burrascano *et al*., 2011; Burton *et al*., 2011; Cutini *et al*., 2015). Shrub layers of forest ecosystems change dynamically and respond sensitively to the environmental changes (Chipman and Johnson, 2002; Rees and Juday, 2002). They are strongly related to the composition and structure of the overstory (Klinka *et al.*, 1996; Palik and Engstrom, 1999). The shrub community is affected by light availability when the canopy is closed (Légaré *et al*., 2002), leading to negative correlations of shrub species richness and/or cover with the increase of tree basal area (Hutchinson *et al.,* 1999). Shrub species play a major role in the cycles of some essential nutrients, including the dynamics of carbon,nitrogen and potassium (Gilliam, 2007). The shrub layers are directly contributes to forest biodiversity (Kerns and Ohmann, 2004; Čermák *et al.,* 2008), including compositional and structural diversity, enhancing the aesthetics of forest ecosystems and helping to protect watersheds from erosion (Alaback and Herman, 1988; Halpern and Spies, 1995; Muir *et al.,* 2002). The consequences of serious oak decline cause notable changes in the light and stand thermal conditions of forest community which led to structural changes of the shrub layer (Chapman *et al.,* 2006).

Misik *et al*. (2014) described the dynamics behind the increase in the sizes of some woody species and the structure of the new subcanopy layer below the oak canopy. This paper focuses on the following questions: (1) how have the new foliage layer developed after oak decline? (2) Which woody species are the most frequent in this layer? (3) Finally, how have the mean sizes of these species changed?

MATERIAL AND METHODS

Study area The nature reserve research site (Síkfõkút Project) was established in 1972 by Jakucs (1985) and is located in the Bükk Mountains (47°552 N, 20°462 E) in the north-eastern part of Hungary at an altitude of 320-340 m a.s.l. (Figure 1A). Mean annual temperature is 9.9 °C and mean annual precipitation typically about 500-600 mm. Descriptions of the geographic, climatic, soil conditions and vegetation of the forest were described in detail by Jakucs (1985, 1988,). The common forest association in this region is *Quercetum petraeae-cerridis* (Soó, 1963) (sessile oak-Turkey oak) with a dominant canopy of *Q. petraea* and *Q. cerris*. Both oak species are important native deciduous tree species of the Hungarian natural woodlands. Long-term dynamics of shrub community are described among others in works of Misik *et al*. (2013, 2014, 2017, 2020). The plots under study were made up of evenly aged temperate, mixed species deciduous forest that was at least 110 years old and had not been harvested for more than 55 years.

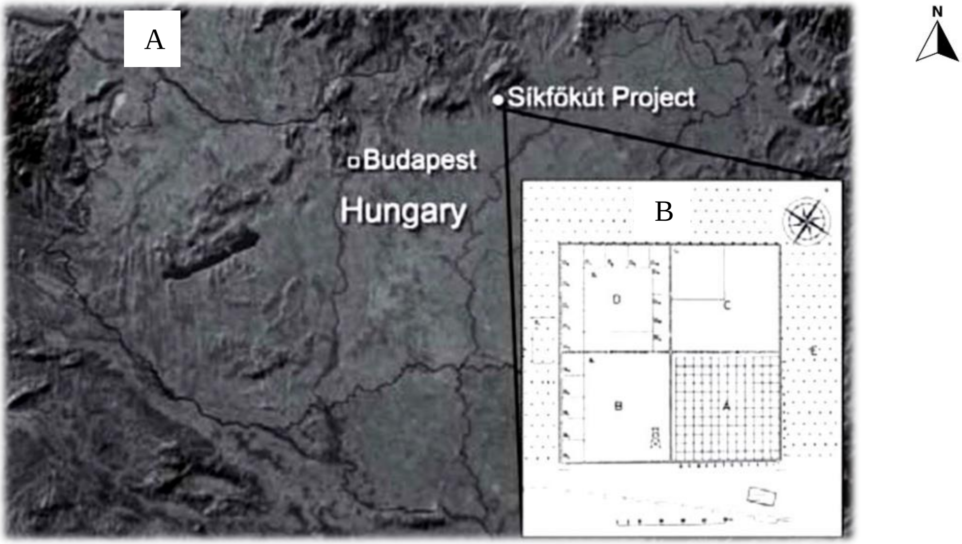

Figure 1.A. Location of the study area in Hungary. B. Study site location with plots.

Sampling and data analysis The condition of the subcanopy layer was monitored on an "A" monitoring plot at the research site, 48 m × 48 m in size; the plot was subdivided into 144 4 m × 4 m permanent subplots (Figure 1B). The "A" plot with many subplots can be found at the bottom right of the Figure 1B. The plot was established in 1972; the new layer inventories took place in 1982, 1988, 1993, 1997, 2002, 2007, 2012 and 2017, during the growing seasons.

In the new foliage layer were classified as subcanopy individuals when between 8.0–13.0 m in height. In monitoring plot the following measurements were carried out: species composition, species density; height, diameter and cover of each subcanopy species. The species' density was also determined in plot and the data was extrapolated for one hectare. Plant height was measured with a scaled pole with an accuracy of 5.0 cm and shoot diameter at 5.0 cm above the soil surface with a digital caliper and the measurement results were averaged. The foliage map was developed in a GIS environment. Based on the digitized map we estimated the foliage area of subcanopy trees with the Spatial Analysis Tools - Calculate Area function of the GIS.

The experimental data were analysed by correlation analysis to investigate the possible effects by the density, height, diameter and cover of subcanopy species on oak tree density (SPSS Statistics 19, Tulsa, USA). We used only the measured oak canopy density to the statistical analysis, because canopy cover has only been measured twice since 1972. On the other hand, oak trees have got typically monolayer foliage and they cannot significantly increase their foliage size in the gaps. In other words, the decreasing oak trees density is strong related to the oak canopy cover Statistical analysis was performed using the PAST statistical software and significant differences for all statistical tests were evaluated at the level of $^{*}P < 0.05$; $^{**}P \leq 0.01$; $^{***}P \leq 0.001$. There was no significant correlation found between the test variables at $^{n.s.}P \geq 0.05$.

RESULTS AND DISCUSSION

From the starting of oak decline, 3 native woody species were identified across the entire study area in the new subcanopy layer; *Acer campestre* L. (field maple), *Cornus mas* L. (European cornel) and *Acer tataricum* L. (Tatar maple) were present continuously as subcanopy woody species in the forest stand. *A. tataricu*m and *C. mas* individuals were not detected between the subcanopy and oak canopy layer in the last 35 years. The total density of woody species in these foliage layers increased remarkably, from 79 to 299 individuals in 3 decades. Woody species with the highest mean density in the new foliage layer was *A. campestre* with 141 shoots per hectare; followed those *C. mas* and *A. tataricum* with 17 and 11 ind. ha^{-1} (Table 1). Despite the decreasing oak density new and/or invasive species could not establish themselves in the forest community, because some native species of the understory would respond positively to change of light, thermal and moisture condition.

Table 1. Species composition and density (ind. ha^{-1}) in the subcanopy layer and under the oak canopy on the monitoring plot between 1982 and 2017.

species	**subcanopy layer (8.0-13.0 m)**							
	1982	**1988**	**1993**	**1997**	**2002**	**2007**	**2012**	**2017**
A. campestre	61	84	113	187	204	139	204	139
A. tataricum	9	4	0	13	13	17	17	13
C. mas	9	4	4	26	13	22	17	44
species	**between subcanopy and canopy layer (> 13.0 m)**							
	1982	**1988**	**1993**	**1997**	**2002**	**2007**	**2012**	**2017**
A. campestre	0	4	13	9	13	104	61	91
sum	79	96	131	235	243	282	299	287

Mean height values of subcanopy woody species changed between 8.03 and 11.40 m in the monitoring plot. The lowest and highest parameters both were recorded by A. tataricum on the basis of a few individuals. Mean height of A. campestre - as the most common species in the subcanopy – shows continuous increasing; there are two exceptions: year of 1997 and 2012. It was observed similar decreasing in height and diameter sizes of all woody species in the subcanopy layer in 2012. The size values of A. campestre increased considerably, but mean height and diameter of A. tataricum and C. mas decreased with minor stops in the last decades, especially in the last 10 years (Table 2). Acer species and C. mas responded positively to the serious oak decline. In the study site, oak decline resulted decreasing canopy cover size and led to the notable height growth of the three woody species from the shrub layer; this phenomenon is called the "Oskar"-strategy (Silvertown, 1982). Acer species typically is a genus that displays this characteristic in such circumstances.

Table 2. Long-term tendency of species' sizes (mean ± S.D.) in the subcanopy layer on the monitoring plot between 1982 and 2017.

species	**mean height in the subcanopy layer (m ± S.D)**							
	1982	**1988**	**1993**	**1997**	**2002**	**2007**	**2012**	**2017**
A. campestre	8.97±0.7	9.46±0.9	9.79±1.8	9.63±1.4	9.92±1.5	10.32±1.6	9.59±1.2	10.45±1.6
A. tataricum	8.23±0.3	8.18±0.0	-	8.73±1.2	8.03±0.0*	11.4±0.0*	8.49±0.4	8.48±0.4
C. mas	9.25±0.4	9.34±0.0	9.00±0.0	8.29±0.6	8.40±0.5	8.22±2.1	8.05±0.0	8.39±0.4
species	**mean diameter in the subcanopy layer (cm±S.D)**							
	1982	**1988**	**1993**	**1997**	**2002**	**2007**	**2012**	**2017**
A. campestre	9.37±1.9	10.11±1.3	10.43±2.0	12.64±2.8	14.48±6.0	14.44±4.9	14.37±5.4	12.19±3.3
A. tataricum	6.82±0.8	7.45±0.0	-	8.53±0.6	10.03±3.2	10.75±0.0	9.50±3.0	6.55±0.3
C. mas	6.74±1.3	7.86±0.0	8.90±0.0*	9.03±2.5	9.69±2.0	13.07±4.9	9.64±1.4	8.54±0.9

*On the basis of a single individual.

Mean cover of *A. campestre*, *C. mas* and *A. tataricum* woody species increased rapidly after the beginning of oak decline. Subcanopy foliage cover is most often dominated by *C. mas*, especially from 2002. Cover values increased continually with *C. mas* individuals, but the mean cover of maples fluctuated (lonely *A. tataricum* individuals were not considered) between 11.20 and 18.30 m^2 between 1993 and 2007. Mean cover of *C. mas* displayed high variation over the last one decade. In 2012 extraordinarily large decreasing of mean foliage cover of species was measured in the new layer (Table 3). The most important reason of this foliage loss was the extreme weather condition, because the summer was very hot and extreme dry in 2012 (Sippel and Otto, 2014).

Table 3. Long-term tendency of species' cover (mean±S.D.) in the subcanopy layer and under the oak canopy on the monitoring plot between 1982 and 2017.

species		**mean cover in the subcanopy layer (m^2 ± S.D)**			
		1982	**1988**	**1993**	**1997**
A. campestre	8.0-13.0 m	4.83±2.11	7.75±8.75	11.67±3.43	18.27±6.11
	> 13.0 m	-	-	25.43±5.76	-
A. tataricum		3.43±1.46	4.50±4.00	-	11.23±1.60
C. mas		3.64±1.13	8.50±7.00	10.38±0.00*	18.99±2.23
year		**2002**	**2007**	**2012**	**2017**
A. campestre	8.0-13.0 m	12.00±2.90	14.56±2.81	8.26±6.22	18.10±11.50
	> 13.0 m	30.24±5.32	28.14±6.93		23.33±15.19
A. tataricum		18.19±0.00*	31.74±0.00*	3.47±2.21	9.58±6.37
C. mas		27.03±1.90	34.76±5.99	11.61±5.59	19.52±5.65

*On the basis of a single individual.

We found a non-significant correlation between oak tree density and density condition of the *A. tataricum* and *C. mas* subcanopy species ($P > 0.05$). Low and negative significant relationship was between oak density and field maple and total subcanopy density ($P \leq 0.05$). Mean height and shoot diameter increment of *A. campestre* and mean height of *C. mas* correlated to oak tree density ($P \leq 0.05$). Mean sizes of *A. tataricum* and mean diameter of *C. mas* specimens increased, but non-significantly after tree decline ($P > 0.05$). The regression analysis did show a low significant association between oak canopy density and mean cover of *A. campestre* ($P \leq 0.05$). The statistical analysis recorded that after large-scale oak decline, the mean cover of *A. tataricum* and *C. mas* woody species had increased remarkably but not significantly ($P > 0.05$) (Table 4).

Observations from mature *Quercus*-dominated forests suggest throughout the eastern United States that an important part of these forests are undergoing significant compositional transformation. *Quercus* spp. are being replaced in the understory by species such as *Acer rubrum* L. (red maple) and *Acer saccharum* Marshall (sugar maple); these mesophytic, relatively shade-tolerant species are likely to become canopy dominants if current trends continue in the future (Shotola *et al*., 1992; Galbraith and Martin, 2005; Nowacki and Abrams, 2008). Röhrig and Ulrich (1991) paper described that *A. campestre* is a relatively drought tolerant species. Moreover, according to Banks *et al*. (2019) *A. campestre* shows significant seasonal drought tolerance variation. Oak species cannot successfully compete with these species (McDonald *et al*., 2002; Zaczek *et al*., 2002). At the study site, oak decline remarkably decreased canopy cover and led to the notable height growth of three species - especially *A. campestre* individuals - from the shrub layer; this phenomenon is called the "Oskar"-strategy (Silvertown, 1982). Our results support these statements, because in our study site maples showed a considerably increase in size and cover, and a low regeneration potential of canopy oak species due to detected abiotic and biotic shading effects (Jakucs, 1988; Misik *et al*., 2017).

