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ENVIRONMENTAL PROBLEMS ASSOCIATED WITH SMALL SCALE MINING IN AFRICA: GHANA'S PERSPECTIVE

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Abstract

The review seeks to present an overview of the environmental problems or impact associated with small scale mining in Africa, taking Ghana as the case study and to recommend ways for alleviating problems. The environmental problems of small scale mining in Africa include land degradation, river and channel erosion, noise pollution, air and mercury pollution. African governments could roll out effective regulatory policies, support education and enhance corruption-free environmental protection initiatives to manage the impacts of small scale mining activities in Africa. In Ghana, the effects of small scale mining activities on land, water, soil and air quality cannot be overlooked. Small Scale mining is characterized by higher and quicker income earnings which makes the activity attractive to most people, especially in the rural communities. The environmental problems of small scale mining in Ghana could be managed if all key stakeholders including regulatory authorities, chiefs and environmental

agencies effectively play their roles and promote community participation in environmental decision-making.

Keywords: environmental, policies, pollution, Small scale mining, regulate

Összefoglalás

Munkánk áttekintést kíván nyújtani Ghána példáján keresztül az afrikai kisüzemi bányászattal kapcsolatos környezeti következményekről és hatásokról, továbbá javaslatokat kívánunk megfogalmazni a problémák enyhítésére. A kisüzemi bányászat által okozott legjelentősebb környezeti káros hatások Afrikában a talajromlás, a folyók és csatornák eróziója, a zajszennyezés, valamint a levegő és higanyszennyezés. A környezeti károk enyhítésére az érintett afrikai kormányok feladata a hatékony szabályozási politika kidolgozása, az oktatás fejlesztése és támogatása, valamint a korrupciótól mentes ügyintézés biztosítása kiemelve a környezetvédelmi kezdeményezéseket. Ghánában nem lehet figyelmen kívül hagyni a kisüzemi bányászati tevékenységek szárazföldi, víz-, talaj- és levegőminőségre gyakorolt hatását. A kisüzemi bányászatot a relatíve magas és gyors jövedelemtermelő képesség jellemzi, amely vonzóvá teszi a tevékenységet a legtöbb ember számára, különösen a vidéki közösségekben. A ghánai kisüzemi bányászat környezeti problémáit akkor lehetne kezelni, ha minden kulcsfontosságú érdekelt fél, beleértve a szabályozó hatóságokat, a vezetőket és a környezetvédelmi ügynökségeket, hatékonyan ellátná szerepét és elősegítené a közösség részvételét a környezeti döntéshozatalban.

Kulcsszavak: környezetvédelem, politika, szennyezés, kisüzemi bányászat, szabályozás

Introduction

The contribution of minerals to Africa's economic growth and development is undisputable. Ghana, formerly called the Gold coast, is the second largest gold producer in Africa, contributing about 40% of the country's gross foreign exchange earnings (Amankwah and Anim-Sackey, 2003). Aside these importance, the exploitation or mining of these minerals in Africa widely consists of small scale mining activities which present serious environmental problems or impact. This review seeks to explain the environmental problems or impact of small scale mining in Africa, taking Ghana as a case study and discussing possible ways of mitigating environmental problems of small scale mining.

Minerals and Small scale mining countries in Africa

Small scale mining has become a fast growing sector in rural sub-Saharan Africa. Burkina Faso, Ghana, Mali, Sierra Leone and Tanzania are among countries with higher number of people dependent on the sector. In Tanzania, there are more than one million small scale mining operators (Bryceson and Geenen, 2016). The operations of small-scale gold has increased since 1984. Today, between 5,000 and 10,000 individuals can be found at a single site (Tráore, 1997). It is estimated that heavy mining activities contributes to the clearing of 100,000 hectares of land each year in Zimbabwe (Maponga and Anderson, 1995).While gold is the focus of most of these operators, other commodities mainly gemstones and diamonds also engage a significant number of people in countries like the DRC, Madagascar and Sierra Leone (Hentschel et al., 2002). Figure 1 below illustrates minerals of small scale mining regions and list of African countries.

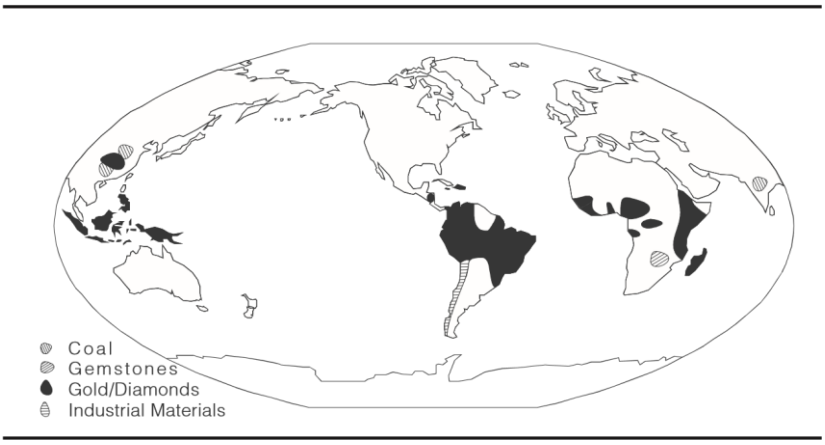

Figure 1. Minerals of small scale mining regions
Africa: Ghana, Kenya, Tanzania, Zambia, Zimbabwe, Ethiopia, Guinea, Liberia, Nigeria, Gabon, Central African Republic, Burundi and Madagascar
(updated from Dorner et al., 2012.)

Environmental impact in Africa

Environmental awareness is mostly low throughout the industry and has few effective environmental safeguards in situ. Few miners regrade excavated land, which is usually left exposed to erosion agents (wind, rain, groundwater, etc.). The fundamental environmental impacts of intensive small-scale mining activity include sedimentation, river and channel erosion, dust and noise pollution. However, the foremost pressing environmental problems in African small-scale mining regions are pollution from mercury and land degradation (Lacerda, 1997).

Mercury which is employed for amalgamation in gold mining, is often dispensed untreated into the atmosphere and waterways where it is then transformed by microbes into toxic methylmercury. Above the recommended level, methylmercury threatens the health of virtually every invertebrate, human, bird and mammal (Wolfe et al., 1998). It is now a widespread contaminant throughout the continent because it is used carelessly. For example, in Victoria Fields, Tanzania, where the yearly input of mercury to gold mining operations is approximately 6 tons, an estimated 24 loads of gaseous mercury has been released into the atmosphere since

1991 (Lacerda, 1997). In another study which was conducted within the Victorian Goldfields, during which samples of water, soil, river sediments and mine tailings were analyzed for mercury content, it had been discovered that mercury had heavily bio accumulated in the natural environment. The findings suggest that the careless handling of mercury could after all, adversely affect the health of many Tanzanian miners (Ikingura et al.,1997).

Small-scale mining, as a migratory industry, has caused substantial land degradation throughout the continent. Thousands of pits and trenches are dug within the process of excavating for prospective ore bodies, many which have since been filled with water and now function as breeding grounds for malaria-infected mosquitoes (Aryee et al., 2003). Furthermore, as a result of intense prospecting and excavation activity, the pristine African rainforest has been removed, vegetation trampled and large areas of earth upturned (Kevin, 2017).

The case study of Small scale mining in Ghana

The activity of individuals engaging in illegal small scale mining in Ghana is locally known as "*galamsey*". The number of people who engaged in illegal mining expanded from 30,000 in 1995 to about one million in 2006 (Bawa, 2006). Some of these people are farmers who cultivate cocoa and other cash crops but eventually abandon their activities to join illegal small scale mining in their quest to get quick money (Figure 2). Morever, Boateng et al. (2014) reported that as a result of quicker and better earning in mining activities contrast to earnings from livestock rearing and crop farming explains the reason for low labor turn out for agricultural activities.

Although the government has long regulated small scale mining, requiring prospective applicants to follow a series of streamlined regulations to get a concession, ineffective policies and bureaucratic inefficiency have impeded formalization, making the illegal activity more

appealing. Factors that have been raised as a propulsion turning thousands of Ghanaians to *galamsey* communities in search of labor include increased rate of unemployment (Hilson and Potter 2005). Another aspect of the debate relates to miners being trapped in a very vicious cycle of poverty and so being unable to readily abandon their activities. It is unfortunate that what has further reinforced this perception in Ghana is the poor response of miners to efforts being made by the government and private sector partners to develop alternative income earning activities in rural areas (Hilson and Banchirigah, 2008). The waste emissions from small-scale mining resulting from the method of extraction and processing causes serious environmental problems that affect the health and livelihoods of residents of most mining communities (Agyemang, 2010). Small scale mining activities also violate the human rights of residents of mining communities and sometimes adjoining communities (Niber, 2008).

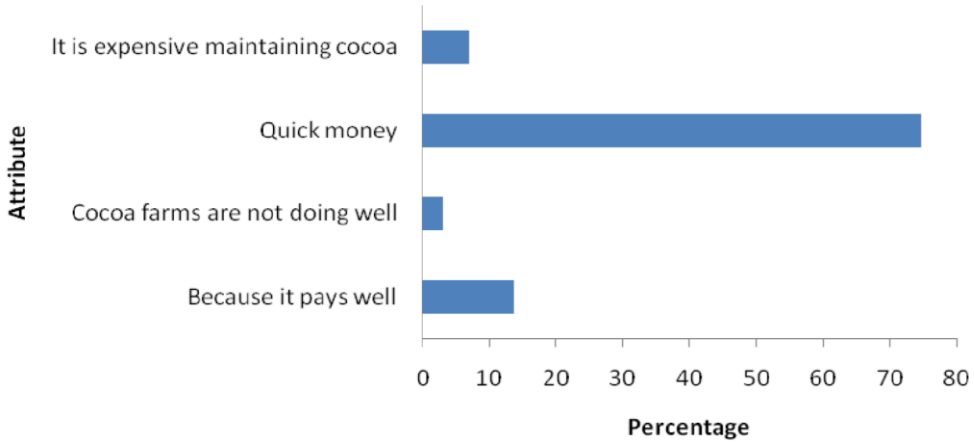

Figure 2. Views on why farmers go into "Galamsey" (Boateng et al., 2014)

Effects of small scale mining in Ghana

Effect of small scale mining on Land

Land degradation is one of the major impacts of small scale mining in Ghana. Flora and fauna are destroyed in the process of mining (Coomson, 2004). The mining of deep deposits typically produces wider openings than shallow deposits. The openings are often not backfilled after the ore extraction. The resultant repercussions are massive gullies, excessive runoff, heavy erosion,

loss of land productivity, reduced soil infiltration, reduction in groundwater recharge and consequent loss of viability for agricultural purposes. These openings also leads to loss of habitat for birds and other animals, and have the tendency of trapping or killing farmers and animals (Akabzaa and Darimani 2001). Examples of trenches and pits created as a result of small scale mining activities are shown in Figure 3. Figure 4 illustrates a degraded land devoid of vegetation cover resulting from gold mining activities in Prestea, Ghana.

Figure 3. Illegal small-scale mining activities (popularly known as "galamsey". Source: Internet)

Figure 4. Degraded land devoid of vegetation cover resulting from gold mining activities in Prestea. Source: Fieldwork, June (2014)

Effects of small scale mining on water

Small scale mining of alluvial gold is a major contributing factor of river water pollution in Ghana. Because of the dredging activities and therefore the washing of alluvial gold in the water, siltation is common in major rivers and streams where the miners operate. The operations have also changed some water course of streams and rivers (Oblokuteye, 2010), depriving downstream users of their only source of water. Fish and other aquatic organisms die-off can occur, altering the food cycle significantly (Aryee et al., 2003). The Bonsa River, for example, which was relied on by farmers and some community members for their source of drinking water and fish has been stricken by illegal small scale mining. Figure 5 shows a section of the

Bonsa River where the mining activities occur. Piles of excavated materials are heaped along the river bank with trees felled into the river.