Table 4. Statistical relationship between the structural condition of the subcanopy and the oak canopy density on the monitoring plot over the period of 1982-2017.

woody species	*r*	*P*	R^2
		density	
A. campestre	-0.77	0.02*	0.60
A. tataricum	-0.37	$0.36^{n.s.}$	0.14
C. mas	-0.46	$0.24^{n.s.}$	0.22
subcanopy	-0.79	0.02*	0.62
		height	
A. campestre	-0.75	0.03*	0.56
A. tataricum	-0.08	$0.86^{n.s.}$	0.59^{-2}
C. mas	0.74	0.03*	0.55
		diameter	
A. campestre	-0.73	0.04*	0.53
A. tataricum	-0.22	$0.60^{n.s.}$	0.05
C. mas	-0.60	$0.11^{n.s.}$	0.36
		cover	
A. campestre	-0.71	0.47^{-1}*	0.51
A. tataricum	-0.36	$0.38^{n.s.}$	0.13
C. mas	-0.62	$0.99^{-1n.s.}$	0.39

CONCLUSIONS

Our results confirm that the decreasing oak trees density led to the size and foliage cover condition changes in the shrub community in the last decades. The response of the shade and relatively drought tolerant woody species, as maples and European cornel in Síkfőkút is strong and rapid once to the oak decline; this phenomenon is so-called "Oskar"-strategy. The conclusions to be derived from the studied site are as follows: (1) the new foliage layer, the subcanopy layer was developed and improved from 1982. It was continually increasing the total density of subcanopy from 1982. (2) Three native woody species, two maples and *C. mas* composed this layer. *A. campestre* was the most common species in this foliage layer. (3) A non-significant association was obtained by the size condition of subcanopy species after the oak decline. Only mean size values of *A. campestre* and C. mas' height increased significantly over the period of 1982-2017. Better understanding understory development and possible interaction between the subcanopy layer and the oak canopy layer is critical to achieving forest management goals in the Hungarian oak forest stands, as this knowledge helps explain stand developmental patterns and predict future stand structures.

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Original Scientific paper
10.7251/AGRENG2202095H
UDC 633.15:632.9(659.4)

INSECTICIDE RESISTANCE MANAGEMENT FOR FALL ARMYWORM IN MAIZE FIELDS OF ISRAEL

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ABSTRACT

Fall Armyworm (*Spodoptera frugiperda*) (FAW) is endemic to tropical and subtropical regions of North and South America. FAW larvae, if not well managed can cause significant yield losses to various important crops, such as maize, rice and cotton. In Israel, populations of FAW have been found since 2018 damaging many maize fields. Management of FAW relies mainly on the use of insecticides; however, this pest has evolved high resistance levels to many insecticides, worldwide. To prevent or delay the development of insecticide resistance, resistance management strategy should be employed to decrease FAW exposure to insecticides. Our study is focusing on resistance management of FAW and field resistance monitoring to main control agents along with the use of biorational-selective insecticides and other non-chemical methods. The objective of the current study was to establish a baseline susceptibility of FAW larvae to insecticides such as diamides, IGRs and *Bt.* We rear a reference population, without exposure to any pesticide, on artificial diet under standard controlled room conditions. To date, we have assayed various recommended insecticides against third instars of FAW. During May 2022, we have collected larvae of FAW from maize fields, located in the eastern warm valley of Israel and they were tested for their susceptibility to various insecticides. We intend to assay late-season FAW populations as well. An outcome of this study is to form an IPM-IRM strategy that will have the ability to decrease FAW exposure to insecticides and to increase the use of other environmentally-friendly pest control practices.

Keywords: *Spodoptera frugiperda, Maize, Resistance management, Biorational-selective insecticides, IPM.*

INTRODUCTION

Fall Armyworm (*Spodoptera frugiperda*) (FAW) is endemic to tropical and subtropical regions of North and South America. However, recently it has rapidly invaded many regions worldwide due to its outstanding migration and dispersal ability (Johnson, 1987). In 2016, it was first appeared in Africa and then in India,

Thailand, Australia and China; in addition, it is likely that this pest might migrate into southern Europe either from North Africa or Middle East, or via commercial trade. Currently, this pest is presented in many countries of the Old and New World (FAO, 2022). FAW is an economic pest that attacks maize and other important crops such as rice, sorghum, sugarcane, vegetable crops and cotton; and its host range includes more than 100 plant species (Pogue, 2002). In Israel, populations of FAW have been found since 2018 damaging many maize fields.
Management of FAW relies mainly on the use of insecticides; however, this pest has evolved high resistance levels to many insecticides (including the new diamide group), worldwide (Bolzan et al. 2019; Mota-Sanchez and Wise, 2021); and also, it has developed moderate resistance to *Bt*-maize (Omoto *et al*., 2016). To prevent or delay the development of insecticide resistance, resistance management strategy should be employed to decrease FAW exposure to insecticides (IRAC, 2021). Hence, it is important to evaluate the susceptibility of insecticides against this pest. In this study, we have assayed various new insecticides against a susceptible strain of FAW under standard controlled room conditions; at second step, we intend to monitor FAW resistance and insecticide susceptibility in maize fields of Israel in order to form a sustainable resistance-management strategy.

MATERIALS AND METHODS

Insects

A field strain of *S. frugiperda* was collected in June 2021 from maize fields near Negba (located in the northern Negev desert, near the city of Ashkelon) and was reared, without exposure to any pesticide, on mixture of maize leaves and premixed diet (Ward's Stonefly Heliothis Diet, USA) under standard room conditions of 27°C, 55% RH and 14 h photoperiod. Later on, we developed another diet that is based on polenta (boiled and ground cornmeal) plus some additives; this diet was comparable to the premix.

Table 1. Insecticides used for the bioassays against FAW

Brand name and formulation	**Active ingredients**	**type**	**Resistance group, IRAC**	**Producer**
Sparta Super 60 SC	Spinetoram	Contact and stomach	5	Dow AgroSciences
Uphold (Armada) 360SC	Spinetoram (60) + methoxyfenozide (300)	Contact and stomach	5+18	Dow AgroSciences
Coragen 200 SC	Chlorantraniliprole	Contact and stomach	28	FMC
Rimon 100 EC	Novaluron	Insect growth regulator	15	Adama
Avaunt 160	indoxacarb	Contact and	22	FMC

EC		stomach		
Dipel DF	*Bt* var kurstaki	Bio-insecticide	11	Valent BioSciences
Probit DF	*Bt* var. kurstaki and var. aizawai	Bio-insecticide	11	Certis

Bioassays

Leaves of maize were dipped in aqueous concentrations of tested compounds (Table 1) or in water, as control. After 15 seconds of dipping, the maize leaves were allowed to air dry for 2 h. Subsequently, *S. frugiperda* larvae (third instar, 8-10 mm), reared under controlled room conditions were put inside Petri dishes on the treated maize leaves; in addition, filter papers also placed into the dishes (to avoid excess moisture). The experiments were done with 10 larvae for each concentration and repeated at least three times, on separate days. Mortality of the larvae in comparison with the control was determined five days after exposure to the insecticides. To obtain a concentration- mortality line, at least four concentrations were used for most of the bioassays. LC values were estimated by probit analysis (POLO, 1987). Control mortality was corrected using Abbott's (1925) formula.

Maize field collections

To detect insecticide resistance, we have collected field populations of FAW in May-June 2022. Field populations of FAW larvae were collected from commercial maize fields located in the eastern valley of Israel, and they transferred on the same day to the lab location in SW Israel. One generation was reared under standard controlled room conditions and thereafter, similar bioassays were conducted as mentioned above. Resistance ratios (RR) were calculated by dividing the LC_{50}- and LC_{90}-values of the respective field populations by LCs of the reference strain.

RESULTS AND DISCUSSION

Table 2 and figure 1 present the effect of the various recommended insecticides on third instar *S. frugiperda*. Accordingly, the insecticides spinetoram, chlorantraniliprole, novaluron and uphold® exhibited quite similar effectiveness against susceptible larvae; indoxacarb was less effective. To control third instars of FAW with *Bt* formulations, we had to use higher concentrations compared with the other compounds, mentioned above. Other studies on susceptible populations reported similar results (Tay *et al*., 2022). Up to now, we did not detect significant insecticide resistance in the early-season field collections.

Bt-maize technology is an efficient control option for FAW; however, so far, Israel is GMO (Genetically Modified Organism)-Free. In addition, this technology would not carry sustainable options for long time; hence, *Bt*-maize should be combined with effective insecticide programs (IRAC, 2021).

Prior to developing an IRM (Insecticide Resistance Management) strategy of FAW, we have to establish baselines susceptibility of a reference (“susceptible”) and field strains. After defining the laboratory baseline (Table 2, Fig. 1), we intend to collect maize-field populations in early- and late- growing seasons, in accordance with the Cotton IPM-IRM strategy that has been implemented in Israel, mainly, as countermeasures for delaying the evolution of resistance in the whitefly *Bemisia tabaci* (Horowitz and Ishaaya 1992; Horowitz *et al*., 2020).

Table 2. Comparative toxicity of various compounds on *Spodoptera frugiperda* (L3)

Compounds	n	**Slope ±SEM**	**LC_{50} mg a.i./liter (F.L.)**	**LC_{90} mg a.i./liter (F.L.)**
Spinetoram	160	1.44±0.11	0.05 (0.03-0.07) a*	0.35 (0.19-0.97) a
Chlorantraniliprole	180	0.86±0.07	0.03 (0.01-0.07) a	1.03 (0.37-6.28) abc
Novaluron	195	0.79±0.06	0.06 (0.03-0.12) ab	2.51 (0.80-20.7) abc
Uphold**	165	1.29±0.11	0.11 (0.09-0.14) b	1.08 (0.75-1.75) ab
Indoxacarb	160	1.19±0.09	0.38 (0.29-0.48) c	4.46 (3.00-7.46) bc
Bt- formulations***			in gr./0.1 liter (F.L.)	in gr./0.1 liter (F.L.)
Dipel DF	165	3.48±0.29	0.21 (0.14-0.30)	0.49 (0.33-1.29)
Probit DF	160	2.60±0.29	0.25 (0.21-0.29)	0.78 (0.65-1.01)

Mortality curves were constructed from 4-5 concentrations each and untreated control. Each concentration was conducted with 3-4 replicates. LC values were determined according to probit regression, using POLO-PC analysis procedure. * Failure of 95% FL (Fiducial Limits) to overlap at a particular lethal concentration indicated a significant difference. **Uphold (Armada) 360 SC is a mixture of Spinetoram (60g/L)+methoxyfenozide (300g/L);
*** Concentrations of *Bt*- formulations are in percentages.

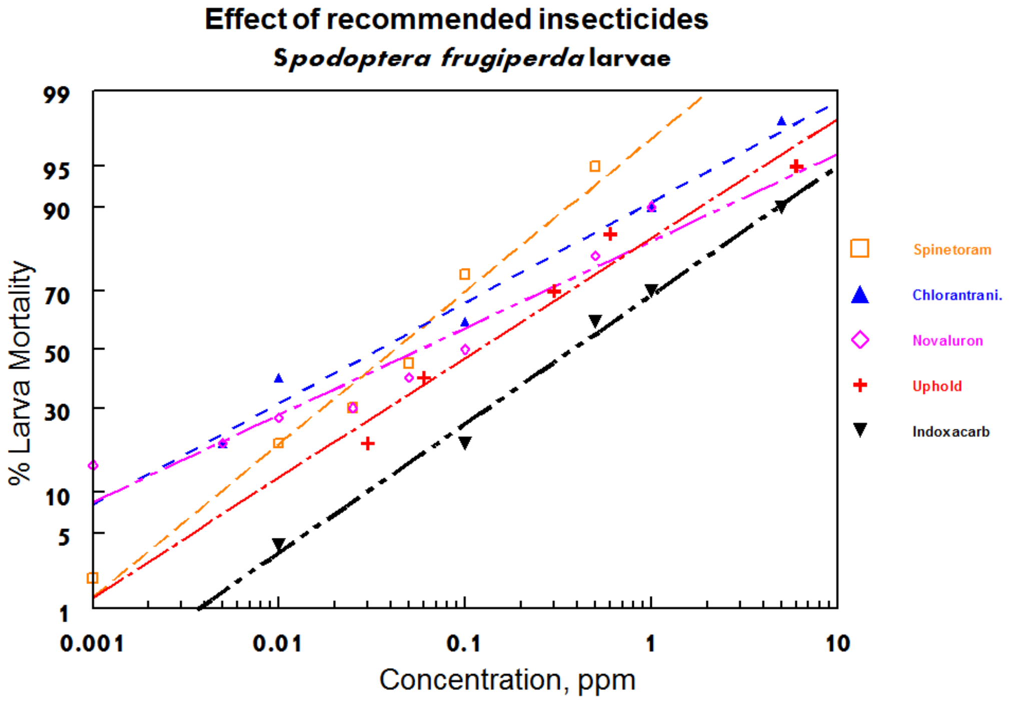

Figure 1. Log dose-response curve (on a probit scale) of the effect of various insecticides on *Spodoptera frugiperda* 3rd instars (reference strain)

IRM strategies are based on the assumption that the ratio between resistant and susceptible genotypes can be manipulated by various factors such as application frequencies, insecticide rotation with different modes of action and exposure of just one pest generation to the insecticide. These factors may help in diluting the resistant genotype by immigration of susceptible individuals from untreated fields, by reducing the fitness of the resistant strain in the absence of insecticides or both (Georghiou and Taylor 1986; Sawicki and Denholm 1987; Horowitz and Ishaaya 1992). Moreover, it is very important to enhance nonchemical control methods using IPM practices (e.g., biological control, crop plant resistance and pheromone mating- disruption techniques). The integration of these methods would contribute to improving the combat against FAW, and thus, helping to form sustainable managements in the future.