Figure 5. Highly impure Bonsa River as a result of small scale mining in Ghana

Effects on air quality, poor ventilation and noise pollution

Air pollution resulting from mining related activities comes from the production of dust and emission of mine gases during drilling, blasting, grinding and crushing of ore. The ambient air quality is deteriorated by fine particulates released from the sieving of crushed stones during small scale mining activities (Al-Hassan and Amoako 2014). In terms of underground mining operations, the confined nature of the operation compels dust to be generated within the stopes and accumulate, serving as a possible health threat to the miners. Most of those same stopes are used as openings for entry and exit without any ventilation system in place. This is as result of ignorance on the part of the operators (Aryee et al., 2003). Small scale mining activities can generate loud noise that can result in hearing impairment of the miners and nuisance to the residents of the encircling communities. The loud noise produced by crushing machines during blasting can affect the hearing of the operators who often operate the machines without any form of hearing protection. People who live near the operations see small scale mining activities as major source of nuisance.

Mercury Pollution and impact on Ecosystem

The use of mercury for processing of ore has serious adverse effects on human life and the ecosystem, unfortunately not much attention has been given to mercury contamination in Ghana (Donkor et al., 2006). For example, a research was supported by the United Nations Industrial Development Organization to determine the environmental impacts of mercury on river water, soil and fish samples obtained from Dumasi, (a small scale mining village with about 2000 people) in the Western Region of Ghana. The results showed significant contamination of soil sediments. Most of the fish fillets were also found to have accumulated mercury levels that exceed the United States Food and Drug Agency (US-FDA) action level. The fish from the rivers were reported to be unfit for consumption (Essah, 2000).

Impact on Soil Quality and Agricultural activities

Soils are adversely affected by surface mining. Since miners employ heavy machinery during mineral's extraction, vital soil organisms are destroyed, stable soil aggregates break apart, eventually depriving the soil of organic matter. The soils or newly created substrates are often inhospitable to vegetation due to a combination of physical, chemical and microbiological factors resulting in low yield of crops grown on these soils and subsequent reduction of income generated (Mensah, 2015). Soil substrates from mined areas have very low levels of macro-nutrients especially nitrogen, phosphorus and potassium which can tend to limit tree growth (Sheoran et al., 2010).

Agriculture is an important source of livelihood in mining communities. Crop farming, livestock rearing and fishing are affected by small scale mining activities. For instance, the clearing of shea trees by miners during gold extraction affect shea nut production since such trees are cut down. The bioaccumulation from polluted water as a result of mining activities

makes the aquatic environment not conducive for fishes, thereby reducing their population (Ontoyin and Agyeman, 2014). This occurrence reduces the quantity of fish obtained by fish farmers and the stock of fish obtained can easily go bad within a short period of time. Small scale mining activities has also led to death of livestock, theft of animals and low farm labour productivity (Obiri, 2012).

Mitigating the impact of small scale mining

The government should make conscious efforts to tackle the weakness in environmental policies on small scale mining and strengthen their enforcement in order to achieve sustainability of the environment. In the absence of a workable environmental regulatory framework, sustainability cannot be achieved since the policies will provide the avenue for guiding the overall environmental behavior (Hilson, 2000).

Environmental policies and their enforcement are actions taken to manage human activities with the view of preventing, reducing, or mitigating harmful effects on nature and natural resources. Land reclamation is an adaptive tool to manage degradation and openings created as a result of small mining activities. Regulatory bodies could conduct regular inspection to see to it that, openings and shallow deposits are backfilled after mining activities. The addition of local manure to the soil after backfilled would help to rejuvenate and enhance land formation.

In case of the effect on water, the activities of small scale miners should be guided and properly regulated. Miners should be banned from washing their mineral ore in a whole flowing water body. Miners also should clean or get rid of their unwanted excavated materials to prevent them from getting into nearby rivers and water course. Those that are found culpable should be punished by law to serve as a deterrent to others.

Regulatory authorities should help to make sure that, miners embrace innovative ways of carrying out mining related activities in a manner that drastically reduces loud noise and pollution of fresh air. Miners should also be educated on the dangers associated with the use of mercury and its toxicity to both aquatic and human lives. The government in collaboration with environmental regulatory bodies could organize training workshops for miners on improved techniques, introduce mercury abatement technologies and to help raise overall environmental awareness (Hinton et al., 2003). Regulatory authorities could also resort to the use of local radio stations to disseminate information to miners since the medium has been proven to be one of the effective ways of reaching out to miners (Heemskerk and Olivieira, 2004).

Mitigating the impacts of small scale mining on Soil and Agricultural activities

Soil conservation is important for the cultivation or growing of food that feeds the people. The responsible regulatory bodies should enforce laws that would desist miners from using illegal machinery that excessively distort the soil living and organic components. Miners should be educated on the importance of giving excavated portions of the soil time to heal or recover from previous excavations. Continuous mineral excavation should be discouraged. Regular monitoring by regulatory bodies would help to ensure that, miners stick to places where they have been given consignments, so as to refrain from the forceful clearing of farm trees and food crops of farmers (Aryee et al., 2003; Banchirigah, 2006). Miners should also be admonished by regulatory authorities to avoid undertaking activities that kill livestock and excessively pollute water bodies. Provision of some relief packages, government's commitment and support would help to increase farm labor and enable farmers to thrive in their activities instead of abandoning their farms to go into mining activities (Boateng et al., 2014).

There is a need to organize environmental awareness campaigns and education in various small scale mining communities as a medium of ensuring sustainable use of the environment. Effective community participation in environmental decision making is necessary for natural resources management practices. Participation should not be based on only public consultations but also by creating avenues for open exchange of ideas, transparency, mutual learning, informed and representative decision making (Bastidas, 2004).

Conclusion

Small scale mining is causing huge environmental problems in Africa, for which irrespective of the benefits the industry presents, leaders must first prioritize environmental conservation. Small-scale mining operations in Africa have caused considerable environmental problems such as chronic soil degradation, water and air pollution. African governments, when deciding what approach to adopt to tackle pressing environmental problems in small-scale mining regions, can draw heavily from deep environmental impact assessment in the mining sectors of the countries. For marked improvements to occur, initiatives must be corruption free and also protect the interests of people living around these mining areas. It is suggested that primary emphasis be placed on improved regulation, expanded support and education on the environmental impact of illegal small scale mining activities.

In Ghana, poor monitoring of small scale mining operations, lack of policy implementation and regulatory enforcement by regulatory authorities have led to series of environmental pollution and threats including noise and mercury pollution as well as the effect on the land, water, and air quality. The underground mining operations is characterized by unsafe acts and practices, improper choice of tools, absence of personal protective equipment for drilling and blasting. To deal with the environmental problems associated with small scale mining in Ghana, regulatory

authorities such as the Minerals Commission has to improve its monitoring activities, enforce regulatory requirements and organize workshops to educate miners on the environmental and health risks. Other key stakeholders such as the Environmental Protection Agency, the security agencies, chiefs, landowners, the local and national government must play their roles to mitigate the negative impacts of the sector in Ghana.

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FLOWER PREFERENCE OF BRACHYCERA SPECIES IN A DRY GRASSLAND WITH A *PULSATILLA GRANDIS* POPULATION

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Abstract

The aim of our study was to investigate the importance of *P. grandis* flowers for Brachycera communities. We also studied the attractiveness of other plant species flowering together with *P. grandis*. We aimed to measure the effect of temperature and precipitation on the number of Diptera visitors of *Pulsatilla grandis* in two consecutive years, so we compared the data of the present study with our former results. 135 individuals were collected, 46% of them were female and 54% male. The highest number of *Brachycera* individuals occurred on *Potentilla arenaria*. The ratio of males and females was various. 91% of the collected individuals represented the Bombyliidae family, rest of the individuals were member of Tachinidae (6%), Syrphidae (2%) and Calliphoridae (1%) families. Mean precipitation values and mean daily temperature correlate with the number of flies of *Pulsatilla grandis* flowers.

Keywords: Bombyliidae, female, fly, male, precipitation

Összefoglalás

Tanulmányunkban arra a kérdésre kerestük a választ, hogy a *P. grandis* virágai milyen mértékben fontosak a Brachycera közösségek számára. Fel kívántuk tárni, hogy a gyepszintben egyidejűleg virágzó fajok milyen mértékben vonzzák a legyeket, és milyen mértékben látogatják ezzel párhuzamosan a *Pulsatilla* virágokat. Kíváncsiak voltunk továbbá arra, hogy a *Pulsatilla grandis* virágok légy látogatóinak száma két egymást követő évben hogyan változik a hőmérséklet és a csapadék függvényében. Kutatásunk során összesen 135 egyedet fogtunk be, melynek 46%-a nőstény, 54%-a pedig hím volt. A legtöbb Brachycera egyed a *Potentilla arenaria* fajon fordult elő. A hímek és a nőstények aránya változó volt. Az összes begyűjtött egyed 91%-a tartozott a Bombyliidae családba, ezután a Tachinidae (6%), a Syrphidae (2%) és a Calliphoridae (1%) család következett. A gyűjtési idő alatti átlag csapadék mennyiségek és napi átlaghőmérsékletek korreláltak a begyűjtött legyek számával.

Kulcsszavak: Bombyliidae, csapadék, hím, légy, nőstény

Introduction

Dry grasslands are species rich and threatened habitats in Europe, containing many endemic plant and animal species. Their area considerably decreased in the last 200 years (Csecserits et al., 2018). The abandonment of grassland management can result in litter accumulation, spread of competitor species, succession and loss of valuable species (Valkó et al., 2012). *Pulsatilla grandis* Wender. is one of these valuable and endangered species, which provides important nectar and pollen resources for insects in early-spring.

The aim of our study was to investigate the importance of *P. grandis* flowers for Brachycera communities. We also studied the attractiveness of other plant species flowering together with *P. grandis*. We aimed to measure the effect of temperature and precipitation on the number of

Diptera visitors of *Pulsatilla grandis* in two consecutive years, so we compared the data of the present study with our former results.

Members of the Diptera order has only one pair of membranous wings, the second pair evolved into mechanosensory organs (halteres). The order is very species rich, its representatives are characterized by various morphology and ecology. Most Diptera taxa (flies and mosquitos) lay eggs and undergo complete metamorphoses. About 120 000 Diptera species has been described worldwide. In Hungary the estimated number of species is 10000. But the fauna is still not well-described (Tóth, 2009). The number of described species in Hungary is about 6000 (Papp, 2001). Dipterans play important role in decomposition, sewage cleaning, pest reduction, pollination etc. Damages caused by Diptera is related to the various feeding of their larvae.

Former studies in the Bakony Mts found *Platycheirus fulviventris* (Macquart, 1829) and *Rhingia campestris* Meigen, 1822 on *Pulsatilla nigricans* Störck (Tóth 2001). According to our study in 2018 the flowers of *P. grandis* on the Vörös Hill and Csatár Hill were mainly visited by hoverflies (*Brachypalpus laphriformis* (Fallén, 1816), *Brachypalpus valgus* (Panzer, 1798), *Chrysotoxum elegans* Loew, 1841, *Criorhina asilica* (Fallén, 1816), *Eristalis tenax* (Linnaeus, 1758), *Eupeodes luniger* (Meigen, 1822), *Scaeva pyrastri* (Linnaeus, 1758), *Scaeva selenitica* (Meigen, 1822), *Sphaerophoria* scripta (Linnaeus, 1758)), but representatives of Calliphoridae (*Calliphora vicina* Robineau-Desvoidy,1830, *Pollenia vespilo* (Fabricius, 1786), *Pollenia rudis* (Fabricius, 1786)), Stratiomyidae (*Nemotelus pantherinus* (Linnaeus, 1758)) Tachinidae (*Blondelia nigripes* (Fallén, 1810), *Germaria ruficeps* (Fallén, 1820)) were collected too.

All hoverfly species collected in our study had already been reported from the Bakony Mts (Tóth, 2001), but no hoverflies have been observed on *Pulsatilla grandis* flowers in that region so far. So our former study recorded 9 new hoverfly visitors of that species in the Bakony Mts (Mészáros and Tóth 2020).

Materials and methods

The study area can be found on the Csatár Hill near Veszprém city (Hungary). The 0.6 ha study site lies at 328–335 m a.s.l.. Its vegetation can be classified as *Chrysopogono-Caricetum humilis* Zólyomi (1950) 1958 association. According to our former studies this is a diverse dry grassland with high nature conservation value, we have recorded 9 protected plant species on the area: *Anacamptis pyramidalis* (L.) Rich., *Erysimum odoratum* Ehrh., *Jurinea mollis* (L.) Rchb., *Linum tenuifolium* L., *Orchis morio* L., *Plantago argentea* Chaix in Vill., *Polygala major* Jacq., *Pulsatilla grandis* Wender., *Stipa pennata* L. (Mészáros et al., 2018).

Diptera were collected in a 24 hours long period between 14-29 March 2020. We collected the Diptera visitors of every flowering plant species. Only *Pulsatilla grandis* Wender was flowering on the first and second study days, later the following species were flowering too: *Alyssum montanum* L., *Muscari neglectum* Guss. ex Ten. s. l., *Potentilla arenaria* Borkh. and *Pulsatilla nigricans* Störck. Insects were collected with a butterfly net 30 cm in diameter, but the original net had been replaced by a dense and transparent tulle net, which is suitable for Diptera collecting. The site was scanned continuously.

The species have been identified by Sándor Tóth according to Mihályi (1979; 1986) and Tóth (1977; 2017).

We used our data from 2019 (Mészáros and Tóth 2020) to compare the number of Diptera visitors of *Pulsatilla grandis* in two consecutive years. Mean daily temperature and precipitation data was shared by the Hungarian Meteorological Service.

Results and discussion

135 individuals were collected, 46% of them were female and 54% male. Although 5 early-spring species were flowering on the study area *(Alyssum montanum, Muscari neglectum,*

Potentilla arenaria, Pulsatilla grandis, Pulsatilla nigricans), flies were collected only from 3 species *(Muscari neglectum, Potentilla arenaria, Pulsatilla grandis)* (Table 1).

Table 1. Diptera visitors collected on the Csatár Hill

Species	*Potentilla arenaria*			*Muscari neglectum*			*Pulsatilla grandis*		
	Male	Female	Total	Male	Female	Total	Male	Female	Total
Bombylius discolor Mikan, 1796	4	3	7	23	15	38		1	1
Bombylius major Linnaeus, 1758	30	27	57	10	5	15			0
Bombylius medius Linnaeus, 1758		1	1	1	3	4			0
Brachypalpus laphriformis (Fallén, 1816)		1	1			0			0
Brachypalpus valgus (Panzer, 1798)	1		1			0			0
Calliphora vicina Robineau-Desvoidy,1830		1	1			0			0
Gonia divisa Meigen, 1826		1	1			0			0
Gonia ornata Meigen, 1826	1	1	2			0			0
Melanostoma mellinum (Linnaeus, 1758)						0		1	1
Meriania puparum (Fabricius, 1794	1	2	3			0			0
Tachina lurida (Fabricius, 1781)	2		2			0			0
Total:	39	37	76	34	23	57	0	2	2

The highest number of *Brachycera* individuals occurred on *Potentilla arenaria* (76) and *Muscari neglectum* (57). Only 2 individuals were collected from *Pulsatilla grandis*. The ratio of males and females was various: *Potentilla arenaria* and *Muscari neglectum* had more male visitors, while only females were collected from *Pulsatilla grandis*. 53% of all individuals (135) were *Bombylius major*, and 34% were *Bombylius discolor*. *Bombylius major* was the most frequent visitor of *Potentilla arenaria* (57), *B. discolor* had the second highest number with significantly less individuals (7). Their frequency was different in the case of *Muscari neglectum*, 38 *Bombylius discolor* and 15 *B. major* were collected. From the 11 visitors only *Bombylius discolor* was collected on all the 3 plant species. *B. major* and *B. medius* visited 2 plant species, the other species were only collected on *Potentilla arenaria*. 91% of the collected

individuals represented the Bombyliidae family, rest of the individuals were member of Tachinidae (6%), Syrphidae (2%) and Calliphoridae (1%) families (Table 2).

Table 2. Diptera families collected on the Csatár Hill

Family	*Potentilla arenaria*			*Muscari neglectum*			*Pulsatilla grandis*		
	Male	Female.	Total	Male	Female	Total	Male	Female	Total
Bombyliidae	34	31	65	34	23	57		1	1
Calliphoridae		1	1			0			0
Syrphidae	1	1	2			0		1	1
Tachinidae	4	4	8			0			0
Total:	39	37	76	34	23	57	0	2	2

Bombyliidae are medium-sized, usually hairy flies with long proboscis. Some species hover in the air over the flowers and use their proboscis to reach the nectar (Tóth, 1977). Panov (2007) studied the gut content of Bombylidae species and observed that females consumed much more pollen than males. They found no difference in the pollen consumption of long-proboscid and short-proboscid species. Both sexes are capable of pollen digestion. According to our observations they touched the flowers of *Pulsatilla grandis* with their proboscis only for moments, whereas they spent more time on *Potentilla* and *Muscari* plants. They reach the nectar with their long proboscis (without mowing in the flower), therefore they role in pollination is negligible. Adult Syrphidae (hoverflies) mainly feed on nectar, honeydew and pollen, thus can play important role in pollination (Tóth, 1977). Adult *Tachinidae* live in forests and bushy habitats, feeding on nectar and sugar containing sap. Among Diptera Tachinidae have the highest importance in forest ecology, therefore they belong to families with great economic value. They help pest control in forestry (Tóth, 2014). They occurrence on the study site can be expected as the grassland is bordered by forest on two sides.

All hoverfly species collected in our study had already been reported from the Bakony Mts (Tóth, 2001), on different plant species. Our study reports one new hoverfly visitor (*Melanostoma mellinum*) of *Pulsatilla grandis* in the Bakony Mts. On *Pulsatilla grandis* we did not recorded the occurrence of *Platycheirus fulviventris* and *Rhingia campestris*, which were observed on *Pulsatilla nigricans* Störck in the Bakony Mts in a former study (Tóth, 2001). Neither *Brachypalpus laphriformis* nor *Brachypalpus valgus* had not been observed before on *Potentilla arenaria* in the Bakony Mts, so these are 2 new records for the Bakony Mts too. No hoverflies were found on *Muscari neglectum* flowers, former studies in the Bakony Mts have recorded also only 4 hoverfly species on *Muscari racemosum*.

The yellow flowers of *Potentilla arenaria* attracted the most visitors, thus we can conclude that colour is more important than the size of flower for Brachycera species in flower selection. *Potentilla* was abundant in the whole grassland, so the little groups of blue *Muscari* flowers were not so conspicuous, not to mention the solitary, purple flowers of *Pulsatilla*. It is also probable that the length of the stem play a role in flower selection as well. Flies can safely visit the *Potentilla* flowers on short stems even in stronger airflow. *Muscari* has longer stem so its flowers are not so stabile in windy conditions. The huge, bell shaped flowers of *Pulsatilla* sway in the wind.

The results of the two study years show that the number of Diptera visitors of *Pulsatilla grandis* has considerably decreased. In 2019 15 individuals were collected from the same *Pulsatilla grandis* population between 16-24 March (Mészáros and Tóth 2020). Although the length of our study was nearly the same in 2020, only 2 individuals were collected. According to the data of the Hungarian Meteorological Service (Table 1) the average of mean daily temperature decreased in the second year (7.52 and 5.30 °C). Mean daily temperature values were 2.9–13.5 °C in the first year, and -4.1–13.4 °C in the second. The mean of daily total

precipitation values was significantly higher in the first year compared to the second (0.28 and 0.05 mm).

The decrease of mean daily temperature in the study period is interesting because *Pulsatilla* flowers were in the same phenological stage in both years. Buds were gradually opening, than slowly wilting after full blossoming. So pollinators could visit flowers in the same phenological stages in both years. In early spring insects limitedly visit flowers due to unfavourable weather (Kratochwil, 1988), thus lower temperature could also cause the decrease of *Pulsatilla* visitors in the second year. The overlap of phenological stages of plants and insects was less optimal in 2020. In 2020 mean daily temperature values fluctuated widely (Figure 1). The difference between the lowest and highest mean daily temperature was 10.6 °C in 2019 and 17.5 °C in 2020 (moreover the mean daily temperature was under 0 °C on some days in this year). The fluctuation between nights and days was considerable too, which also could have unfavourable effects on the visiting activity of flies.

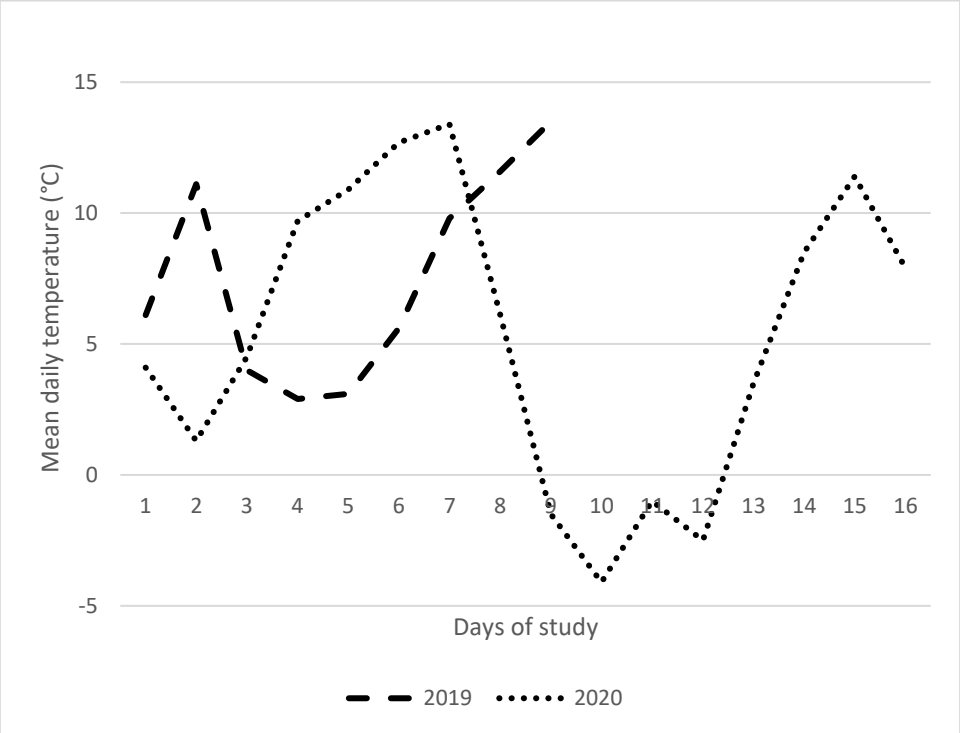

Figure 1. Mean daily temperature values during the study period (Kab Hill) according to the data of the Hungarian Meteorological Service

Mean precipitation values correlate with the number of collected flies, the lack of precipitation also has negative effects on the presence of Brachycera individuals.