CONCLUSION

Our study is focusing on resistance management for FAW and field resistance monitoring to various recommended control agents along with the use of biorational-selective insecticides and other non-chemical methods. The integration of these methods would contribute to improving the combat against FAW, and thus, helping to form sustainable management in the future.

Prior to developing an IRM strategy for FAW, we ought to establish baselines susceptibility in a reference and field strains. After we have defined the laboratory susceptible baseline, we intend to collect maize-field populations in early- and late-growing seasons, in accordance with the cotton IPM-IRM strategy that has been implemented in Israel.

ACKNOWLEDGEMENT

The authors are grateful to Dr. Isaac Ishaaya for critical reading the manuscript. The study was partial supported by ‘The Plants Production & Marketing Board’.

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Original Scientific paper
10.7251/AGRENG2202102N
UDC 637.4

RESEARCH ON LAYING HEN CROSS DOMINANT BIOLOGICAL EGG COMMERCIAL PRODUCTION USING VARIOUS TYPES OF FEED

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ABSTRACT

The demand for ecologically-friendly food produce, including those of animal nature, e.g. hen eggs, is increasing continuously in Latvia as well as all across the world. Research objective is to determine the most productive and most suitable for Latvian environment laying hen crosses that can be kept for bio-egg commercial production, as well as to decide upon most suitable biologically produced and economically efficient feed for these hen crosses. The research was held in Kandava municipality, Kandava rural territory, "Kurzemes olas" Ltd. laying hen farmstead "Upkalnu ferma". The research involved three Dominant laying hen crosses: Dominant Barred D 959, Dominant Tinted D 723 and Dominant Red Barred D 459. All in all, there were 6 groups, each of them consisting of 100 birds. One group of each hen cross was fed with manufactured complete bio-feed (group numbers D723K, D459K and D959K), while the second group – with home-made bio-feed designed for laying hens (group numbers D723S, D459S and D959S). The maximum results that were reached during the entire research period were as follows: D723K – 71% in September (26-29 weeks old), D723S – 82% October (30-34 weeks old), D459K – 56% November (35-38 weeks old), D459S – 58% October (30-34 weeks old), D959K – 54% November (35-38 weeks old) and D959S – 62% October (30-34 weeks old). The best productivity indicators on average were demonstrated by laying hen cross D723 (p<0.05). The cross D459S produced eggs with higher average weight and better proteins, correspondinly by 3.81 g and 11.15 Haugh units than D459K. The amount of dry matter, crude protein and fat in egg mass was equivalent and met physiological norm indications. Reproductive tract organs in all groups but D459K were developed evenly. Liver mass in all groups showed no visible pathologies.

Keywords: *Laying hen, egg production, egg quality, reproductive tract, liver mass.*

INTRODUCTION

The demand for ecologically-friendly food produce, including those of animal nature, is increasing continuously in Latvia as well as all across the world. One of the produce varietes that make customers' choice, which is based upon production sustainability and produce origin, are hen eggs. As the least harmful to both human health and the natural environment, are considered the eggs produced at bio-farms, though this type of production at a larger scale is rather restricted in Latvia, as there is a lack of information about the most suitable hen breeds and crosses for bio-production. Traditionally, for commercial production, they use various laying hen crosses which were bred using precise selection, with the purpose to develop a certain characteristics, e.g. egg quantity, shel colour, egg size, feed application efficiency, start of laying, etc. It should be noted that there are certain complications for bio-farms to secure and maintainin productivity which are related to complete animal feed supply that complies with scientifically recommended feed rations (Vītiņa, Jemeļjanovs, Mičulis, 2004; Jemeļjanovs, Mičulis *et al.,* 2004). The problem is often caused by bio-feed availability in large quantities and its relatively high price for both complete feed mixes and raw materials, especially for protein sources like beans, peas, soy beans, etc. Research objective is to determine the most productive and most suitable for Latvian environment laying hen crosses that can be kept for bio-egg commercial production, as well as to decide upon most suitable biologically produced and economically efficient feed for these hen crosses.

MATERIALS AND METHODS

The research was held in Kandava municipality, Kandava rural territory, "Kurzemes olas" Ltd. The research involved 3 laying hen crosses: Dominant Barred D 959, Dominant Tinted D 723 and Dominant Red Barred D 459, starting with one-day old chicken till the beginning of laying cycle. In each cross, the birds were divided into two groups, where the first one was fed with commercially produced complete bio-feed for chicken, growers and layers (K – commercial; group numbers D723K, D459K un D959K) from JSC "Dobeles dzirnavnieks". The other group was fed with home-made complete bio-feed for chicken, growers and layers (S- home-made; group numbers D723S, D459S un D959S) made after especially designed recipes. All in all, there were 6 groups, 100 birds in each. The research was held from May 2019 till March 2021. 24-hour-old chicken were purchased from internationally recognised Czech enterprise Dominant Genetika official representatives in Latvia farmstead "Veckūkuri". The testing farm's designed bio-feed mix included the following ingredients: barley, rye, pea meal, soy cakes, fodder yeast, fish meal, calcium carbonate mineral feed (Profimix) and natrium chloride. JSC "Dobeles dzirnavnieks" comercially produced complete bio-feed contained the following ingredients: wheat, barley, oats, soy beans, beans, soy cakes, calcium carbonate, fodder yeast, pre-mix and other feed additives.

Table 1. Supply of nutrients to laying hens, %.

Analytical components	Home-made bio-feed mix	Commercially produced complete bio-feed mix
Dry matter	88.76	88.00
Crude protein	17.17	17.34
Crude fiber	4.24	4.58
Calcium	3.18	3.91
Phosphorus	0.83	0.67
Lysine	0.85	0.85
Methionine	0.31	0.24
Threonine	0.58	0.61
Tryptophan	0.18	0.22

Keeping conditions were the same for all groups of layer crosses. Upon reaching laying age, the birds were moved to the permanent accomodation for egg laying in separate compartments in the same 6 groups. Those were secured with all the free-range keeping conditions necessary for layers, including easy outdoor access through special entrance/exit doors. For feeding the birds, feeding equipment was used, while drinking water was freely accessible from the round drinking bowl. Throughout the entire growing period, the bords were kept on the floor in separate boxes, using wooden chips as manipulable material. Hen laying quality was evaluated in compliance with internationally accepted methodology, i.e. determining the amount of eggs and calculating laying intensity from the existiing number of hen (Yilmaz Dickmen *et al.*, 2016). Egg quality was determined using morphological indicators (shell mass, shell thickness, shell durability, egg white's height and yolk's colour) as well as biochemical indicators, such as dry material, crude protein and fat, taking as samples 30 eggs from D459K and D459S hen groups. Upon slaughtering, there was a random choice of hens from each group, and internal organs were taken for lab analysis. Liver and reproductive tract were assessed macroscopically. The liver was measured for its mass using Casbee MW-1200, US (precision 0.1 g), while the reproductive tract was exposed to various measurements (Gongruttananun, 2011). Oviduct length was measured with electronic vernier limit (precision 1 mm) as well as its mass. Ovaries were weighed, follicules tat are 10 mm or bigger were determined, counted and weighed (Waddington *et al.*, 1985). Feed chemical analysis was performed by Latvia University of Life Sciences and Technologies Biotechnology scientific laboratory following the accredited ISO standard methods. Feed recipe development involved a special computer program "Hybrimin". Egg quality analysis was performed by JSC "Balticovo". Chemical laboratory, using standard methods. The acquired data were analysed using descriptive statistics method, calculating data mean value and standard deviation, and inferential statistics. To determine the two measured parameters' correlations, with no relation to a certain research group, Pearson's correlation test was applied. To compare the results across the groups, Mann-

Whitney U test was used, while T test – for comparing laying intensity across the groups. Calculations and analysis are performed using MS Excel, while Mann-Whitney U test used SPSS data analysis program.

RESULTS AND DISCUSSION

Layers crosses Dominant started laying rather early – 13 to 17 weeks old (in June). Early beginning of laying period is related to the birds' fast growing and maturity, that brings along heavier body mass than of those chicken of the same age, but which are grown at a normal pace
(Robinson *et al*., 2001).
Dominant CZ cross standard (Dominant CZ Programs) determines age when laying productivity is 50% as one of the characteristic features, and this age for Dominant layer crosses begins at about 22-23 weeks. During the trial, 50% productivity was determined: for cross D 723 in July, when the chicks were 17 to 21 weeks old, and for D 459 and D 959 crosses in August, correspondingly (at the age of 22 to 25 weeks) and September (26 to 29 weeks).

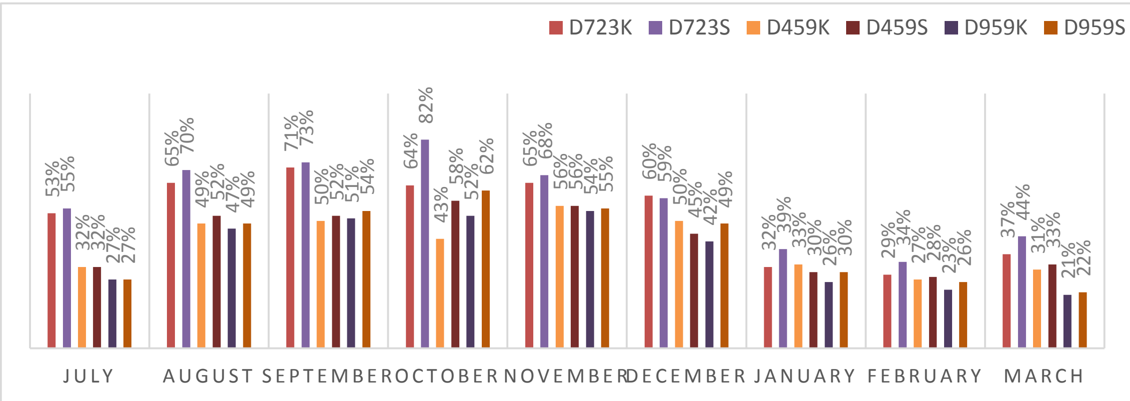

Figure 1. Productivity of layers, %.

Material differences among crosses, that were fed with different feed types, were not determined. According to standard notions, Dominant cross laying hen reach their max. productivity at the age of 29 to 30 weeks (up to 90%) D 723 - 93-95% and D 459 and D 959 – 91-94%. Maximum results reached during the trial ae as follows: D 723K – 71% in September (26-29 weeks old), D 723S – 82% in October (30-34 weeks old), D 459K – 56% in November (35-38 weeks old), D 459S – 58% in October (30-34 weeks old), D 959K – 54% in November (35-38 weeks old) and D 959S – 62% in October (30-34 weeks old).
Assessing the egg quality morphological indicators in group D 459S, aerage egg weight was 66.01 g, i.e. 3.81 g heavier than in D 459K group. There is physiological correspondence when at lower laying intensity the eggs have bigger mass (Vītiņa, 2006). Another important egg quality indicator is egg shell mass and thickness. These indicators determine broken egg quantity (Vītiņa, Latvietis, 2000). During the trial, the egg shel mass in group D495K was a little higher, 8.43 g upon

average, while in group D459S it was 8.39 g upon average. This makes 12.71-13.55% from egg mass, though the difference in per cent was not too relevant $p<0.05$). Egg shell thickness in both bird groups was similar and within optimal norms (0.4-0.6 mm). This indicates that both home-made and commercially produced feed mixes contain ingredients that facilitate the egg shell thickness and durability.

Table 2. Egg morphological and biochemical analyses.

Indices	D 459	
	D 459S	D 459K
Number of eggs, pcs.	30	30
Average egg weight, g	66.01±5.29	62.20±2.81
Shell mass, g % of eggs mass	8.39±0.96 12.71	8.43±0.80 13.55
Shell thickness, mm	0.49±0.08	0.51±0.04
Shell strength, Newtons	40.30±14.57	45.30±10.38
Albumen height, mm	6.84±0.93	5.36±1.27
Albumen height, Haugh units	80.41±6.22	69.26±13.13
Yolk color, Roche scale	2.80±0.62	2.80±0.48
Dry matter, %	23.48±0.86	24.19±0.85
Crude protein, %	11.65±1.06	11.96±1.29
Crude fat, %	8.61±0.63	9.50±0.88

Egg shell quality is basically determined by the bird's health, feed ingredients aand its quality aspects, as well as its accessibility and availability. However, highly producttive layers in their 2nd laying phase (45 weeks old and later) may experience fatigue of the reproductive system which is responsible for egg-forming from the nutritive elements. This is especially important for egg-shell formation. According to various researchers (Rama Rao, Nagalakshmi, Redoly, 2002; Juan Gomez – Basauri, 1998; Robert, Schwartz, 1997), egg shell quality is determined not only by major elements – calcium, phosphorus and vitamin $D_{3,}$ but also the amount of manganese and chloride in the feed mix. Egg shell quality is also determined by various stres factors, such as: transporting, lodging refurbishment, pecking agression from other birds, etc. The stress traces are still felt 8 to 10 days after averting the problem. Also, such an issue as bird inflammation with such diseases as avian encephalomyielitis, infectious bronchitis, Newcastle disease, and mycoplasm leave a negative effect on the egg shell quality. Mycotoxins that penetrate into feed mixes (e.g., *Fusarium moniliforme* and *Fusarium proliferatum*), which produce fumonisin B_1, that is is strongly toxic and completely blocks metabolic processes performered by vitamin D, deteriorated the egg shell quality (Grant Richards, 1998; Dewegowda, Aravind, 2002). Shell strenght in group D 459S was within optimal ranges (at least 40 N), but the indicators were a little higher in group D 459K – 45.30 N, upon average, 5 N higher than in group D 459S. Albumen height was bigger in group D 459S – 6.84 mm (optimal is 6 to 10 mm) and 80.41 Haugh units (optimal is 80 to 100 Haugh units), correspondingly, the indicators were higher by 1.48 mm and 11.15 Haugh units, which shows better egg quality than in group D 459K. Egg yolk colour was equally pale (2.80 grades) after Roche scale. Egg yolk quality depends on protein and vitamin content in the feed. A vitamin is transformed unevenly, i.e. increasing the concentration of vitamin A will result only in a fair increase in colour. Increasing vitamin A proportion in egg yolk will decrease the amount of carotenoids (paler yolk), as well as the concentration of vitamin B_2 (Nudiens, 1999). Biochemical indicators of eggs were not affected by either feeding layers with commecially produced or home-made feed mixes. Dry matter, crude protein and crude fat in eggs were well-balanced and complied with physiologic normative indications.

After reproductive tract organs measurements, one can determine the hens' recent, present and further laying potential (Rahman, 2018). The measurements identified the longest oviduct in group D 959K – average 626 mm, while the shortest – in group in group D 459K – 382 mm. The heaviest oviducts were found in group D 723S – 51.5 g, the lightest – in group D 459K – 19.0 g. The majority of birds that demonstrated heaviest oviducts were those that were fed with home-made feed mix. The highest ovary mass was indicated in group D 723S – 53.7 g, and the lowest – in group D 459K – 16.4 g. Groups D 723S and D 959K had more big follicles – 6.3 and 6.5 pieces correspondingly, while the lowest amount was detected in group D 459K – 2.0 cases.

Table 3. Reproductive tract and organs measurements.

Definite organ parameter	Grupa					
	D 723K	D 723S	D 459K	D 459S	D 959K	D 959S
Oviduct length, mm	527±84	563±68	382±149	587±44	626±36	592±50
Oviduct weight, mm	28.3±15.6	51.5±9	19.0±23.1	43.7±16.7	40.0±12.4	44.5±13.4
Ovary weight, g	22.0±9	53.7±7.1	16.4±18.5	41.8±11.1	49.3±10	43.5±7
Big folliculle amount, pcs.	3.0±1.5	6.3±0.5	2.0±2.8	5.2±1.2	6.5±1.8	5.7±0.5
Big follicle mass, g	12.6±10.4	43.2±6.1	11.1±16.8	31.1±11.1	41.2±12.1	35.4±6.7
Liver weight, g	44.7±7.1	48.2±5.3	44.4±10.9	52.2±4.6	53.8±4.4	46.5±4.2

Comparing reproductive organ development across various feeding groups, average oviduct and ovary weight, big folicle amount and their mass indicators were significantly higher ($p<0.05$) for the hens that were fed with home-made feed mix. The research determined a strong positive correlation between oviduct length and its weight (r=0.755, p<0.05), as well as average correlation between ovary weight and the amount of big follicles (correspondingly r=0.89, p<0.05 un r=0.91, p<0.05). Layers that have more active ovaries that produce more folicles, also have longer oviducts, while those birds that have not yet reached the reproductive age, and their ovaries are not active, or older birds that have scarse ovulation, have relatively shorter oviducts (Rahman, 2018).

Reproductive tract organs for all hen groups, except for group D 459K, were well-developed. Group D 459K birds had smaller measurements of all. As a reason, there were birds with smaller reproductive tract organs which reached hatching phase that could have influenced the size of reproductive tract organs, but were deprived of laying (Gongruttananun, 2011).
Visual assessment of hen liver did not identify any macroscopically identifiable pathological changes of tissues in either group. The highest liver weigh was determined in group D 959K – 53.8 g, the lowest – in group D459K, 44.4 g. Comparing the bird groups that were fed with home-made feed mix with those that were were fed with commercially produced feed mix, there were no statistically notable differences identified in liver mass (p>0.05).

CONCLUSIONS

In all layer groups, where the birds were fed with home-made biological feed, showed higher laying intensity in comparison to those that were fed with commercially produced bio-feed. During the research preiod, layer Dominant crosses did not reach the maximum potential laying intensity, yet the highest productivity indicators were shown by layer cross Dominant Tinted D 723.
D 495S hen group got larger and more qualitative eggs with average weight of 66.01 g and higher albumen height of 80.41 Haugh units than the birds from group D 495K. The amount of dry matter, crude protein and crude fat was equal in both groups D 495S and D 495K and complied with the physiological norm specifications.
Reproductive tract organs for all groups but group D 459K, were developed evenly. Liver mass across the groups was above average, though macroscopic analysis did non reveal any visible pathologies.

ACKNOWLEDGMENTS

The research was carried out within the framework of European Agricultural Foundation Latvian Rural Development Programme 2014-2020 event 16 “Cooperation”, sub-16.2 “Support for development of new products, methods, processes and technology development”. Project No.18-00-A01620-000025 „Apropriateate layer breed research for organic egg commercial production in Latvia using different feed mixes.

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Review paper
10.7251/AGRENG2202110S
UDC 634.8:543.645

RESVERATROL IN GRAPES - BENEFITS AND POTENTIAL USE

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ABSTRACT

Resveratrol (3,4',5-trihydroxystilbene) is one of the most important polyphenol compounds present in the human diet. From the aspect of agricultural production, resveratrol is also an important component. Grapevine plants produce it as a secondary metabolite that is synthesized to the greatest extent in stressful conditions. These conditions include abiotic and biotic factors such as a lack or excess of water, the content of micro and macro elements in the soil, agrotechnical measures, infections caused by phytopathogenic microorganisms, the presence of chemical stressors such as heavy metals, intensive application of plant protection products (PPP), uncontrolled application of synthetic fertilizers, etc. The most important stressor is infections caused by phytopathogenic organisms, especially the fungus *Botrytis cinerea*, whose presence is a trigger for the synthesis of resveratrol. With the intensive resveratrol production, plants activate the induced defense system, which inhibits the growth and further progression of pathogens. Often, the concentrations of synthesized resveratrol in infected plants are not sufficient to completely suppress pathogens, but they certainly slow down and keep infections at a low level, to provide additional time for us to use adequate protection measures. In addition to the plants that produce it, its application is also possible in the form of a water solution of pure resveratrol that can be applied during the storage of agricultural products to preserve their freshness as long as possible and to reduce the use of PPP in storages. In this paper, an overview of the last references was given to gain an overall picture of how important resveratrol is and how much potential it has from the aspect of human health and plant protection.

Keywords: *resveratrol, phenols, grapevine, Botrytis cinerea Pers.*

INTRODUCTION

The grapevine (*Vitis* spp.) is considered one of the oldest plant species. It has a long history of cultivation of about 7000 years. Due to the method of cultivation, the nutritional value of the fruits, and the economic profitability it gained great importance in agriculture. According to the latest data, around 7.6 million ha are under vineyards in the world, with Europe accounting for 65% of those areas. The annual production of grapes reaches almost 78 million tons, and the largest producers of grapes and wine are Spain, France, and Italy (Korać et al., 2016). In the Republic of Serbia, a tendency to decrease the area under vineyards has been observed (in the past few decades, it decreased by almost 50%). The reason for this is an insufficiently developed market, insufficient competitiveness, and a changing assortment where autochthonous varieties have been neglected. All of this represents a major obstacle on the way to strong and modern viticulture in Serbia, and it can be overcome by returning to the old, autochthonous grape varieties and exploiting all their qualities. The content of nutritional components in grapes is at a high level. The mentioned components include vitamins, minerals, colors, sugars, tannins, and polyphenols as the most abundant compounds (Korać et al., 2016). The mentioned substances have a positive effect on human health with their anti-inflammatory and antimicrobial effects. In addition, they affect the properties of fruits and their products, giving them color, smell, taste, pungency, etc. Polyphenols are secondary plant metabolites present in cereals, vegetables, fruits, and their products (Pandey and Rizvi, 2009). In addition to affecting the physical properties of the fruits in which they are represented, they have multiple positive effects on human health such as anti-inflammatory action, inhibition of platelet aggregation, antimicrobial activity, and antioxidant activity the most important among them. They are the most widespread antioxidants in the plant world, and their consumption has a beneficial effect on the human body, protecting cells from oxidative stress (Đekić et al., 2008). The reason for this is their susceptibility to oxidation due to the presence of a hydroxyl group and an unsaturated double bond (Rice-Evans et al., 1997). Among the plants that contain large amounts of polyphenols, and which are present in a variety of human diets, the grapes stand out. The presence of polyphenols in grapes is influenced by many factors such as climatic conditions (De Pascali et al., 2014), physical and chemical properties of the soil, stress, and agrotechnical measures (irrigation, tillage), health status of the vine (Pantelić et al., 2016). Polyphenols in grapes can be roughly divided into four groups, depending on the number of phenolic rings they contain and based on the structural elements that connect these rings, namely: phenolic acids, flavonoids, stilbenes, and lignins (Pandey and Rizvi, 2009). In the non-flavonoid class of phenolic compounds, stilbenes (1,2-diarylethenes) stand out because of their defensive effect on plants. They contain two phenyl moieties connected by a methylene bridge of two carbon atoms (Moreno and Peinado, 2012). The synthesis of stilbene, which includes resveratrol, takes place in the skin and seeds of the berries, but it has also been detected in the stem, winter buds, shoot tips, petioles, roots, and leaves of the vine, in concentrations of 0.2-16.5 mg. /kg (Colica et al., 2018). Resveratrol stands out as the most important polyphenol (stilbene) in grapes. Over the past several decades, this compound has been the subject of much research in the fields of medicine, pharmacy, plant physiology, biochemistry, and agriculture. Its synthesis in vine shoots, especially in berries, is stimulated by stressful conditions such as infections with phytopathogenic microorganisms, unfavorable environmental conditions, mechanical damage to plants, UV radiation, etc. Economically significant phytopathogenic fungi such as *Botrytis cinerea* Pers, *Plasmopara viticola* Berk. & M. A. Curtis, Berl. & De Toni, *Uncinula necator* Schwein, *Guignardia bidwellii* Ellis stand out as the most intensive trigger of resveratrol synthesis. Therefore, it is of great importance to assume that resveratrol forms a significant part of the induced immune system of the grapevine, which is activated upon the occurrence of stressful conditions. In addition, its consumption has a beneficial effect on health, and all the mentioned benefits of resveratrol, discovered so far, speak in favor of the perspective of further study of this compound.