The population of *Pulsatilla* decreased significantly in the two years of the study because of game damage. In the second year we observed that the number of damaged plants increased every day. Sauberer and Panrok (2015) found in their study in Lower Austria and Vienna that some *P. grandis* individuals were damaged by red deer or European hare. The results of Kerekes (2013) also confirm that roe deers consume the shoots of *P. grandis* in early spring. We found in our former study that 61% of game damage happened when flowers were in buds (Mészáros and Galambos 2017). When the achenes have formed, we did not recorded new game damage. This can be the result of appearance of other, more preferred plants. Although every part of the plant is poisonous game species often feed the *Pulsatilla grandis* shoots with buds and flowers, and preferred the former phenological stage. Despite the above mentioned game damage the number of *Pulsatilla* flowers were still high, thus the lower number of flowers could not be the main reason of the decrease of Brachycera individuals in the second year.

Flies are mainly not studied as pollinators but they role in plant-insect relations is indisputable. These relations are very complex and diversified, deep and detailed knowledge is needed for their better understanding. The aim of our study was to improve this knowledge.

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EFFECTS OF TEMPERATURE AND PRECIPITATION ON DIPTERA SPECIES, AND FLOWER PREFERENCE OF DIPTERA SPECIES IN AN *ADONIS VERNALIS* POPULATION

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Abstract

One aim of our study was to examine the effects of temperature and precipitation on Diptera visitors of *Adonis vernalis*. Another aim was to describe flower preference of Diptera species. *Adonis vernalis* visitors were studied between 3–13 April 2020 in the slope steppe of the Csatár Hill near Veszprém city. Diptera individuals found on every flowering plant species were collected. Data of *Adonis vernalis* visitors was compared to our results from the previous year. Meteorological data was shared by the Hungarian Meteorological Service. The number of Diptera visitors of *A. vernalis* decreased significantly in the second year. Our results show that compared to temperature precipitation has stronger effect on the flower visiting activity of Diptera taxa. Six plant species were flowering on the study area, but flies were collected only on *Adonis vernalis* and *Lamium purpureum.* The total number of collected flies was 19 representing 5 species. *Lamium purpureum* was visited only by Bombyliidae species. The

number of male flower visitors was higher on both plant species. Although none of the collected fly species was flower specific, we still found differences in their flower preference. Fly species are not primarily mentioned as pollinators and pollination studies mainly focus on bees, it would be important to carry out further studies on the role of Diptera species in pollination.

Keywords: Bombyliidae, flower visitor, fly, *Lamium purpureum*

Összefoglalás

Kutatásunk egyik célja az volt, hogy megállapítsuk, hogy az *Adonis vernalis* Diptera virághoz látogatóit milyen mértékben befolyásolja a hőmérséklet és a csapadék. Másik célunk az volt, hogy felmérjük, hogy az egyidejűleg nyíló kora tavaszi virágok közül a Diptera fajok melyikeket részesítik előnyben. A megfigyeléseket 2020. április 3-13. között végeztük a Veszprém melletti Csatár-hegy lejtősztyeppjében. Az összes Diptera egyedet begyűjtöttük, melyek a terület virágaira szálltak. Az *Adonis vernalis* virágok esetében kapott eredményeket előző évi gyűjtéseinkkel hasonlítottuk össze, melyhez az Országos Meteorológiai Szolgálattól kapott időjárási adatokat használtuk fel. Az *A. vernalis* Diptera látogatóinak száma a második évben jelentősen csökkent. Kutatásunkkal rámutattunk arra, hogy a Diptera fajok korai tavaszi virághoz látogatási tevékenységét nagyobb mértékben befolyásolhatja a csapadék, mint a hőmérséklet. A területen egyidejűleg 6 faj virágzott, de legyeket csak az *Adonis vernalis* és a *Lamium purpureum* virágokon gyűjtöttünk. Az összesen begyűjtött legyek száma 19 volt, melyek 5 fajhoz tartoztak. A *Lamium purpureum* kizárólag Bombyliidae látogatókkal rendelkezett. Mindkét növényfaj esetében a hím virághoz látogatók voltak többségben. Bár a gyűjtött légyfajok egyike sem virágspecifikus, mégis különbségeket találtunk a fajok virágválasztásában. Habár a hazánkban előforduló légy fajok elsődlegesen nem pollinátorokként vannak számon tartva, és a megporzó rovarokat vizsgáló kutatások is főleg

méhekre irányulnak, a legyek megporzásban betöltött szerepét fontos lenne tovább kutatni és feltárni.

Kulcsszavak: Bombyliidae, *Lamium* purpureum, légy, viráglátogató

Introduction

Conditions of dry grassland species are changing continuously nowadays, thus some species become threatened. Agricultural expansion, regression of grazing or even overgrazing, afforestation, natural succession are all threatening factors (Forycka et al., 2004, Łuszczyński and Łuszczyńska, 2009). *Adonis vernalis* L. still has a large distribution area, but the abovementioned factors threaten the survival of some populations. Due to its decreasing populations *Adonis vernalis* L. became a potentially endangered species and is listed in red data books of the relevant countries (Cites, 2000). The IUCN Red List evaluates it as a Vulnerable species (Schnittler-Günther, 1999). It is an early-flowering species important for insects too, providing food especially for Hymenoptera species. In beekeeping literature it is mentioned as an important pollen producing plant (Denisow and Wrzesień, 2006). Our former studies confirmed that *A. vernalis* is mainly pollinated by Aculeata species (Mészáros and Józan, 2018; 2020). The flowers of *A. vernalis* are nectarless, and provide only pollen as a reward for flower visitor insects (Denisow et al., 2014). The results of Chittka et al. (1999) show that nectarless species have fewer visitors than those producing nectars, even if they flower at the same time. The species which can not offer high caloric reward ensure pollen transfer with alternative strategies. The dichogamous flowers of *A. vernalis* are partly protogynous. Dichogamy depends on the activity of pollinators as well (Denisow et al., 2014). Stigma receptivity starts about one day before the anthers of the same flower start to shed pollen. Pollen viability is increasing during the life-span of the plant (Lloyd and Webb, 1986).

According to our observations, flowers are visited by Diptera individuals as well. The role of Diptera species in pollination is underrated, but while they are moving in the flower pollen can stick on their body so they can carry the pollen to the stigma. Our former studies showed that *Adonis vernalis* flowers are mainly visited by Syrphidae taxa, but Empididae, Anthomyidae, Tachinidae, Culicidae and Tephritidae species were collected too (Mészáros and Tóth, 2020). One aim of our study was to examine the effects of temperature and precipitation on Diptera visitors of *Adonis vernalis*, based on two-year results. Another aim was to describe flower preference of Diptera species.

Materials and methods

Adonis vernalis visitors were studied between 3–13 April 2020 (22 hours long period in 6 days) in the slope steppe of the Csatár Hill near Veszprém city. The area of the study site is 1200 m^2. About 130 flowering *Adonis vernalis* individuals can be found in the grassland. According to our former study the grassland can be classified as a Chrysopogono-Caricetum humilis Zólyomi (1950) 1958 association. Besides *A. vernalis* two more protected species was found: *Erysimum odoratum* Ehrh. and *Stipa pennata* L. (Mészáros et al., 2018). The co-flowering species during the study period: *Adonis vernalis* L., *Euphorbia seguieriana* Neck., *Lamium purpureum* L., *Muscari neglectum* Guss. ex Ten. s. l., *Potentilla arenaria* Borkh. and *Taraxacum officinale* agg.

Diptera individuals found on every flowering plant species were collected. The site was scanned continuously. Insects were collected with a butterfly net 30 cm in diameter, but the original net had been replaced by a dense and transparent tulle net. The species have been identified by Sándor Tóth according to Mihályi (1975) and Tóth (1977; 2017).

Data of *Adonis vernalis* visitors was compared to our results from the previous year (Mészáros and Tóth, 2020). Meteorological data was shared by the Hungarian Meteorological Service.

Results and discussion

Although our aim was to study both Diptera suborders (Nematocera and Brachycera), in 2020 only Brachycera individuals were collected. Six plant species were flowering on the study area, but flies were collected only on *Adonis vernalis* and *Lamium purpureum* (Table 1).

Table 1. Flower visiting fly species collected on the Csatár Hill (2020)

Species	*Adonis vernalis*			*Lamium purpureum*		
	Male	Female	Total	Male	Female	Total
Bombylius discolor Mikan, 1796				4	4	8
Bombylius major Linnaeus, 1758				1	5	6
Bombylius pictus Panzer, 1794					2	2
Musca autumnalis De Geer, 1776		1	1			
Sphaerophoria taeniata (Meigen, 1822)	2		2			
Total:	**2**	**1**	**3**	**5**	**11**	**16**

The total number of collected flies was 19, representing 5 species. Two species were collected on *Adonis vernalis* (3 individuals of 2 families) and 3 on *Lamium purpureum* (16 individuals of 1 family) (Table 2). The number of male flower visitors was higher on both plant species. The two species had no common Diptera visitors (Table 1).

Table 2: Flower visiting fly families collected on the Csatár Hill (2020)

Families	*Adonis vernalis*			*Lamium purpureum*		
	Male	Female	Total	Male	Female	Total
Bombyliidae				5	11	16
Muscidae		1	1			0
Syrphidae	2		2			0
Total:	**2**	**1**	**3**	**5**	**11**	**16**

24 individuals of 18 species were collected in 2019, and only 3 individuals of 2 species were recorded in 2020, so the number of Diptera visitors of *A. vernalis* decreased significantly in 2020, despite the fact that the length of the study period was nearly the same in both years (20 and 22 hours). In 2020 *A. vernalis* was flowering earlier than in 2019 (between 20 April- 3 May). In 2020 we had to study the Diptera species earlier because our aim was to investigate the visitation activity of insects in the same phenological stage of *A. vernalis*. Probably, insects could not adapt so fast to warmer weather. The mean daily temperature varied between 0.5 and 17.3 °C, the average of mean daily temperature was 10.6 °C. The values fluctuated widely, the temperature was not balanced (Figure 1).