RESVERATROL IN GRAPES

The history of resveratrol (3,4',5-trihydroxystilbene) can be traced back more than 2000 years since the grape extract was first used to treat heart disease and other health problems (Huang et al., 2019). It was isolated for the first time, as a pure compound, in 1939 from the roots of the plant *Veratrum grandiflorum* O. Loes (Takaoka, 1939; Pezzuto, 2019). Later, in 1963, it was isolated from the roots of *Polygonum cuspidatum* Siebold & Zucc., a plant used in traditional Chinese medicine (Yang et al., 2019; Huang et al., 2019). It is present in at least 72 plant species, including families such as *Vitaceae*, *Myrtaceae*, *Dipterocarpaceae*, *Cyperaceae*, *Gnetaceae*, *Fabaceae*, *Pinaceae*, *Moraceae*, *Fagaceae*, *Liliaceae*, and 31 genera of spermatophytes (Colica et al., 2018). Extraction of resveratrol can be successfully carried out with plant species such as *Arachis hypogeal* L., *Eucalyptus gunnii* Hook, *Morus rubra* L., *Vitis vinifera* L., *Veratrum grandiflorum* O. Loes, *Rheum rhaponticum* L., *Polygonum cuspidatum* Siebold & Zucc, *Gnetum montanum* Markgr., *Picea abies* L., *Pinus silvestris* L., and others. (Aggarwal et al., 2004; Colica et al., 2018; Huang et al., 2019). When it comes to the content of resveratrol, fruits, and vegetables that contain it in significant quantities, and are part of the everyday human diet should be especially highlighted. It is important to emphasize that grapes contain more resveratrol than their other natural sources (Hasan and Bae, 2017). Resveratrol is one of the most important polyphenolic compounds in grapes and wine (Đekić et al., 2008; Cvejić et al., 2010; Yang et al., 2019; Huang et al., 2019). It is a natural phytoalexin, one of the carriers of the induced defense mechanism of plants. In nature, this compound is present in two isomeric forms, trans and cis. The trans isomer has greater biological activity due to the presence of the 4'-hydroxystyryl group (Soleas et al., 1997). The cis form occurs as a consequence of the transformation of the trans isomer by UV radiation or throughout the processing of grapes during the vinification, and all due to the presence of a C—C double bond (Tian and Liu, 2019), whereby it never reaches the concentration of the trans isomer (Sato et al., 1997; Soleas et al., 1997). Both forms of resveratrol are present in grapes, with the cis form present in very small amounts that are often difficult to detect (Cvejić et al., 2010; Colica et al., 2018; Đekić et al., 2008; Yang et al., 2019). Resveratrol originates from the acetate-malonate and phenylpropanoid pathways of the plant's primary and secondary metabolism. Condensation of p-coumaroyl CoA with three molecules of malonyl

CoA is achieved by stilbene synthase activity. Four molecules of CO_2 are released for every molecule of resveratrol synthesized. The synthesis of resveratrol in grapes is then catalyzed by the enzyme stilbene synthase, which uses p-coumaroyl-CoA and malonyl CoA as a substrate (Colica et al., 2018). Accumulation of resveratrol in grapes varies depending on the grape variety, genotype, location, environmental conditions, and ripening period. Different amounts of resveratrol have been detected in grape skin, seeds, shoots, buds, and roots, however, a relatively higher amount of resveratrol can be found in the skin of grapes, and less in the pulp and end products such as juice and wine (Hasan and Bae, 2017). Expressed as a percentage, the distribution of resveratrol in the berry is uneven, and about 60–70% of the total resveratrol is found in the seeds, 28–35% in the skin, and only 10% in the pulp (Gođevac et al., 2010). The synthesis of resveratrol was also monitored during the ripening of different vine varieties. The amounts of resveratrol were relatively high in unripe fruits, and low in ripe berries. It was observed that resveratrol is synthesized more intensively and to a greater extent in the skin cells of the berries, while it is almost absent in the mesocarp of the fruit. Furthermore, a clear negative correlation was established between the content of resveratrol in the grape skin and its stage of development (Jeandet et al., 1991). Although it is naturally present in grapevine plants in limited quantities, its synthesis can be induced by exposing plants to stress, i.e. various biotic and abiotic factors, among which the most prominent are ultra-violet (UV) radiation, ozone, anoxic treatment, mechanical damage, phytopathogenic infections organisms (Hasan and Bae, 2017), jasmonic acid (JA), salicylic acid (Tassoni et al., 2005), H2O2 and AlCl3 (Wang et al., 2013). In addition to the fact that resveratrol belongs to the chemical group of stilbenes, it is also included among phytoalexins (Pandey and Rizvi, 2009). The term phytoalexin is related to antimicrobial compounds of low molecular mass produced by plants, in response to infection with phytopathogenic organisms or other forms of stress. In the interaction with phytopathogenic microorganisms, they have an inhibitory effect and their intensive synthesis in plants begins with the interaction between the plant and the microorganism. This is the reason why their presence is always associated with the resistance of plants to phytopathogenic fungi, although they can also occur in conditions of abiotic stress. On the other hand, the term "metabolite of stress" introduced by Stoessl et al. (1976) includes antifungal and non-antifungal compounds produced in response to the presence of various stressors, which may or may not be involved in the plant's defense mechanism. Whichever group it belongs to, the importance of resveratrol in protecting plants and preventing the progress of established infections is not diminished (Langcake and McCarthy, 1979).

HEALTH BENEFITS OF RESVERATROL

At the beginning of the nineties of the last century, when during epidemiological studies it was discovered that resveratrol has a cardioprotective effect, the attention of scientists from the fields of medicine and pharmacology was increasingly directed towards this compound. Since then, interest in resveratrol and grapevine, as its most important source in human nutrition, has grown exponentially. Resveratrol is an activator of SIRT1, one of the forms of proteins from the sirtuin group, characteristic of mammals (Chung et al., 2010). SIRT1 deacetylates histone and non-histone proteins, and in addition functions as a transcription factor for many physiological processes. The pathway regulated by SIRT1 affects metabolism, stress resistance, cell lifespan, cellular senescence, immune system function in inflammation, endothelial function, and circadian rhythm. Resveratrol has been shown to activate SIRT1, which indicates the possibility of exerting positive effects in diseases caused by abnormal metabolic control, inflammation, and cell cycle defects (Berman et al., 2017). Resveratrol inhibits cellular events associated with tumor initiation, promotion, and progression. Resveratrol was also found to affect tumor initiation by dose-dependently inhibiting free radical formation when cells (HL-60) were treated with 12-O-tetradecanoylphorbol-13-acetate (Sharma et al., 1994). The inhibitory effect of resveratrol on the development of preneoplastic lesions in the mammary glands treated with a carcinogen and the tumorigenesis of skin cancer was proven in mice (Yang et al.,1997). This drew attention to the fact that resveratrol, as a common ingredient in human nutrition, has great potential in medicine and pharmacology as a potential chemopreventive agent in the treatment of cancer in humans, because it affects all three stages of carcinogenesis: initiation, promotion, and progression (Berman et al., 2017).Considering the positive effect of resveratrol against cancer (Jang et al., 1997), a great effort was made to investigate its biological and pharmacological properties and activities in more detail (Garg et al., 2005; Huang et al., 2019). Therefore, in addition to the anti-cancer activity of this compound, antioxidant, anti-inflammatory, immunomodulatory, regulatory, neuroprotective, and additional cardiovascular protective effects have been determined (Poulsen et al., 2013), and a positive effect in various chronic diseases such as liver diseases, obesity, diabetes, Alzheimer's and Parkinson's disease (Meng et al., 2020). Resveratrol has been shown to affect multiple molecular targets associated with cardioprotective effects. For example, resveratrol promotes endothelial function that may help prevent atherosclerosis and coronary artery disease (Cho et al., 2017; Berman et al., 2017). Also, in research conducted on mice, a positive effect in the treatment of hypertension was found, by causing oxidative activation of protein kinase 1-(PKG1), which leads to the normalization of blood pressure (Prysyazhna et al., 2019; Meng et al., 2020). Several neuroprotective effects of resveratrol have also been demonstrated, including protection against neuronal damage, improvement of cognitive dysfunction, and enhancement of learning and memory abilities (Weiskirchen and Weickirchen, 2016). When it comes to inflammatory processes in the body, resveratrol acts by inhibiting the production of pro-inflammatory cytokines and modifies the immune response against pathogens such as viruses, fungi, and bacteria (Liu et al., 2014; Meng et al., 2020). In the treatment of bacteria, resveratrol can change the expression of virulence factors, reduce biofilm formation, reduce motility and increase the sensitivity of bacteria to different classes of conventional antibiotics (Vestergaard and Ingmer, 2019). Based on all than mentioned, it can be concluded that resveratrol acts in several ways, namely: antioxidant (de la Lastra and Villegas, 2007), an inhibitor of cyclooxygenase and lipid modification, inhibitor of LDL oxidation and platelet aggregation, vasodilator and antiviral agent (Campagna and Rivas, 2010; Cvetković et al., 2015). Considering that it is a bioactive compound present in grapes (and its products), the consumption and utilization of resveratrol represented in the diet through natural sources are very interesting. A large number of in vitro studies have been performed by monitoring the consumption of resveratrol at concentrations that greatly exceed even the highest amount that could be ingested through the diet. Today, various dietary supplements of resveratrol can be found on the market, in which its content varies, and is expressed in milligrams or even grams. An example of this is the daily intake of resveratrol doses of a few grams, while it is known that e. g. a liter of wine contains very small amounts expressed in mg/L (Guilford and Pezzuto, 2011). However, there are doubts that the body cannot use the total amount of resveratrol introduced by supplements, but only a small part, which is a problem for further pharmacological research. This gives priority to the intake of resveratrol through natural sources and highlights the intake of resveratrol through resveratrol-rich foods. We should certainly not forget the fact that it is a very valuable natural compound that has a beneficial effect on several target sites and whose potential needs to be explored and used to the maximum (Pezzuto, 2011; Pezzuto, 2019).

RESVERATROL AND PHYTOPATHOGENIC FUNGI

Phytopathogenic fungi represent the main threat to vine growing and achieving healthy and high yields. With their way of life, they limit the development of plants, cause degeneration of different plant organs, and cause changes in plant tissues during the growing season, thereby permeating all developmental stages of the vine. During evolution, as well as through genetic engineering, grapevine varieties with a certain level of resistance to certain phytopathogenic fungi have been developed, where autochthonous varieties stand out the most. A significant factor that affects the aforementioned resistance is the amount of resveratrol present in healthy plants, as well as the intensity of its synthesis during stressful conditions, which infections certainly are. The presence of infection encourages the creation of resveratrol in larger quantities than it is naturally present, where it acts on pathogens by limiting them and preventing the further progress of the infection until the environmental conditions become unfavorable for further development and reproduction of the pathogen. It is believed that resveratrol is directly involved in the resistance of the grapevine to some of the most economically important pathogens such as *Botrytis cinerea*, *Plasmopara viticola*, *Uncinula necator*, *Guignardia bidwellii*. The concentration of resveratrol in cases of infection with the mentioned pathogens increases around the spot of infection. Localized synthesis and accumulation of resveratrol help to some extent in limiting the infection and preventing its further spread. The synthesis of this compound begins immediately after recognizing the presence of the pathogen, and long before the formation of visible symptoms of infection (Hasan and Bae, 2017). Detection of infection includes the recognition of signaling molecules, i. e. elicitors (Montero et al., 2003). Elicitors are molecules capable of stimulating plant defense mechanisms, participating in various oxidative and physiological responses to abiotic stress including the activation of several secondary biosynthetic pathways such as the one leading to the synthesis of phenolic compounds (Gutiérrez-Gamboa et al., 2021). Salicylates, jasmonates, and ethylene play the role of elicitors in grapevine plants, and in response to their presence, plants synthesize phytoalexins, and among them resveratrol to the greatest extent (Montero et al., 2003). The phenomenon of the dependence of resveratrol synthesis on the presence of infections with phytopathogenic organisms is the subject of many studies conducted over the past decades, with a large number of them supporting the existence of a positive correlation. The established opinion is that resistant varieties of grapevines, in contrast to sensitive ones, initially contain more resveratrol and can produce it in greater quantity when they find themselves in stressful conditions. When it comes to infections with phytopathogenic fungi, they spread from the point of penetration into the plant to the surrounding tissues. In that situation, the concentration of resveratrol in the surrounding tissues increases simultaneously with the growth and spread of the pathogen, reaching several times higher concentrations than the initial one, present before the infection. In this way, resveratrol forms a chemical barrier to limit the progression of pathogens. Among the already mentioned phytopathogenic fungi, *B. cinerea* stands out the most and its influence on the synthesis of resveratrol in grapevine plants is the most studied. During research on the production of trans-resveratrol in grapes in response to infection with this fungus, was concluded that the grapevine in sensitive stages such as ripening in the presence of infection cannot synthesize the required amount of trans-resveratrol sufficient to inhibit the growth and development of the pathogen. However, it is certainly possible at earlier stages, especially during the first stages of berry development (Jeandet et al., 1995). In the period from BBCH 71 to BBCH 75 (from the “phase young fruits begin to swell” to “berries pea-sized”) the concentration of trans-resveratrol decreases, indicating a decrease in resveratrol levels during ripening. Healthy fruits contain less resveratrol than infected ones. When comparing sensitive and resistant varieties of grapevine (Huxelrebe and Castor) to the mentioned pathogen, it is important to point out that a significantly higher amount of trans-resveratrol (up to 50% more) is synthesized in the berries of resistant varieties. Accumulation of this compound increased during the first four days after infection and then decreased until the sixteenth day. Also, it was found that leaves of less sensitive varieties produce higher total amounts of resveratrol per lesion in response to infection (Baveresco et al., 1997). By creating new, high-quality, wine and table grape varieties, the natural ability to defend against various stressors has been somewhat impaired. Therefore, today, more and more vine growers are returning to old, autochthonous varieties with high resistance to various types of stressors, in which synthesis of resveratrol is represented to a greater extent.

RESVERATROL AS A FUNGICIDE

Nowadays when intensive agricultural production is at its peak, the application of synthetic fungicides is an inevitable step in protecting plants from diseases caused by phytopathogenic fungi. According to the Law on plant protection products (Službeni glasnik Republike Srbije, 41/09), their application is regulated and limited, due to the harmful effects on human health, non-target organisms, the environment, and the increasingly frequent development of resistant populations of various phytopathogenic fungi. Therefore, finding new solutions in the form of biological, natural fungicides is of great necessity. Resveratrol has proven to be a good partner in the fight against the most important phytopathogenic fungi of the grapevine. In case of infections of lower intensity, during unfavorable meteorological conditions for the development of pathogens, the effect of resveratrol is reflected in the limitation and inhibition of development, and therefore in the suppression of pathogens. When it comes to severe infections, the effectiveness of resveratrol is not enough to completely suppress the pathogens. In the mentioned situations, intensive synthesis limits the further progression of pathogens and provides the additional time necessary for implementing suitable protection measures. The non-specific antifungal effect of resveratrol and its accumulation in infected tissues makes it a suitable natural fungicide, which, with its natural presence in grapevine plants, contributes to its resistance to fungal infections. However, the problem of protecting the vine does not end after the harvest, because the grapes also need to be protected during storage. A special threat is *B. cinerea*, as the most destructive storage pathogen, not only of grapes but also of other agricultural products. Since resveratrol is a natural antioxidant, it is assumed that it may have positive effects when it comes to preserving stored products. Grapes naturally contain it, and additional, external applications could greatly enhance its effectiveness and prevent fruit decay. This is supported by the research of Montero et al. (2003) who reported the activity of trans-resveratrol and its correlation with ethylene released from grapes infected with *B. cinerea* at room temperature. Ethylene is an indicator of the ripening and aging of fruits, and if it is present in the atmosphere of the storage area, it can contribute to the faster decay of fruits. In this case, the content of trans-resveratrol and ethylene showed the opposite behavior, because the high content of trans-resveratrol corresponded to a low concentration of released ethylene. In grapes infected with *B. cinerea*, ethylene emission started to increase after 48 h when the content of trans-resveratrol started to decrease. The activity of trans-resveratrol as a natural fungicide was also tested by exogenous application on grapes. A short immersion of bunches in trans-resveratrol solution delayed the increase in ethylene emission for about 48 h and led to a decrease in its emission. The tested compound had a positive effect on the preservation of grapes during storage, doubling its usual shelf life at room temperature. During the other efficacy tests, the fruits of different plant species were immersed in an aqueous solution of trans-resveratrol of different concentrations. During the test carried out on grapes, after ten days the fruits treated with trans-resveratrol kept their freshness, while the untreated fruits were dehydrated and infected with phytopathogenic bacteria and fungi. Treated fruits kept freshness twice as long compared to untreated ones. Apart from the purpose of protecting grapes during storage, its effectiveness has also been tested during the storage of apple fruits, which today represents a significant challenge (Ureña et al., 2003). Considering a large number of chemical treatments of apples during the growing season, there is an increasing effort to find adequate solutions for the protection of fruits during storage, while avoiding as much as possible the use of synthetic PPP such as fungicides based on fludioxonil or thiophanate-methyl. By monitoring the condition of treated and untreated fruits of the Golden Delicious variety for 75 days, it was observed that the fruits treated with trans-resveratrol kept a freshness without damage and external signs of decay, while the untreated fruits were completely rotten after 75 days (Ureña et al., 2003). Also, it has been proven that in vitro conditions, the use of resveratrol in the concentration range of 60-140 μg/ml significantly reduces the growth of *B. cinerea* mycelia, while the concentration of 90 μg/ml inhibits the growth of *B. cinerea* mycelia by 50%. In addition, the mentioned concentrations of resveratrol cause cytological deformations of the mentioned pathogen, which results in the formation of secondary and tertiary germ tubes in conidia, cytoplasmic granulation, and the formation of deformed and curved germ tubes (Hoos and Blaich, 1990; Adrian et al., 1997). In addition to *B. cinerea*, agricultural products are exposed to other phytopathogenic fungi and bacteria during storage. During the research, when observing the treated fruits, no signs of infection with phytopathogenic bacteria that were present in the control were observed, which is in favor of resveratrol. When it comes to the action of resveratrol on phytopathogenic bacteria, its mechanism of action is based on damaging the bacterial cell membrane and preventing biofilm formation (Chen et al., 2016). In addition to its effect on *B. cinerea*, resveratrol also showed positive results when it comes to suppressing and inhibiting the fungus *Bipolaris oryzae* and the bacterium *Xylella fastidiosa* (Yu et al., 2013; Paulo et al., 2010). All of the results support the fact that resveratrol is a potential part of the antifungal and antibacterial strategy for the control of not only grapevines, but also other cultivated species, both in the field and during the storage.

CONCLUSION

Studying the metabolism of grapevine plants and their responses to stressful conditions has made it possible to discover another compound that has many positive properties. In addition to all the benefits for human health, which inevitably exist, many others have been discovered that make resveratrol a human ally. The problem of phytopathogenic fungi and the development of their resistance is one of the main limiting factors in growing vines, therefore any new option in their control is desirable, especially if it is natural and environmentally acceptable. Resveratrol is an initial compound of great potential which can improve vine protection in the future. All previous research on resveratrol provided a very significant contribution to the overall knowledge of this plant metabolite and opened the door for many new aspects of research.

ACKNOWLEDGEMENT

This study is a part of the project No. 451-03-68/2022-14/200117, funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia.

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Zakon o izmenama i dopunama Zakona o sredstvima za zaštitu bilja (2021). „Službeni glasnik RS”, broj 41/09.

Original Scientific paper
10.7251/AGRENG2202123H
UDC 636.594

EFFECTS OF PLUMAGE COLOR MUTATIONS ON GROWTH, FEED EFFICIENCY, AND CARCASS CHARACTERISTICS IN JAPANESE QUAIL

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ABSTRACT

The aim of this study is to determine the performance traits of the quail plumage color mutants (Golden, Italian, Black, White, and Wild) with the Gompertz growth function. A total of 100 birds from plumage color mutants (Golden, Italian, Wild, White, Black) with mixed sexes were used in the study. All quails were wing banded and then weighted weekly hatching to six weeks of age. The feed consumption of quails was determined on a weekly basis, and they were slaughtered at six weeks of age. It was determined that the difference in body weight between plumage color mutation groups first appeared between the 14-21[st] days and continued to exist until the end of the trial. The higher live weight (197.64 g) on day 42 is in quail with the golden color mutation than mean values (163.33-177.31 g) of other genotypes ($P<0.05$). Although similar results were observed in terms of carcass weight and abdominal fat weight ($P<0.05$), there was no difference between genotypes in terms of feed efficiency ($P>0.05$). As a result, it was determined that Japanese quails with different feather colors differed in terms of their performance characteristics.

Keywords: *Japanese quail, Plumage color, Body weight, Feed efficiency, Carcass*

INTRODUCTION

Japanese quail has been increasingly used as a source of animal protein for human consumption in recent years. Quail, which was produced only in small quantities in the past years, was formerly produced for hobby purposes and was used as a model animal in scientific studies. Today, there are herds with high productivity because of genetic improvement studies for meat and egg production. However, almost all of these studies were carried out in wild type Japanese quails. The advantages of quail breeding are many, these are short generation intervals, good feed conversion, disease resistance, high reproductive capacity, keeping a large number of animals in small areas. The widely available Japanese quail, known as the wild type, is a bird that can be sexed based on its grizzled feathers and appearance. Besides, there are many plumage color mutations in the Japanese quails. There are studies indicating that the high egg yield in white genotype, and high body weight on the Golden and Italian genotypes. The growth performance and other economically traits of color mutations reported in quail are very limited. Information on growth, egg production and carcass characteristics of mutations other than wild type feather color is insufficient to evaluate their use in commercial production. The associated effects of Japanese quail plumage genetic variants on performance traits have been studied for only a few mutations. There are reports of the roux mutation that can be used for sex discrimination at the exit, and there is information about the curly mutation associated with high body weight (Minvielle, 1999; Minvielle, 2005). The aim of this study was to determine the growth performance, feed consumption, feed efficiency, slaughter-carcass characteristics of plumage color mutants in Japanese quails.

MATERIAL AND METHODS

This study was performed in the Poultry Research Unit of Namık Kemal University, Turkey. Japanese quail (*Coturnix coturnix japonica*) were used as animal material. A total of 100 birds from plumage color mutants (Golden, Italian, Wild, White, Black) with mixed sexes were used in the study. All chicks were wing banded and then weighted weekly hatching to six weeks of age. Chicks were housed in heated brooding cages (82.56 cm^2/quail) for first three weeks. Then, they were transferred to grower cages (150 cm^2/quail). The diet was supplied containing 24% CP and 2900 kcal of ME/kg and *ad libitum* feeding and a 23 h lighting program were applied from hatch to the end of the experiment (Narinç, 2016). The feeds given to the quails were measured as a group for the first three weeks and then individually. In determination of the difference between genotypes in terms of body weight measurements at a time point, profile analysis method was utilized (Alkan , 2012; Narinç , 2010). The Gompertz growth function was used to compare the growth samples of genotypes which is known the best fitted model (Aggrey 2002; Korkmaz & Üçkardeş 2013; Üçkardeş, 2013). Expression, growth rate and inflection point coordinates of Gompertz function is presented in Table 1.

Table 1. Gompertz model expression and coordinates of inflection point

Model	Gompertz
Y_T	$\beta_0 e^{-\beta_1 e^{-\beta_2 t}}$
IP_T	$ln(\beta_1)/\beta_2$
IP_W	β_0/e

β_0 parameter is the asymptotic (mature) weight, β_1 is a shape paremeter, β_2 is growth rate parameter. Model parameters were analyzed using with SAS 9.3 software NLIN procedure Levenberg-Marquardt iteration method (Karaman, 2013).

All quails in the experimental groups were sent to slaughter at the end of the six-week fattening period. Before slaughter, the feed was removed for 4 hours, and the slaughter weight of the quails was determined. All weight measurements during cutting were carried out with a digital scale with a precision of 0.01 g. Following slaughter, wet plucking and evisceration, hot carcass weights were determined, including neck and abdominal fat, excluding edible internal organs.

In the statistical analysis of the continuous data obtained from the study, the variance analysis technique was used for the variables that met the parametric test assumptions, and the Kruskal Wallis test for the variables that did not meet the assumptions, and whether there was a difference between the experimental groups at 0.05 significance level. In case of statistically significant difference between the groups, Duncan multiple comparison test was used for parametric tests and Mann-Whitney U test was applied for non-parametric tests in order to determine from which group or groups the differences originated. All statistical analyzes were performed using SAS 9.3 statistical software.

RESULTS AND DISCUSSION

The results of variance analyses and mean values of performance traits of genotypes are presented in Table 2. As can be seen from Table 2, the highest body weight averages at both five and six weeks of age were found in the Golden genotype, followed by the Italian genotype ($P<0.05$). White quails have the lowest body weight averages in both weeks. ($P<0.05$). In terms of feed consumption averages, Golden and Italian genotypes have higher values ($P<0.05$), while there is no statistical difference between the feed consumption of other genotypes.

Table 2. The mean values of body weight, feed intake, feed efficiency by genotypes and gender

Genotype		BW 35	BW 42	FI 35	FI 42	FCR 35	FCR 42
Golden		169.91^{a}	201.41^{a}	588.43^{a}	779.34^{a}	3.65	4.05
Italian		160.21^{b}	179.01^{b}	569.18^{a}	721.98^{b}	3.75	4.24
Wild		151.68^{c}	171.24^{c}	496.05^{b}	645.02^{c}	3.45	3.96
White		146.96^{d}	160.32^{d}	515.13^{b}	665.11^{c}	3.72	4.40
Black		150.86^{c}	170.18^{c}	497.55^{b}	642.65^{c}	3.48	3.96
Sex							
Female		163.97	186.85	565.64	729.07	3.65	4.12
Male		147.88	166.01	500.90	652.57	3.57	4.13
Interaction							
Golden	Female	175.81	210.79	595.30	799.69	3.57	3.97
	Male	164.02	192.02	581.57	759.00	3.73	4.12
Italian	Female	176.31	195.94	618.39	773.21	3.69	4.13
	Male	144.11	162.07	519.97	670.75	3.80	4.34
Wild	Female	158.01	179.83	537.19	690.04	3.58	4.05
	Male	145.35	162.65	454.91	600.00	3.31	3.88
White	Female	149.57	168.12	526.47	681.02	3.78	4.32
	Male	144.35	152.52	503.79	649.21	3.66	4.48

Black	Female	160.14	179.55	550.84	701.41	3.63	4.11
	Male	141.58	160.81	444.26	583.90	3.32	3.81
SEM		1.87	1.87	8.49	9.07	0.05	0.06
Variation Source		P Value					
Genotype		0.001	0.000	0.001	0.000	0.253	0.068
Sex		0.000	0.000	0.000	0.000	0.422	0.907
Interaction		0.173	0.478	0.223	0.430	0.466	0.479

As can be seen in Table 2, there was no statistically significant difference between genotypes at the ages of five and six weeks in terms of cumulative feed efficiency (P>0.05). The results of the profile analysis carried out to determine the effects of the plumage color mutation are presented in Table 3.