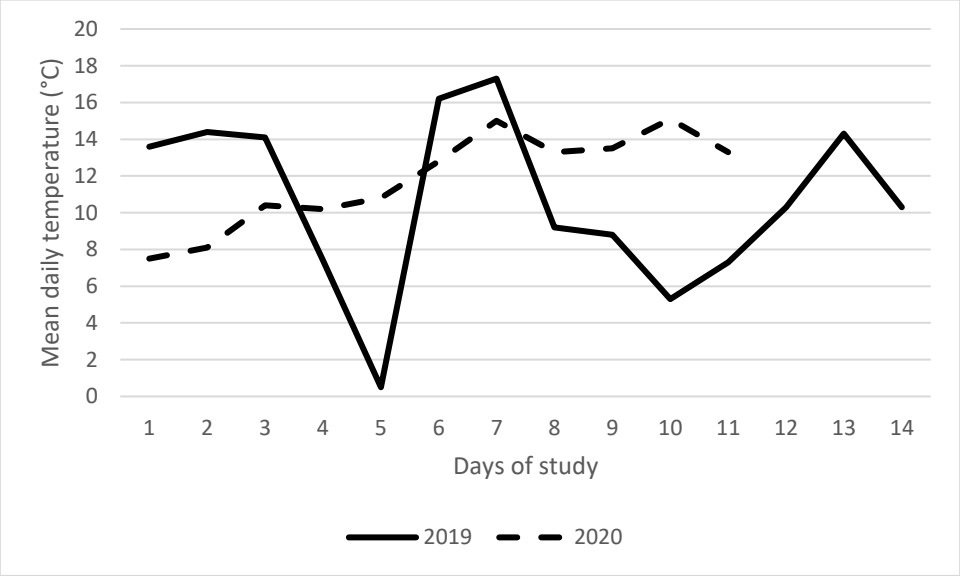

Figure 1: Mean daily temperature values (Kab Hill) according to the data of the Hungarian Meteorological Service

In the second year mean daily temperature altered between 7.5 and 15.5 °C, the average of mean daily temperature was 11.8 °C. Although temperature values were more balanced, the number of Diptera individuals still decreased significantly. According to precipitation data of the Hungarian Meteorological Service it rained seven times during the study period (8.1 mm in total) in 2019. In 2020 it rained only once (2.7 mm), on the last day of the study period, thus it

had no effect on our observations (Figure 2). The lack of precipitation obviously could cause the drastic decrease of Diptera species (Figure 2).

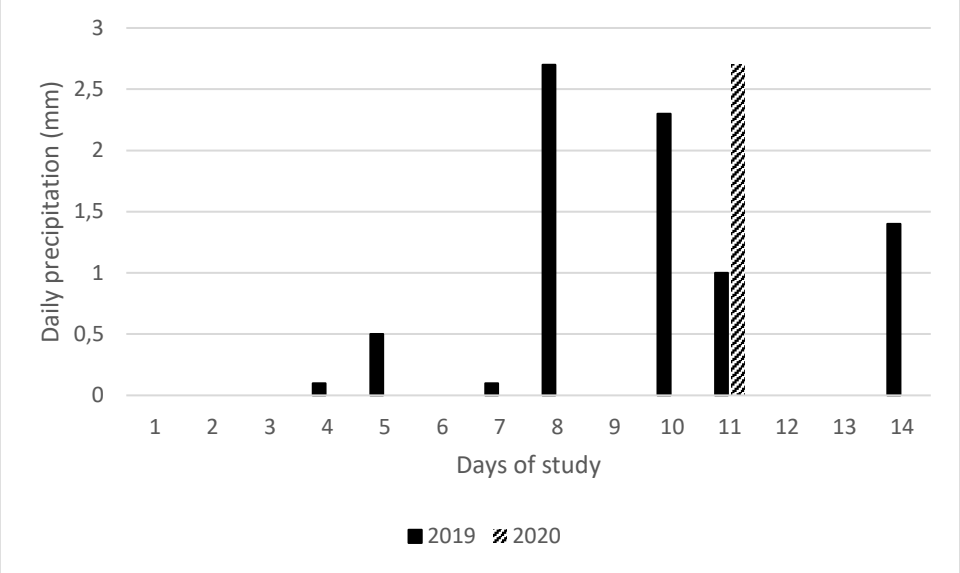

Figure 2: Daily precipitation values according to the data of the Hungarian Meteorological Service

Syrphidae species were dominant in both years. Adults mainly feed on nectar, honeydew and pollen, they have a role in plant pollination. In contrast to 2019 one Muscidae individual was found in 2020. Some common and frequent Diptera taxa are members of this family. Their body is strongly bristled. They have various feeding behaviour, they feed on manure, nectar, sugar containing materials, fruits, but adults can be predator as well. Some species are hematophagous, others lick nasal discharge and sweat (Mihályi, 1975). *Musca autumnalis* is native to Europe and Western Asia, it feeds on the eyes and lip of horses and cattle. The larvae develop in horse and bison manure (Krafsur and Moon, 1997). Its occurrence on the Csatár Hill can be explained by the presence of riding halls near the study area. As this species does not feed on pollen, its occurrence on *Adonis vernalis* proves the fact that flowers not only function as pollen and nectar resources for insects but provide place for resting and warming as well.

Our study on the flower preference of Diptera taxa in 2020 showed, that insects visited only 2 from the 6 flowering species (*Adonis vernalis* and *Lamium purpureum*). *Lamium purpureum*

was visited only by Bombyliidae species. The members of this family feed on nectar and pollen as well. Some species hover in the air over the flowers and use their long, straight proboscis to reach the nectar. Other species land on flowers to feed (Panov, 2007; Tóth, 1975; 1977). In early spring we can observe *Bombylius major* individuals sucking nectar mainly from *Pulmonaria* and other Lamiaceae species (Tóth, 2014).

Our results show that compared to temperature precipitation has stronger effect on the flower visiting activity of Diptera taxa. Although none of the collected fly species was flower specific, we still found differences in their flower preference. Fly species are not primarily mentioned as pollinators and pollination studies mainly focus on bees, it would be important to carry out further studies on the role of Diptera species in pollination.

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SOME CLIMATIC ASPECTS OF APPLE GROWING

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Abstract

Malus domestica Borkh is the most widely grown fruit in the temperate climate (worldwide only bananas and citrus fruits precede it). The regional impact of global warming has already been manifested in extreme weather events today. Signs of climate change are also reflected in the intensity and frequency of temperature and precipitation extremes. The number of heat waves and hot days will increase throughout Europe, including Hungary, which will be accompanied by the rarer extremes of cold and frosty days, which - supported by previous researches - may result in changes in apple production. The behavior of plants is influenced by various ecological processes, of which weather factors and climatic conditions are of great importance. Individual weather conditions, especially temperature, affect all aspects of apple growing.

Keywords: *Malus domestica*, global climate change

Összefoglalás

Az alma (*Malus domestica* Borkh) a mérsékelt égövben a legnagyobb mennyiségben termesztett gyümölcs (világviszonylatban csupán a banán és a citrusfélék előzik meg). A globális felmelegedés regionális hatása már napjainkban is megnyilvánult a szélsőséges időjárási eseményekben. A változó éghajlat jelei a hőmérsékleti- és csapadék szélsőségek intenzitásában és gyakoriságában is megmutatkoznak. Európa-szerte, köztük hazánkban is a hőhullámok és forró napok száma nőni fog, ami a hideg és fagyos napok szélsőségének ritkábbá válásával párosul, mely -eddigi kutatásokkal alátámasztva- változásokat eredményezhet az almatermesztésben. A növények viselkedését a különböző ökológiai folyamatok együttesen befolyásolják, mely hatások közül nagy jelentőségűek az időjárási tényezők és az éghajlati adottságok. Az egyes időjárási viszonyok, különösen a hőmérséklet, hatással van az almatermesztés valamennyi aspektusára.

Kulcsszavak: alma, globális klímaváltozás

Global climate change

One of the main topics of current climatological researches are the recognition of global climate change. Global trends in climate change indicate that global temperature has risen by about 0.4–0.8 °C on average in the 20th century (Panel on Reconciling Temperature Observations, 2000). As the environmental effects of this phenomenon are likely to be most pronounced in the proliferation of extreme weather events (primarily temperatures) rather than in changes in temperature mean, understanding the processes behind extremes may be important for both observation and analysis of their effects (Jones et al., 1999; Katz and Brown, 1992). The extent and frequency of global temperature extremes on a global scale - including the probability of heat waves - has been rising since the middle of the 20th century. According to forecasts, hot

extremes will become more frequent in most terrestrial areas, cold extremes may occur less frequently, and global average surface temperatures are also expected to rise (IPCC, 2014).

Changes in the concentrations of greenhouse gases and aerosols, as well as the transformation of the surface by humans and the development of incoming solar radiation, are changing the energy balance of the climate system. The increase in the greenhouse effect is arguably real and helps regulate the Earth's temperature. Without the natural greenhouse effect, the average temperature of Earth would be around -18 °C instead of the current 15 °C. In contrast, increased greenhouse gas emissions will cause further warming and long-term changes in all components of the climate system, increasing severe and irreversible environmental impacts (IPCC, 2014).

According to forecasts, changes in precipitation will not be uniform at regional as well as local levels, as while annual average precipitation is likely to increase at high and medium latitudes and in the Pacific equator, in many medium-latitude and subtropical arid areas, precipitation is expected to decrease. According to IPCC (2014), special weather events associated with precipitation will become more intense and frequent for the most part of the mid-latitude and rainy tropics.

The effects of climate change are most comprehensively experienced in natural systems. The increasing rate, magnitude of global warming and other changes in the climate system increase the risk of severe, comprehensive and sometimes irreversible adverse effects. Some risks affect only certain regions, while others should be taken into account in most parts of the world. Extreme warm temperatures affect phenology in many fruit species. The phenological phases are mainly determined by the genetic conditions, while the environmental factors influence the speed and rate of the processes. Numerous researches have been published to map the effects of weather events on the phenology of fruit trees in many parts of the world, including Europe (Legave, 2013), North America (Nemani et al., 2001), Asia (Sugiura et al., 2012) and the

southern hemisphere (Grab and Craparo, 2011). As described by these authors, each of the phenological changes was consistent with an increase in the number of days with extreme warm temperatures (Miraglia et al., 2009).

Fruit-bearing plants have a defined need for their environment at each stage of development (Soltész, 1997). Assessing the effects of climate change on fruit production allows researchers to predict changes in crop yields (Olesen et al., 2011), as climate change is expected to have a significant impact on global agricultural production (Slingo, 2009). Apple is a fruit species that is particularly exposed to the effects of climate change. Exposure to strong radiation or extremely high temperatures during the growing season can lead to a reduction in fruit production and a negative effect on certain content characteristics.

Malus domestica

Malus domestica Borkh is an important and popular temperate fruit, one of the oldest cultivated fruits (Morgan and Richards, 1993), native to many parts of Europe and Asia (Sandor, 2008). Apple prefers cooler climates (Lenti, 2011) and has a high ability to adaptation. It can generally be grown between latitudes of 25° and 52° (Ferree and Warrington, 2003), but under ideal environmental conditions it can be grown in other areas as well. High yield and good quality are expected at balanced temperature, free from extreme weather events.

Conditions of radiation

In case of apple, stressful conditions - such as strong solar radiation, high temperature and low relative humidity - subserve physiological disorders, such as sunburn (Schrader et al., 2001, 2003). Conditions of radiation also affect photosynthesis and fruit color. In terms of photosynthesis, the shading effect is significant. Leaves inside the canopy that receive only 50%

of the radiation reach only 25–30% of the assimilation compared to leaves exposed to direct sunlight (Avery, 1975).

The temperature of the fruit skin is generally higher than the air temperature, because fruits have very limited cooling capacity through transpiration. Sun damage, or sunburn symptoms on fruits range from white spots to dark brown, depending on variety and environmental conditions (Hernandez et al., 2014). Schrader et al. (2001) identified two main types of sunburn injuries. In the first case, sunburn necrosis results from heat death of the shell cells, as indicated by the breakdown of cell membranes. If the shell temperature reaches 52 °C in 10 minutes, necrosis develops. This temperature does not affect the integrity of cell membranes. Sunburn occurs when the surface of the fruit reaches 46-49 ºC, but the intensity of solar radiation also plays a decisive role in its formation. During sunburn tanning, the membranes of surface cells are less damaged. Furthermore, it has been found that UV-B radiation plays a higher role in the development of sunburn symptoms, than visible light. However, the side of apples exposed to sunlight has a high sun protection ability (Ma and Cheng, 2003). Natural defense mechanisms provide some protection against sun damage, such as epicular layer thickness and wax composition (Wünsche et al., 2004), accumulation of antioxidant compounds and sunscreen pigments (Felicetti and Schrader, 2009), all of them can affect sunburn. However, fruits are more sensitive to pathogen attack in the affected area (Racskó et al., 2005). Brooks and Fisher (1926) reported that sunburn injuries can occur if surface temperature of the fruit is 14 ºC higher than air temperature. In their view, damage to the surface can be caused by high temperature rather than sunlight.