Table 3. Profile analysis results for quail with different plumage color

Time (day)	P value
1-7	0.865
7-14	0.785
14-21	0.034
21-28	0.001
28-35	0.001
35-42	0.002
Wilks' Lambda	0.001

Different results have been obtained in studies carried out to determine the live weights of Japanese quails with different feather colors. Minvielle et al. (1999), Genchev et al. (2008), Oğuz and Minvielle (2001) and Marks (1990) reported that the difference in quail feather color had a significant effect on body weight and wild type quails had higher body weights. According to Tarhyel et al. (2012) determined that the white color mutation causes lower body weight, while Nasr et al. (2017) reported that white colored quails had a higher body weight. The results of this study were found to be consistent with the findings reported by Tarhyel et al (2012).

According to the MANOVA test statistic (Wilks' Lambda) in the corresponding Table 3, genotype profiles do not show parallelism (P<0.05). As a result of the analyzes carried out, it was determined that the difference in body weight between plumage color mutation groups first appeared between the 14-21st days and continued to exist until the end of the trial. The difference in favor of females in terms of body weights of female and male quails is species specific and emerged from the third week due to reverse dimorphism to other birds. A similar situation has been reported by many researchers (Oğuz et al. 1996; Toelle et al. 1991; Sezer et al. 2006). Non-linear regression parameters of Gompertz function were presented in Table 4.

Table 4. The mean values of Gompertz growth curve parameters by genotypes and gender

Genotype		β_0	β_1	β_2	IP_t	IP_w
Golden		275.42[a]	3.78	0.064	21.97	101.32[a]
Italian		240.33[c]	3.68	0.065	20.76	88.41[c]
Wild		260.05[b]	3.74	0.057	24.18	95.67[b]
White		217.42[d]	3.68	0.069	20.42	79.98[d]
Black		244.22[c]	3.70	0.062	22.46	89.84[c]
Sex						
Female		263.82	3.70	0.061	22.18	97.05
Male		231.16	3.73	0.065	21.73	85.04
Interaction						
Golden	Female	316.59	3.67	0.054	24.51	116.47
	Male	234.25	3.90	0.073	19.42	86.17
Italian	Female	268.47	3.75	0.063	21.55	98.77
	Male	212.18	3.62	0.066	19.96	78.06
Wild	Female	269.66	3.72	0.057	23.81	99.20
	Male	250.44	3.76	0.057	24.55	92.13
White	Female	225.54	3.69	0.066	20.52	82.97
	Male	209.31	3.68	0.071	20.32	77.00
Black	Female	238.82	3.68	0.066	20.52	87.86
	Male	249.63	3.71	0.059	24.41	91.83
SEM		5.98	0.04	0.002	0.56	2.20
Variation Source		P Value				
Genotype		0.031	0.852	0.254	0.306	0.031
Sex		0.008	0.662	0.208	0.691	0.008
Interaction		0.084	0.526	0.078	0.106	0.084

In terms of the β_0 parameter of the Gompertz growth model, the mean found for the Golden genotype was higher than the others. The mature weight parameter (β_0) was found between 204 and 281 g in most of the studies examining the growth of Japanese quails with the Gompertz model (Akbaş and Oğuz 1998; Kızılkaya et al. 2005; Narinç et al. 2009; Alkan et al. 2009; Narınç et al. 2010b). The results of this study are consistent with previous reports.

In Gompertz model, β_1 and β_2 are constants related to the shape of the growth curves were in the range of 3.62 to 3.90, and 0.054 to 0.073, respectively. In current study, age and weight at the point of inflection of Gompertz curve were determined to be from 19.42 to 24.55 day and 77.00 to 116.47 g for different plumage color grups. Similar results reported by Alkan et al. (2009) who estimated ages and weights at point of inflection using Gompertz model for selected and control lines. They reported that the mentioned parameters in selection line were found 113 g for female, and 108 g for male. Also, 82.3 g for female quail, and 75 g for male were found for control line. However, Kızılkaya et al. (2005) reported that ages and weights at point of inflections of Gompertz model were found between 16.19 and 17.05 day, and from 81.57 to 82.96 g respectively. As shown here, growth curve parameters of quail can be affected from both the selection and environmental conditions. As a result, it was determined that Japanese quails with different feather colors differed in terms of their growth characteristics.

Table 5. The mean values of carcass characteristics by genotypes and gender

Genotype		CP	BRP	LP	WP	AFP
Golden		66.25[b]	23.66[b]	15.47	5.50[c]	0.98[a]
Italian		68.17[ab]	25.21[a]	16.21	5.68[bc]	0.75[ab]
Wild		68.87[ab]	25.57[a]	16.96	6.12[ab]	0.61[b]
White		69.60[ab]	25.96[a]	16.86	6.55[a]	0.57[b]
Black		71.57[a]	26.76[a]	16.72	6.25[ab]	0.69[b]
Sex						
Female		66.74	24.69	15.74	5.95	0.67
Male		71.04	26.17	17.15	6.09	0.77
Interaction						
Golden	Female	65.04	22.52	13.90	5.31	0.78
	Male	67.45	24.80	17.05	5.68	1.18
Italian	Female	64.63	23.84	15.58	5.52	0.83
	Male	71.72	26.58	16.84	5.84	0.67
Wild	Female	67.78	25.49	16.73	6.20	0.64
	Male	69.95	25.65	17.18	6.03	0.58
White	Female	68.68	26.24	17.12	6.77	0.52
	Male	70.52	25.69	16.61	6.33	0.61
Black	Female	67.55	25.37	15.38	5.95	0.57
	Male	75.58	28.15	18.07	6.56	0.80
SEM		0.53	0.25	0.21	0.09	0.04
Variation Source		P Value				
Genotype		0.018	0.002	0.119	0.001	0.006
Sex		0.000	0.004	0.001	0.430	0.190

Interaction	0.156	0.112	0.028	0.300	0.121

Carcass characteristics of different color mutant quails are shown in Table 5. As a surprising result in terms of carcass yield, the average value of the black genotype was higher than the others, while the lowest average was found in the Golden genotype. In addition, it was determined that the Golden feather mutation caused an increase in the abdominal fat rate as expected. No study has been found in the scientific literature investigating the effects of plumage color mutations on carcass characteristics in poultry.

CONCLUSIONS

As a result, it was determined that the plumage color mutation affected many performance traits in Japanese quails. Wild genotype was used in all genetic improvement studies in Japanese quails. However, the breeding performance of the lines obtained according to the plumage color mutation is a matter of interest.

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Original Scientific paper
10.7251/AGRENG2202131E
UDC 635.63:631.563

EVALUATION OF QUALITY AND SHELF-LIFE OF FRESH AND FLESHY CUCUMBER STORED IN CHARCOAL COOLER BIN IN THE TROPICS

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ABSTRACT

Adiabatic evaporative cooling is a concept and process adopted for extending shelf-life of fresh fruits and vegetables in tropics. This study evaluated the performance of Charcoal Cooler Bin (CCB) and its effects on the quality and shelf-life of cucumber. The storage microclimate and ambient conditions (air temperature and relative humidity) were measured using data logger developed for the purpose of this research consisting of a micro-control unit (MCU), a logging device and sensors. By monitoring the physiological and quality parameters, the effects of the storage media: CCB, open-air, refrigerator and laboratory conditions on the storability of cucumber, and effectiveness of the CCB for fresh cucumber preservation through percentage weight loss, visual quality, degree of shriveling, colour changes and life expectancy were evaluated. The CCB had an average cooling efficiency of 75.56%, an average relative humidity and temperature of 94.71% and 23.22^{o}C for the storage period. The CCB markedly maintained freshness, reduced weight loss (with weight loss of 3.4696% after the storage period relative to cucumber samples stored in the refrigerator, laboratory and open-air condition with 8.4176%, 9.8260%, and 11.2696% respectively) and extended the shelf-life of cucumbers fruits at CCB environmental conditions. Cucumber stored in the CCB were still acceptable for 14 days with weight loss of less than 5% while refrigerated, laboratory and open-air samples were acceptable for 9, 6 and 5 days having weight loss greater than 5% respectively. Therefore, CCB passive system is an inexpensive and reliable storage medium for reducing postharvest losses in a sustainable manner.

Keywords: *Charcoal cooler bin, cucumber, shelf-life, quality, evaporative cooling system*

INTRODUCTION

Cucumbers (Cucumis sativus L.) are fruit vegetables containing high amount of nutrient, vitamins and antioxidants. According to Dzivama *et al.* (2006), fruits and vegetables should be consumed in a fresh form for maximum usefulness and optimum nutritive value. Fruits and vegetables are generally regarded as essential herbaceous plants with high moisture content in their fleshy forms (Mogaji and Fapetu, 2011). Cucumber have a great importance all over the world today due to the social and economic value and are consumed in several ways. Based on the values and freshness for consumption, it is important to study various ways to maintain the cucumbers freshness after harvest to the point of consumption. The appropriate techniques and technology suitable for rural, small and medium scale farmers and fruits and vegetable dealers is the evaporative cooling storage (e.g. CCB). Charcoal cooler Bin technology is known and used to avert post-harvest losses and deterioration. The relationship between temperature and relative humidity of the air in the storage structure vis a vis evaporation in the storage bin is required in a passive system condition. The evaporative processes of CCB are used for all types of agricultural produce especially tropical fruits and vegetables. According to Ronoh *et al.* (2018), the evaporative cooling process is used to extend shelf-life of agricultural produce by slowing down of the rate respiration. The stored produce tends to take a considerably longer time for deterioration to be evident or undergo structural decay because of a reduced risk of microbial growth facilitated by lower temperatures and a higher relative humidity within the cold storage facility (Wills and Golding, 2016). The use of simple systems like evaporative cooling system (e.g. the CCB) would help in solving preservation problems in such marginalized areas (Liberty *et al*., 2013b). This study tends to proffer solution on postharvest handling of fleshy and fresh cucumbers to extend the shelf life as well as reduce the rate of deterioration of the fruit after harvesting. The objective of this study was to evaluate the quality and shelf-life of tropical cucumbers stored in charcoal cooler bin based on physical, chemical and physiological changes in cucumber during storage.

MATERIALS AND METHODS

Cucumber Preparation and Storage Conditions

Cucumber (Cucumis sativus L.) used for this research and analysis were obtained from a local farm in Nsukka, Enugu state, Nigeria on the 16th of December 2020. The cucumber were properly washed with clean water for eliminating microbial contamination and preventing fungal infections. Before storing the fruits in different medium (CCB, Open-Air, Laboratory and refrigerator), the cucumber were then randomly distributed into four groups of five (5) each for the four different storage medium considered and used in this research and labeled accordingly for recognition.

The cucumber were stored in a CCB, a Scanfrost refrigerator (at 8^{o}C and 80-90% RH), open-air (ambient) and in the laboratory (at average room temperature and relative humidity). The external dimensions of the developed Charcoal cooler Bin are 580 mm long, 540 mm wide, 500 mm high (front side and rear side). The cooler room has charcoal-laden walls of 50 mm thickness held by weld and chicken wire meshes on the inner and by well perforated mild steel metal sheets on the outside. The cooler has a metal door measuring 2 m by 0.7 m. The cooler was developed at the department of Agricultural and Bioresources Engineering, Faculty of Engineering, University of Nigeria, Nsukka (UNN). Figure 1. show the charcoal cooler bin used in this study.

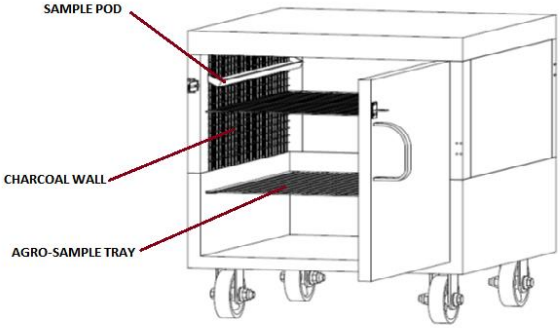

Figure 1. The charcoal cooler bin

Fruits Measurement and Analysis

The Physiological Loss in Weight were calculated as the difference between the initial weight of the fruit and the weight of the fruit at time of measurement and expressed as percentage. Weight losses were determined by weighing the cucumber samples using an electronic weighing balance and recording every day for all storage media calculated as a percent of the initial weight using the following equation (1) (Moalemiyan and Ramaswamy, 2012):

$$WL\ (\%) = 100 \times (W_A - W_B)/W_A \qquad (1)$$

Where: W_A = the weight on the first day of storage [kg] and W_B = the weight on the tested day [kg].

Visual qualities, degree of shriveling and colour changes are important parameters in the evaluation of fruits and vegetables quality and deterioration rates. These quality parameters for different storage conditions were evaluated through the use of the rating charts adopted by Munoz *et al.* (2017) as basis of evaluation. To obtain reliable data on this part of the study, evaluation were conducted with the aid of five (5) students from the University of Nigeria, Nsukka. The data obtained from the result of evaluation were analyzed using a measure of central tendency which is mode, to represent each storage condition and respective quality parameter with respect to storage time on the graph. The temperature and relative humidity were measured for the CCB and ambient conditions and stored in a digital data logger developed for the purpose of this research. The temperature and humidity data were used to analyze the behavior of open-air and CCB setups. All measurements were determined in quintuplicate in all storage media for analyses purpose and the data generated were statistically analyzed using means for weight losses and modes for other quality parameters in Microsoft Excel 2013. The linear regression model in Microsoft Excel 2013 was used for the life expectancy of the cucumber samples stored in CCB.