Temperature, precipitation

Temperature has a high importance in apple growing in several ways. It determines, for example, the length of the growing season, the time and duration of phenological processes, and the occurrence of pests and diseases (Tóth, 2013). The preferred temperature range for apple is 15-33 °C. Moving away from the optimum (in the direction of both extreme hot and extreme cold temperatures), the speed of life processes decreases. When air temperature exceeds 35 °C, the degradation of organic matter from respiration exceeds the amount of organic matter formed during photosynthesis (Lakatos, 2004). The temperature of the orchard can be significantly higher than the temperature of the air. Thorpe (1974) studied an apple orchard on a cloudless day. The air temperature was 27 °C, the surface temperature of the apple exposed to sunlight was 13–14 °C, and the unexposed surfaces were 3 °C warmer than the air. As the number of days with extreme warm temperatures increases, the quality of apples will be damaged.

The growing season of apples lasts from bud burst to the end of foliage. The swelling of the buds starts at a mean daily temperature of 6 °C, if this temperature permanently exceeds it, the vegetative activity is undisturbed and the growth is continuous. The period from bud burst to flowering is closely related to temperature, this phase occurs between 6-18 °C. Above 9-10 °C the plant responds for a 1 °C temperature rise with a phase shift of 4-5 days, while it is only 1-2 days at 14-15 °C. The period between the beginning of flowering and petal death is 12-25 days, of which the flowering phase has a strong temperature dependence. The optimum temperature is between 9-24 °C. At around 10 °C, a 1 °C temperature change causes a 3-4 day phase change, while at 20 °C it is 1 day. The temperature varies between 16-24 °C in the period between the end of petal death and the time of ripening, and it is closely related to the length of the phase. At 17-18 °C, a temperature change of 1 °C results in a phase change of 10-11 days.

A temperature change of 1 °C at or above 20 °C causes a change in phase duration of 5-6 days (Lakatos, 2004).

Analyzes in the Netherlands also show that the rise in temperature and the acceleration of apple development are connected (Poldervaart, 2004), with a 1-2 days earlier trend in phenophases every decade.

Temperature greatly affects anthocyanin synthesis. In apple, high temperature prevents the accumulation of cyanide and UDP sugars (Ban et al., 2009), resulting in a rapid decrease in anthocyanins followed by renewable synthesis at cooler temperature and causing skin color fluctuations (Steyn et al., 2005). Lin-Wang et al. (2011) found that extremely high temperature decreases anthocyanin concentration in the apple skin.

In previous studies, Tukey (1959) found that temperature treatment of branches affected fruit growth. The controlled environment, for example, was set by Ford (1979) by exposing the trees to two opposite temperature systems for three weeks after flowering and showed that temperature strongly influenced the average fruit size. Later, in another study, Tromp (1997) showed that post-flowering temperature treatments significantly affected fruit ripening but had only a small effect on fruit weight at harvest. Bergh (1990) found that in addition to the defined relationship between temperature and fruit size, the timing of exposure to temperature is also significant.

Thus, apple is a deciduous species, it also needs a cold (dormant) period. The length of the frost-free period is of high importance for horticultural production (Anda and Kocsis, 2010). Research in recent decades has shown that the biggest problem was frost during flowering among the extreme weather effects, with frost damage causing much greater loss than all other environmental stressors combined (Tóth, 1982; Flore and Howell, 1987). Apple is sensitive to spring and autumn frosts. The critical value is -4 °C for apple buds, -2 °C for flowers and -1 °C

for fruits. Autumn frosts can cause early foliage, fruit freezing and significant crop loss the following year (Caprio and Quamme, 1999; Lenti, 2011).

The lowest tolerated temperature for the fruit depends on the phenological condition. The extent of damage can be significantly influenced by the degree of cooling, the temperature of the winter period before spring frost damage and the development of the flowers (Zatykó, 1986). Caprio and Quamme (1999) found that low yield averages are largely due to an increase in the frequency of temperature extremes.

Apple is a water-demanding fruit species, with a water demand of 600–800 mm. The half of it is required during the growing season (Tóth, 1997). Its water demand is the highest in summer, which can mostly be provided by irrigation.

Hungary

Hungary is located in the temperate zone. There is only a 3° latitude difference within the country, which cannot cause profound climatic differences between the southern and northern parts of the country, however, the orographic factors are not completely ineffective. Hungary is ranged to cool continental climate, which is suitable for the cultivation of apple, although extreme temperature changes are more common, and weather has a great variability (Péczely, 1998).

In terms of precipitation conditions, the change is significant from year to year, it ensures a water needs at medium level. Although most of the falling precipitation is concentrated in the summer period, in the case of apple growing irrigation may be necessary. There are significant differences between the annual rainfall of some of our landscapes. The west-southwest part of the country is one of the wettest areas, here precipitation is about twice higher (800-900 mm), than in the driest (lowland) districts (480-500 mm). Most rain falls in Hungary between May

and July, which is suitable for apple production, as it requires the most precipitation during the growing season, but sudden, large amounts of precipitation can cause problems (on average 25-40 thunderstorm days occur in Hungary per year) (Péczely, 1998).

In the future, it is likely that the climate of the Carpathian Basin will become more droughty and weather extremes may become more frequent. According to experts studying the changes, as the climate becomes hotter and drier, there will be more and more negative effects in fruit production, both in terms of quantity and quality.

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THE EFFECT OF CLIMATE CHANGE ON DAIRY CATTLE

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Abstract

Cattle farming provides important, basic foodstuffs worldwide, and many body parts that are unfit for human consumption can be utilized through further processing. The impact of global warming has already become apparent today with a more common frequency of extreme weather events. As the number of hot days and heat waves is expected to increase, changes may also occur in animal husbandry. Individual weather parameters, especially temperature, affect all aspects of breeding. Due to the increasing warm periods in recent years, the decline in production is becoming more and more significant, as our farm animals react very strongly to persistently high temperature. In the case of intensively dairy cows, the daily milk production and the reproductive biological indicators decrease due to heat stress.

Keywords: cattle, global climate change

Összefoglalás

A szarvasmarha-tenyésztés világszerte fontos, alapvető élelmiszereket szolgáltat, továbbá - a táplálkozásra nem alkalmas - számos testrészét további feldolgozás útján hasznosítani lehet. A globális felmelegedés hatása a szélsőséges időjárási események gyakoriságának növekedésével már napjainkban is megnyilvánult. Mivel várhatóan a forró napok és a hőhullámok száma nőni fog, változások következhetnek be az állattenyésztésben is. Az egyes időjárási paraméterek, különösen a hőmérséklet, hatással van az állattenyésztés valamennyi aspektusára. Az utóbbi években fokozódó meleg időszakok miatt a termelés-visszaesés egyre jelentősebb, ugyanis gazdasági haszonállataink igen erősen reagálnak a tartósan magas hőmérsékletre. Az intenzíven tejelő tehenek esetében a hőstressz következtében csökken a napi tejtermelés, továbbá romlanak a szaporodásbiológiai mutatók is.

Kulcsszavak: szarvasmarha, globális klímaváltozás

Climate change

Climate is a system that involves the interaction of physical properties and processes in the atmosphere with the environment of a given place (a definable finite element of the atmosphere) and with each other over a long period of time. By extending the concept of climate, it can also be related to weather: the climate of a given place is a system that determines weather changes, as well as the equilibrium state where weather fluctuations take place (Péczely, 1998). It is important to note that the climate change of a given place is not independent of the processes taking place in other areas, so the global climate is not just the sum of local climates (Bártfai et al., 1997). Climate is a combination of elements that includes temperature, humidity, precipitation, radiation, air movement and atmospheric pressure (Johnson, 1987). Climatologists pay close attention to the study of climate change. Evidence for the observed

effects of climate change is the strongest and most comprehensive in natural systems. There are two forms of climate variability: climate fluctuation and climate change. Climate fluctuation occurs when the higher and lower values follow each other, but the fluctuation remains within the extreme values. Climate change occurs when the interval of fluctuations in climatic elements shifts to either higher or lower values, and this condition persists for a longer period of time. This interval is determined by the current lowest and highest values of the given meteorological element, these are the so-called extreme values (Varga-Haszonits et al., 2004).

A sudden rise in global surface temperature was detected in the recent decades, associated with significant changes in the frequency of extremely hot and cold events (Alexander et al., 2006). According to IPCC report (2007), by the end of the 20th century, temperature will rise with 1.1-6.4 °C. Since 1950, changes have been observed in several extreme weather and climatic events, including declining cold temperature extremes, increasing hot temperature extremes, increasing frequency and long-term occurrence of heat waves, rising sea levels and increasing heavy rainfalls in several regions (IPCC, 2014).

Zoometeorology examines the relationships between breeding animals and weather factors. The body of farm animals is strongly connected to the meteorological factors (solar radiation, air movement, humidity, temperature) that affect their production. Global climate change is dramatically increasing the number of extreme weather events, making productive varieties extremely sensitive to external environmental factors.

The effect of sunlight duration

The life processes and production of animals are influenced by the length of illumination. The changing brightness of the Sun is one of the direct manifestations of climate change. Some evidence supports the fact that the brightness of the Sun has increased throughout history

(energy production, rate of fusion, conversion of hydrogen to helium), yet there is little information on changes in the brightness of the Sun on a climatic time scale (Varga-Haszonits et al., 2004). In a study of limousine cattle, Leinonen et al. (2001) found that light perceived by animal's eyes leads to gonadotropic hormone production in the adenohypophysis, which results in the onset of sexual symptoms. Furthermore, pituitary hormone production is also enhanced by light, which controls the duration of pregnancy, biological rhythm, and 24-hour vegetative function through thyroid and adrenal function. Only 10-25% of the radiation is reflected from the animals with a dark outer cover, the rest is absorbed, while in case of light-haired animals 70-80% is reflected (Anda et al., 2010).

Regarding the sensitivity of grazed animals, sunburn and heat stroke are very important, as sunlight is one of the most important heat stress factors (increases the temperature of the environment and body), and shading is one of the major tasks. Calves - young and growing animals - are strongly exposed to this effect. As a result of sunlight, the metabolism and feed uptake increase, the fertilization rate is better, and it also stimulates the mood.

Effect of airflow

Animals are affected by two types of airflow: wind and draft. In the case of wind, gust is dangerous, as the speed of the wind can increase suddenly, thereby mechanical load exponentially rises. Draft is usually common in barns, where it is unfavorable due to its permanence. It is possible to defend passively with windshield boards and forest strips. Actively, animals can run away or group together, which allows to concentrate wind effects on a small surface.

Around the body surface of the cattle - supplementing the beneficial effect of the outer fur - a ~10 cm thick, buffered layer of air is formed, providing some protection. The higher the wind

speed is, the greater the heat dissipates, cold can caught and the buffer air layer can be removed. In addition to the negative effects, however, the wind also has a refreshing, air-exchanging, warm-air-carrying, balancing effect (Anda et al., 2010). Strong flow of air draws heat away from the body, which is especially dangerous for newborn and young animals, as their energy reserve quickly depletes, they die.

Effect of precipitation and humidity

Local precipitation has a mainly negative effect on grazed animals, increases the feeling of cold (but the cooling effect is positive in summer), and can cause deworming, where intermediate hosts quickly appear (Thompson, 1973). During rainy periods, diseases spread, epidemics break out and external and internal parasites multiply. Humidity of 75-85%, coupled with high temperature values, increases respiration and heart rate, which provides growth of viruses, fungi and bacteria. If precipitation and relative humidity occur together, they can strengthen each other's effects. Precipitation can be prevented if the animals hide in natural or artificial shelters, while the modification of humidity is only partially possible, e.g. by reducing water phases in the barn (Anda et al., 2010).

The effect of air temperature

All weather effects are present in mammals via the autonomic nervous system and the hormonal system. The body responds to the changing temperature of the environment with thermoregulation. If a rapid adaptation to weather is required, the autonomic nervous system plays the main role. When it becomes necessary to compensate weather stimulus, hormonal regulation prevails (Makulska et al., 2003). The body of newborn animals shows an increased sensitivity to temperature (Rosenberg et al., 1983). In cold weather, blood circulation speeds

up, blood pressure rises and thus produces more heat, the body is protecting against cooling. In this case, food intake also increases. Cold stress has a negative effect on the body, but animals can tolerate it longer than too high a temperature. In warm weather, the body defends itself by evaporation (in the form of sweating), fluid intake increases while food intake decreases. In serious cases, anorexia can occur (Kovács, 1990). For animals, high temperature soon becomes unbearable because they are less resistant (Dinu et al., 2004). Cattles are particularly sensitive to higher temperatures. Milk loss can be 50% above 30 °C, and at 22 °C feed intake of animals is already reduced. The secretory function of the thyroid gland is reduced, which also slows down the metabolism. The rectification temperature of cows exceeds 39 °C after spending a few hours at 26-30 °C. Due to heat stress, fertilization does not occur due to the reduced biological activity of germ cells. In the case of bulls, the temperature of the testicles also rises as a result of persistent warm temperature, thus producing less and worse sperm (Bártfai et al., 1997).

Effects of heat stress on cattles

The body temperature of a cattle is within a narrow range, like our farm animals. It is a homeotherm species with a constant body temperature, so heat production and heat release are normally in balance. This balance is provided by different thermoregulatory mechanisms (Bárdos et al., 2007). Within this narrow range, the animal does not need to put extra energy into cooling or heating the body (this means a range of 0 - 20 ° C for dairy cattles). Their body temperature is relatively independent of the environmental temperature, but this requires a certain amount of energy to keep body temperature. This is necessary for the proper function of physiological processes.

Subsistence is always more important than production. The energy absorbed by the feed is primarily used for subsistence and in the production energy availability is only additionally utilized. From an economic point of view, therefore, the amount or energy- forced to be used for subsistence – is important. This energy is influenced by a number of factors such as race, body weight, age, sex and production. The most important influencing factor is the temperature of the environment. The temperature range in which the animal uses the least energy to maintain body temperature is called the comfort zone, where the well-being of the animals is also the best (Dankó et al., 2017).

The internal temperature of the body is responsible for the activation of regulatory mechanisms. The thermoregulatory centre is located in the hypothalamus, its frontal core group is called the cooling-, while its hind core the heating centre. The response depends on the temperature of the blood in the hypothalamus. If the blood temperature is higher than normal, the parasympathetic tone increases, if it is lower, the sympathetic tone plays a greater role. At higher temperature, sweating, wheezing, dilation of the skin vessels and increased salivation occur. At lower temperature, the skin vessels constrict, hair ruffles, tremors occur and hormones of thyroid and adrenal flow into the circulation, glycogen and fat stores are mobilized, which actually increases cellular oxidation, resulting heat production (Bárdos et al., 2007).

Bak and Pazsiczki (2008) found that the most preferred temperature range for homeothermic animals is the thermoneutral zone (maximum production with the most efficient energy use). Within the thermoneutral zone, 3 more zones can be separated: cool production zone (-15-0 °C), optimal production zone (0-20 °C) and warm production zone (20-26 °C). In the warm production zone, milk production and feed consumption are already slightly reduced, but this is still an acceptable temperature range for the cow. The thermoneutral zone is surrounded by lower and upper critical temperature values, which are 26 °C and 5 °C for Holstein-Friesian.

It is important to note that these limits may differ (e.g., depending on age, variety, quantity and quality of feed) (Dash et al., 2016).

If the animals are not able to keep their internal body temperature (the two limits are exceeded), they respond by decreasing or increasing the intensity of metabolism, the respiratory rate, the rectal temperature and the number of heartbeats increase. In this case, the balance between heat production and heat release is upset, homeothermia ends and heat stress develops. Decreased growth rate, milk yield and reproductive performance can be observed (Das et al., 2016). The comfort zone of dairy cows is between –15 and + 26 °C (Ribács, 2012). Heat stress occurs when the temperature of the environment exceeds the value that the body can compensate (Kovács and Kovács, 2012). In this case, such environmental conditions are created (due to environmental temperature, relative humidity, solar radiation and air movement) that do not cover the cow's thermoneutral zone.

Dairy cows are very sensitive to heat stress due to their intensive metabolic processes, limited renal and gastrointestinal water retention (Bernabucci et al., 2010). At the level of varieties and individuals, high variability can be observed in heat tolerance and responses to environmental changes, but Kovács and Kovács (2012) found that husbandry technology significantly influences sensitivity to heat stress. Improper feeding practices and deficiencies in husbandry technology can increase the harmful effects of heat stress (Gergácz, 2009). Heat management is playing an increasingly important role in ensuring animals, which is also supported by increasingly strict requirements (nationally and internationally). These laws and directives have a number of climatic implications (Pazsiczki, 2005). The most important solution is to prevent the development of heat stress by reducing or preventing the decline in dry matter uptake, and thus to prevent the deterioration of production indicators. It should also be noted that prevention is intended to reduce negative effects, not to reduce heat stress.

Heat stress is also a serious problem for dairy farms in Hungary (especially in the Great Hungarian Plain, which sometimes has an extremely continental climate). Hungary's climate is expected to warm further due to global climate change. In particular, summer temperatures reflect this warming process. Heat stress reduces the live weight of calves and also has a negative effect on the following lactation performance. The fertilization index deteriorates, the number of oestrus decreases and embryonic death becomes more frequent (Rensis and Scaramuzzi, 2003). In case of persistent heat, rumen acidosis and the associated lameness become more common (Bernabucci et al., 2010). As an effect of temperature change, the heat dissipation is modified. If the environment is much cooler than the cow's body temperature (ie. the air temperature is between 18 and 21 °C), the animal can dissipate 60-70% of its own heat by so-called dry heat dissipation. With wet heat release, the larger part of the excess heat is forced to be released by the animal as air temperature rises. Temperature is not the only environmental factor that influences the intensity of heat stress. It should be taken into account that the heat stress causing factor is also humidity dependent, but in Hungary the limit is usually above 26 °C (Orosz and Latos, 2006), when the rate of wet heat release reaches 80–90%. At this temperature, the humidity in the barn starts to rise, which inhibits wet heat release, causing a thermal stress to the cow. Based on the relationship between temperature and relative humidity, a so-called HPI index (temperature / humidity) can be established, which shows the cow's sense of comfort (Takács, 2013). This index gives the intensity of heat load, which measures the combined effect of environmental temperature and relative humidity (Allen et al., 2013). There are many limit values in the literature, and using the same formula, these present a deviation in the dangerous degree of heat stress. Heat increases pneumonia, especially when coupled with high relative humidity (Morse et al., 1989).

Heat stress can upset the hormonal balance in the body of animals, which affects the levels of sex hormones. Disorders of animal reproduction are the first to appear among the physiological changes induced by heat stress, reducing the length and intensity of oestrus. A certain degree of reproduction seasonality can be shown even in the case of intensively producing varieties, despite the fact that most of our farm animals have lost the seasonal nature of their reproduction. Normal spermiogenesis requires less heat than body temperature. Recent researches have shown that follicular maturation is a temperature-sensitive process. Summer heat stress affects follicular maturation, its dynamics, the development of the corpus luteum and the quality of ovum and embryo. If less mature dominant follicles develop during the sexual cycle, sex steroid production by theca and granulosa cells is disrupted. Estrogen level in the blood will be lower and, due to the nature of the heat stress (acute or chronic), plasma progesterone level will increase or decrease. As a result of hormonal changes, the uterine environment also changes, which also reduces the quality of the embryo. Not only does summer heat stress (July-September) show a lower conception rate on fertility, but it also affects in autumn (October-November). This is due to the prolonged effect that summer heat affects follicles that become dominant follicles in 49–50 days (De Rensis and Scaramuzzi, 2003; Wolfenson et al., 2000).

The central nervous system senses the presence of the stressor, which starts the general adaptation syndrome. Adaptation syndrome has three stages: the alarm response, the resistance phase and the exhaustion phase. Adrenaline - a hormone in the adrenal medulla - plays a key role in this process. It is mainly responsible for the first phase, i.e. the emergency response, while cortisol, which is the hormone of the adrenal cortex, is responsible for the second and third phases. In this case, the hypothalamic – pituitary – adrenal cortex system is activated, and cortisol secretion in the adrenal gland is increased (under the influence of CRF-ACTH

hormones). The effect of elevated ACTH hormones reduces estradiol-induced oestrus symptom (Hansen and Aréchiga, 1999).

At the beginning of lactation, all cows develop a negative energy balance, the metabolic stress, which increases the harmful effects of heat stress. Fat mobility is increased due to the lack of energy, thereby increasing the concentration of keto substances in the blood, which can have a significant negative effect on the reproductive biological performance. In a ketotic animal, follicles exposed to high concentrations of glucose, BHB and NEFA produce less estradiol. They are less sensitive to LH, and are less likely to ovulate and reach the dominant stage (Leroy et al., 2006). Due to summer heat stress, ketosis is 1.4 times more common in summer calving cows than in winter calving ones (Andreu, 2016). Furthermore, Andreu's (2016) studies show that ketosis in the first and second weeks after calving reduces the chance of pregnancy by 50%. He found that the time from calving to conception was nearly 40 days longer than in non-ketotic animals.

A decrease in the number of pregnant days may also be associated with the negative effects of heat stress. Temperature above the prolonged thermoneutral zone in the late stages of pregnancy affects maternal and fetal metabolism, and stress also modifies the functioning of the hormonal system. Calving begins earlier because fetal stress occurs sooner (Collier et al., 1982).

Conclusions

The effects and prevention of heat stress have a very high importance in animal husbandry. In summer, the primary goal is to maintain the homeostasis and production of the cow and to avoid fluctuations in reproductive biology. In dairy cattle farming, especially in intensive milk processing, preventing the harmful effects of heat stress is a daily task during the summer months.

Agriculture is one of the most exposed sectors to environmental impacts. While in crop production plant breeding quickly provides resistant varieties, in the case of farm animals it is a bigger challenge due to the longer generation interval, which is especially true for cattles. As a result, it is important to develop practical methods and technologies that can reduce production losses and maintain their economy even in extreme weather conditions.