RESULTS AND DISCUSSION

The Charcoal Cooler and Environmental Conditions

The CCB attained a relatively high cooling efficiency varying between 50.05% and 100.05% with 75.56% average throughout the period under consideration, in agreement with Babaremu *et al.* (2018) which found a varying cooling efficiency and an average of 86.01%. The temperature of the Open-air (ambient) varied between 18°C to 33°C with an average of 27.84°C while the CCB temperature varied between 18°C and 26°C with an average of 23.22°C throughout the storage period, The relative humidity of the CCB ranged from 93% to 95% (on the average 94.71%) while that of the ambient ranged between 12% and 95% (average of 54.32%). Babaremu *et al.* (2018) also found a significantly varying temperature and relative humidity between an evaporative cooling system and the ambient. Figures 2. and 3. shows the trends in average temperatures and relative humidity for the CCB and open-air conditions respectively. The cooling efficiency (η) of the CCB, indicating the extent to which the dry bulb temperature of the cooled air approaches the wet bulb temperature of the ambient air were calculated as defined in the following equation (2) (Echiegu and Ugwuishiwu, 2015):

$$\eta = {(\boldsymbol{To} - \boldsymbol{Ts})}/{(\boldsymbol{To} - \boldsymbol{To,wb})} \qquad (2)$$

Where: η = Cooling efficiency [%]; TO = Outside temperature [◦C]; TS = the inside air temperature [◦C]; and To,wb = the outside air wet bulb temperature [◦C].

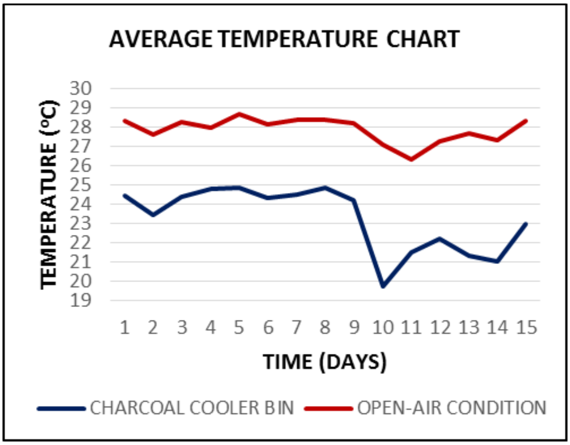

Figure 2. Daily average temperature chart

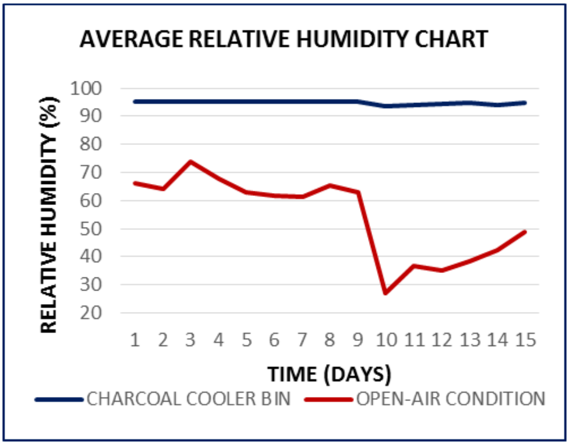

Figure 3. Daily average relative humidity chart

Percentage Weight Loss

The stored cucumber samples in the CCB, refrigerator, laboratory, and open-air (ambient) had an average daily percentage weight loss of 0.2569%, 0.6099%, 0.7458% and 0.8527%. Cucumber samples in the CCB had the least increase in percentage weight loss after the storage period with 3.4696%, compared to 8.4176%, 9.8260% and 11.2696% for refrigerated, laboratory and open-air samples respectively. Figures 4. and 5. below shows the cucumbers weight loss as a function of storage time and temperature, indicating increasing percentage weight loss of the samples during

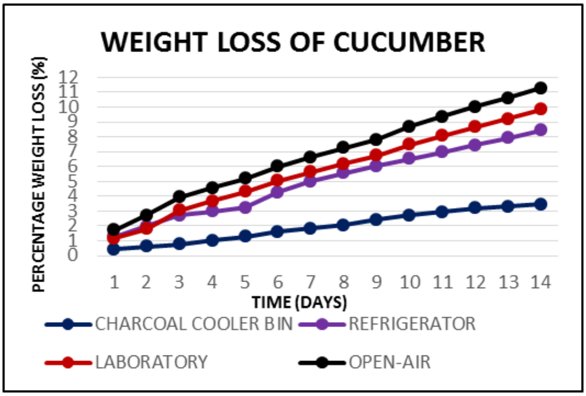

Figure 4: Percentage weight Loss of Cucumber

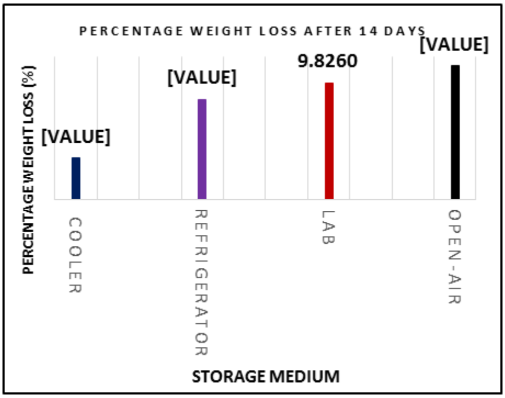

Figure 5: Percentage Weight Loss After 14 Days

the 14 day storage in the media with the storage time. The CCB microclimate (lower temperature and higher relative humidity) reduced the rate of weight loss of samples agreeing with the findings by Basediya *et al*. (2013) that fresh horticultural produce (such as fruits and vegetables) should generally be stored at lower temperatures because of their perishable nature and indicates a lower cucumber weight loss samples stored inside the cooler than those stored outside the chamber. Phal *et al.* (2013) found that the use of evaporative cooling system (ECS) and high density polyethylene (HDPE) with higher relative humidity (93%) than the ambient reduced the weight loss of cucumber from 21% in the ambient to 3.09% during 8 days storage period with fewer changes in other quality parameters.

Quality Parameters

The cucumber samples in the CCB had better visual quality and remained in good condition (with minor defects) compared to samples stored in other storage media which had poor condition (serious defects and limit of usability) as shown in Figure 6, also samples stored in the CCB had minor signs of shriveling, wilting or dryness compared to samples stored in other media which either had evidence of shriveling, wilting or dryness or were severly wilted (Figure 7.). As water is lost from the tissue, turgor pressure decreases, and the cell begins to shrink and collapse, thus leading to loss of freshness (Jadhav, 2018), this can be attributed to the significant loss of freshness for laboratory, open-air and refrigerated fruits due to their increased weight loss. Considering all samples in the different storage medium, cucumbers stored in the CCB maintained the colour of cucumber and had only minor changes in colour comapared to other samples which had either serious evidence of yellowing or major changes in colour at the end of 14 day. The reduced loss of quality and color changes of fruits in CCB may be due to high CO_2 and/or low O_2 levels in the internal atmosphere of the fruits (Moalemiyan and Ramaswamy, 2012).

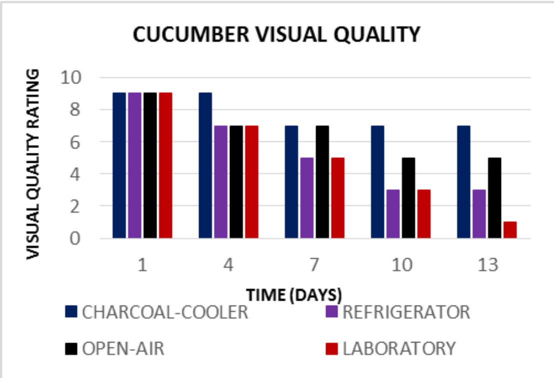

Figure 6: Cucumber visual quality chart

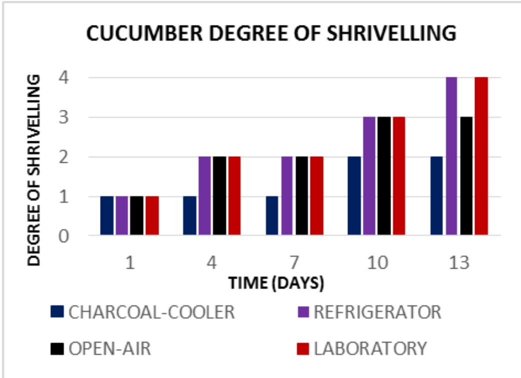

Figure 7: Cucumber degree of shriveling rating chart

8a

8b

8c

8d

Reduced temperature of cucumber lowers their rate of ripening and deterioration (Kays, 1991), this explains why samples in the CCB maintained their dark green colour more than fruits in other storage media. The established fact that the CCB reduced the degree of shriveling (wilting or dryness) and maintained good visual quality of cucumber is in agreement with the results of Munoz *et al.* (2017) that evaporative cooling systems preserve the quality of fruits and vegetables. The images in figure 8 show
the conditions of cucumber samples at the initial and final stages of the experiment clearly showing the colour differences, degree of shriveling and visual quality.

8e 8f 8g 8h

Figure 8: Stored cucumbers at initial and final stages of experiment: (a)Charcoal cooler bin (day one), (b) Laboratory (day one), (c) Open-Air (day one), (d) Refrigerator (Day one), (e) Charcoal cooler (day 14), (f) Laboratory storage (day 14) (g) Refrigerator (day 14), (h) Open-Air (day 14).

Shelf-life and Life Expectancy

After storage period, the data from the weight loss of cucumber stored in the CCB were used to produce a trend line to show the relationship of percentage weight loss with respect to time. These also indicate the possible percentage weight loss of the cucumber samples for the next six days. According to FAO (1989), when the harvested produce losses 5 or 10 percent of its fresh weight, it begins to wilt and soon becomes unusable. As indicated in the graph (Figure 8.), the percentage cucumber weight loss in the CCB will reach 5% after 20 days of storage, agreeing with the results of Munoz *et al.* (2017) that commodities stored in an evaporative cooling system has lesser inclination in the trend line from a linear regression model over time accessed in terms of weight loss. This technique was used to determine the time when the Figure 8 shows the trend line and the equation derived from the regression method. The CCB delayed deterioration and increased the shelf life of cucumber which was detected by observing external appearance, shriveling, the loss of color, and weight loss data. The loss of color, wilting, and weight were greater in cucumbers kept in other storage media than in cucumbers kept in the CCB. Cucumber stored in the CCB were still acceptable for 14 days with weight loss of less than 5% while refrigerated, laboratory and open-air samples were acceptable for 9, 6 and 5 days having weight loss greater than 5% respectively.

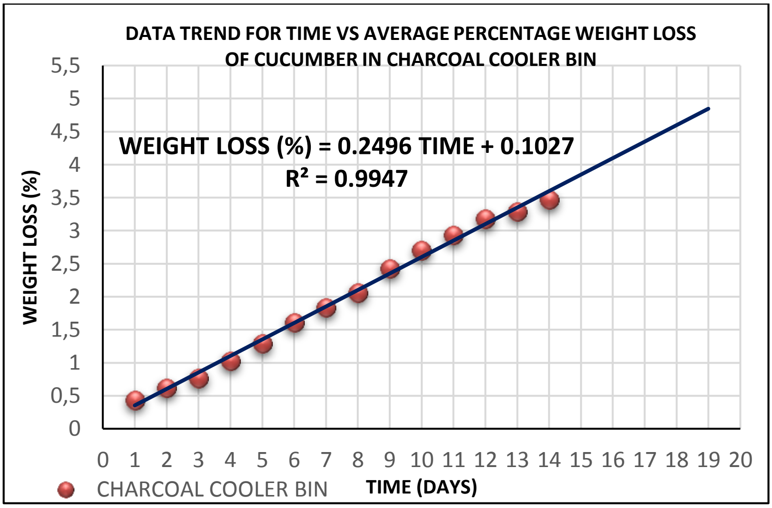

Figure 8: Data trend for time against percentage weight loss of cucumber in the CCB

CONCLUSIONS

This research focused on the evaluation of quality and shelf-life of fresh and fleshy cucumber stored in charcoal cooler bin in the tropics. It can be concluded that the CCB was effective for reducing moisture loss and prolonging the storage life of cucumber. The microclimate of the CCB helped to better retain the different quality parameters by reducing changes in color, degree of shriveling and loss in weight. Commodities stored inside the evaporative cooler show better conditions in terms of weight, visual quality and degree of shriveling compared to those stored in other media. The study proved that the usage of evaporative CCB can prolong the cucumber shelf-life of and perhaps other fruits and vegetables and therefore, proffer a good solution to the issues of postharvest losses.

ACKNOWLEDGEMENTS

The authors would like to thank the Department of Agricultural and Bioresources Engineering, University of Nigeria, Nsukka for approving and facilitating the project implementation. The support received from the Food and bioprocess laboratory section of the department during the study period is also highly acknowledged.

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