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INVESTIGATION OF THE CAUSATIVE AGENT OF A VIRUS-LIKE SYMPTOM IN GRAPEVINE

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Abstract

Our group has been carrying on virus diagnostic surveys since 2013 using a special method: small RNA HTS. In 2018 strange symptoms, suggesting the presence of grapevine red blotch-associated virus (GRBaV), appeared at a Pinot Noir plantation. The virus was described in California in 2013 and its presence, except two descriptions (India and South-Korea) is confide to the North-American continent. To reveal the causative agent of the appeared symptoms we purified RNA from the symptomatic grapevine and made virus diagnostics by small RNA HTS. We confirmed the results using virus specific RT-PCR. Our results show that not the presence of the assumed virus cause the observed symptoms, moreover GRBaV is not present at the investigated vineyard. However, the plantation was infected with several other viruses. We

think that coexistence of several different viruses together with the inhomogeneity in the soil can both contribute in the symptom development, however to clarify this question further investigations would be needed.

Keywords: grapevine red blotch-associated virus, GLRaV-1, small RNA HTS, RT-PCR

Összefoglalás

Kutatócsoportunk 2013 óta végez vírusdiagnosztikai felméréseket szőlőültetvényekben egy új módszer, a kis RNS HTS használatával. 2018-ban egy Pinot noir ültetvényen furcsa, a szőlő vörös foltosodás vírus (GRBaV) jelenlétére utaló tüneteket figyeltek meg. A vírust 2013-ban Kaliforniában írták le, elterjedése az EPPO nyilvántartása szerint 2 kivételtől eltekintve (India és Dél-Korea) az észak-amerikai földrészre korlátozódik. A tünetek okainak felderítésére a jellemző tüneteket mutató szőlők RNS-éből kis RNS szekvenálással végeztünk vírus-diagnosztikát. A kapott eredményeket egy független módszerrel, RT-PCR-rel igazoltuk. Eredményeink azt mutatják, hogy a sajátos tünetek kialakulásában nem a feltételezett vírus játszott szerepet, a GRBaV nincs jelen a vizsgált ültetvényen. Az ültetvény viszont több vírussal is fertőzött volt. A tünetek kialakulásának az oka ez esetben a különböző vírusok együttes fertőzésében, az ültetvény talajának inhomogenitásában keresendő, de pontos megállapításához további vizsgálatok szükségesek.

Kulcsszavak: szőlő vörös foltosodás vírus, GLRaV-1, kis RNS HTS, RT-PCR

Introduction

Grapevine is considered as one of the major fruit crops in the world based on hectares cultivated and economic value. It can be infected with several (more than 80 is described until now) viruses which presence can affect not only its growth, but the quality of important

characteristics (berry weight and colour and sugar content, etc.). Grapevine red blotch-associated virus (GRBaV) was described in California, from a vineyard showing red blotch disease using HTS of ds RNAs (Rwahnih et al., 2013) and was proved to be the causative agent of the disease later (Yepes et al., 2018). It is a member of the Geminiviridae family, having a single circular DNA genome and its presence was proved to have inferior effect on berry development (Blanco-Ulate et al., 2017). The disease symptoms in red varieties include reddening of regions within leaf blades, along with red veins and petioles and delayed fruit maturity. In white varieties, leaves may develop yellow or chlorotic that is similar to leafroll-diseased vines. Asymptomatic vines can remain productive, but they also harbour viruses and act as potential reservoirs for virus spread to susceptible vines. It is graft transmittable, it could originated from a wooded riparian area by a supposed new vector (Cieniewicz et al., 2017). Grapevine virologists highlight that symptoms can be very similar to leafroll disease, with an exception that in GRBaV infected plants margin of the leaf stay flat and instead of green, pink veins appear (Sudarshana et al., 2015). As small RNA HTS can detect the presence of DNA viruses (Pooggin, 2018), we used this method to reveal the causative agent of the observed, virus-like symptoms.

Materials and Method

At the beginning of June 2018, the above symptoms: red blotches and red veins appeared at a Pinot noir plantation at Somogy (South of the Balaton) (Figure 1/A). At the vineyard plants with leafroll symptoms also appeared (Figure 1/B), and these two markedly different symptoms was altered even within a row.

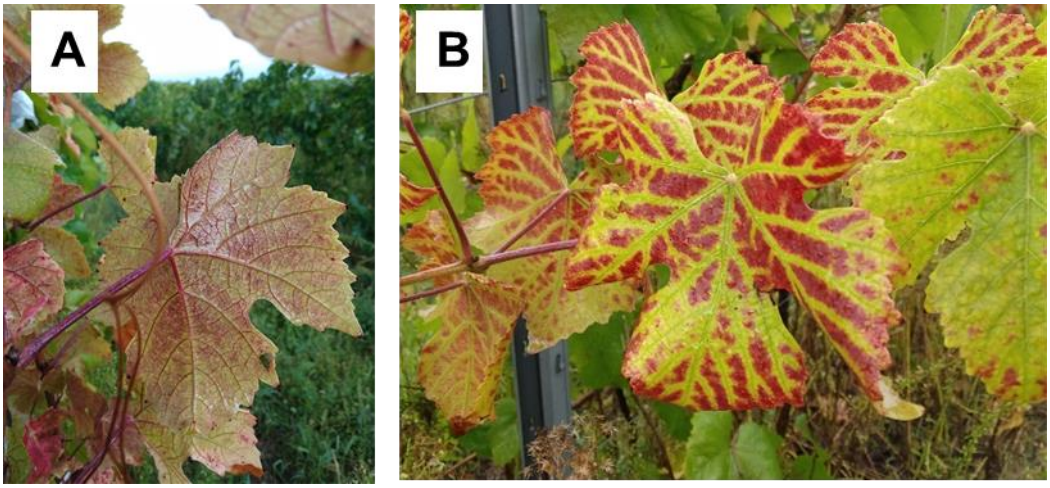

Figure 1. Pictures of the surveyed Pinot noir grapevines showing A/ red blotch-like, B/ leafroll-like symptoms.

Samples were collected from 4 individual plants of the same row, showing either red blotch-like (1, 3) or leafroll-like (2, 4) symptoms. We extracted RNA using a CTAB method and prepared two small RNA sequencing libraries (RB from plant 1 and 3 and LR from plant 2 and 4) according to our adapted protocol (Czotter et al., 2018a).

The sequenced reads were analysed using Qiagen CLC Genomic workbench. The reads were trimmed, both redundant and non-redundant list of sequences were prepared. This later one was used for contig building. Virus diagnostics were done by BLAST search of assembled contigs using all plant hosted viruses in the NCBI. The result list was ordered according to their lowest E-value. The reads (both redundant and non-redundant) were mapped to the GRBaV reference genome, and for other viruses which were present according to the analysis. Based on this analysis the consensus sequences were prepared and the coverage of the viral genome by small RNA reads were calculated. Threshold of virus presence was set to at least 1 virus specific contig, and higher than 60% coverage of the genome.

To validate the results of the small RNA HTS and directly test the presence of GRBaV, RT-PCR was carried out. After cDNA synthesis, an actin test was used to check the cDNA quality. This cDNA was used as a template to validate the presence of the presenting grapevine viruses

by RT-PCR using virus specific primers (for primer sequences please check (Czotter et al., 2018b). We also tested the cDNA with published GRBaV primers (Rwahnih et al., 2013).

Results and discussion

Results of the small RNA HTS show that several viruses: GLRaV-1, GVA, GFkV, GPGV and possibly GVB and viroids: HSVd, GYSVd-1 and 2 were present in the tested plants (Table 1), but neither GRBaV positive contig, nor reads mapped to the GRBaV genome were identified. Moreover, we could not get any product in the RT-PCR reaction using virus specific primers.

Table 1. Summary of the bioinformatics analysis together with the RT-PCR validation. GRBaV: grapevine red blotch-associated virus. Numbers indicate PCR positive samples out of the 2 which served for small RNA library preparation.

	type of analysis	viruses							viroids	
		GRBaV	**GLRaV-1**	**GVA**	**GVB**	**GFkV**	**GRSPaV**	**GPGV**	**HSVd**	**GYSVd-1/2**
RB library	small RNA HTS	0	+	+	0	0	0	+	+	+
	RT-PCR	0/2	1/2	1/2	0/2	1/2	2/2	2/2	2/2	2/2
LR library	small RNA HTS	0	+	+	?	+	0	+	+	+
	RT-PCR	No	2/2	2/2	0/2	2/2	2/2	2/2	2/2	2/2

GLRaV-1: grapevine leafroll associated virus-1, GVA: grapevine virus A, GVB: grapevine virus B, GFKV: grapevine fleck virus, GRSPaV: grapevine rupestris stem pitting-associated virus, GPGV: grapevine Pinot gris virus, HSVd: hop stunt viroid, GYSVd-1 and 2: grapevine yellow speckled viroid-1 and 2.

We have found severe infection with GLRaV-1 and also the presence of several other viruses and viroids (Table 1.) In case of GLRaV-1, GVA, GPGV and the viroids our RT-PCR results verified the result of the small RNA HTS (Figure 2). We could not prove the presence of GVB, but we have found only 1 GVB positive contig and less than 40% coverage of the genome, which indicate a false positive hit during the analysis. In RB library GFkV was not detected by

small RNA HTS, but in RT-PCR one of the plants showed infection. RNA from the other plant could dilute the sample what was used for small RNA sequencing, why we failed to detect it by this method. For GRSPaV we found the same contradiction, but this is what we usually experience in case of this virus. There were very few GRSPaV derived small RNA reads in the sample while the virus was present. One explanation of this can be that it was proved that this virus can have a positive effect on the grapevine physiology why defence mechanism against it could be suppressed during the evolution.

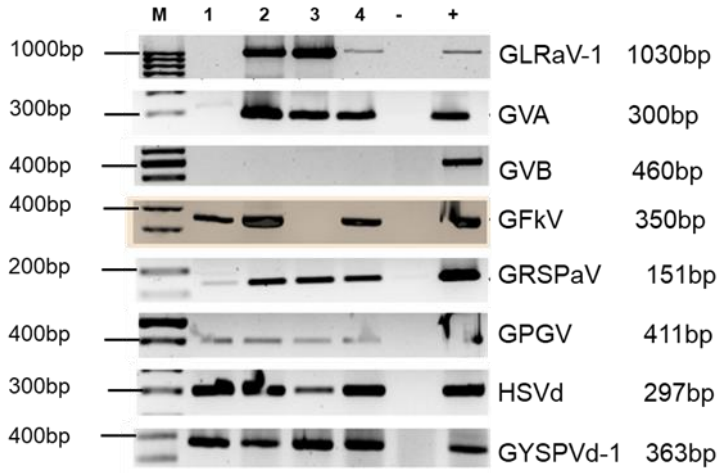

Figure 2. RT-PCR analysis for testing the presence of different viruses in the four plants which small RNA was sequenced.

In summary presence of several different viruses seemed random in the plants, thus we could not correlate any special combination with the appeared symptoms.

Conclusions

Our results showed that although red blotch symptoms appeared, GRBaV was not identified in the investigated Hungarian vineyard. Although it seemed possible for us to detect the presence of several different viruses and viroids, we cannot make any hypothesis about their contribution in the observed symptom development. Distribution of the nutrient in the soil of this vineyard

is very patchy why it is possible that shortage of some of them occurs quite randomly. Combination of these abiotic effects with the virus infections could lead finally or play role in the observed symptom development, but to find out the real causative agent we need further investigations. Moreover, these ambiguous results highlight the importance of the cooperation of classical and molecular virologist to reveal practical importance of the detected virus infections and explain or disclose possible causes of the emerging symptoms.

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