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Monitoring bumblebee pollinator visits to the medicinal plant *Gentiana asclepiadea* L. (Gentianacese) – a comparison between the periods 1990–1994 and 2017–2020

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Abstract

Ever increasing data continue to indicate the decline of bumblebee populations. The key factors causing declines in their abundance and diversity are: 1) habitat destruction, 2) loss of floral resources, 3) emerging diseases, 4) increased use of pesticides (particularly neonicotinoids).

The aim of this study is to monitor bumblebee visits to *Gentiana asclepiadea* L, recording pollinator species, and taking measurements of seed set. This plant species is chosen for two reasons: 1) similar data is available from our previous research in the 1990's and 2) this montane plant species is supposed to be less exposed to hazards from pesticides and habitat destruction. Three study sites were chosen in Mt. Vitosha (SW Bulgaria) where natural populations of *G. asclepiadea* occur in 1990. The observations of bumblebee activity in the flowers of *G. asclepiadea* were conducted during the flowering seasons (August and September) of 2017 – 2020 at the same study sites and compared to the data obtained in the previous period (1990–1994). The free pollination fruit set was tested by monitoring of 100 *G. asclepiadea* flowers each year for development of fruit capsules. The seed set was tested by counting the matured seeds and non-fertilised ovules of 10 fruit capsules each year. A slight decline in bumblebee activity was recorded in 2017 – 2020 in comparison to 1990–1994. This is reflected in the fruit set and the seed set. Our data demonstrates that even in a mountain habitat, where there are fewer direct hazards to bumblebees, that pollination effectiveness has been suppressed over time. This corresponds to a research study which provides evidence that insect biomass fell by 76% in German nature reserves between 1989 and 2016.

Keywords
Bombus, bumblebees decline, monitoring, pollinators

Introduction

Pollinators are a crucial element of biodiversity, since the majority of plant species depend on them for reproduction by seed. However, ever increasing data indicate that pollinating insects, and in particular bumblebees, are in decline. The key factors causing declines in their abundance and diversity are: 1) habitat destruction; 2) loss of floral resources; 3) emerging diseases; 4) increased use of pesticides (particularly neonicotinoids) (Söderman et al. 1997; Sepp et al. 2004; Biesmeijer et al. 2006; Rundlöf et al. 2008; Brown and Paxton 2009; Grixti et al. 2009; Potts et al. 2010; Goulson 2013; Goulson et al. 2015, 2018; Becher et al. 2018). Beside other harm, pesticides reduce bumblebee colony initiation by reducing the number of queens, as well as the flight dynamics and endurance of workers and their activity as pollinators (Baron et al. 2017; Kenna et al. 2019).

For the conservation of wild pollinators, long-term monitoring is necessary (Naeem et al. 2020). There is a need for long-term monitoring especially in montane habitats to determine the role and impact of the different drivers of global change, since bumbles are shifting to higher elevations (Marshall et al. 2020). Bumblebee monitoring technique is based on bumblebee counts based on flower visits and standardised observation routes (Teräs 1976; Söderman et al. 1997; Sepp et al. 2004).

There is no long-term quantitative data about bumblebees in Bulgaria. An efficient approach is by monitoring pollinator visits to one or more plant species, ideally recording pollinator species and also taking seed set measurements (Goulson, personal communication). A good candidate is *Gentiana asclepiadea* L. for two reasons: 1) such data is available from our previous research in the 90's of the last century; 2) this montane plant species is supposed to be less exposed to the hazards such as pesticides and habitat destruction.

Gentiana asclepiadea is a perennial plant. The rhizome is more or less thick and branched. There are sterile and usually several fertile stems, which are straight, non-branched and 35-50 (80) cm tall. There are 1-3-5-merous flowers sitting in nodes, at the base of the leaves. They are of the funnel type. Their size varies between (35) 40-50 mm and the corolla lobes are 3-4 times shorter than the corolla tube (Tutin 1972; Kozuharov and Petrova 1982). The flowers of *G. asclepiadea* are not spontaneously self-pollinated. The main pollinators of this plant are several species of bumblebees and *Thricops* spp. flies (Kozuharova and Anchev 2004).

The aim of this case study is to monitor bumblebee visits to *G. asclepiadea*, recording pollinator species, and also taking seed set measurements.

Material and methods

Three study sites were chosen (Kozuharova and Anchev 2004) in Mt. Vitosha (SW Bulgaria) where natural populations of *G. asclepiadea* grow at altitudes between 1500–1900 m above sea level (Fig. 1). Study sites 1 and 2 were in open woodlands in the coniferous forest belt. Study site 3 was in the subalpine meadows just above the coniferous forest belt.

The field investigations were conducted during the flowering seasons (August and September) of 1990–1994 (Kozuharova and Anchev 2004) and then at the same study sites repeated in 2017 to 2020.

The field observations were conducted over 66 hours during 48 days in different meteorological conditions. The visiting bumblebees were identified in the field. Their visitation rate and behaviour in the flowers were recorded. Since the focus of this study was mainly on visitation rate, we refrained from further collection of specimens as this would affect the results. Although *Bombus wurflenii* (Radoszkowski, 1860) and *B. lapidarius* (L., 1758) belong to different subgenera (*Alpigenobombus* and *Melanobombus*) and ecologically, they are different (*B. lapidarius* is distributed everywhere, while

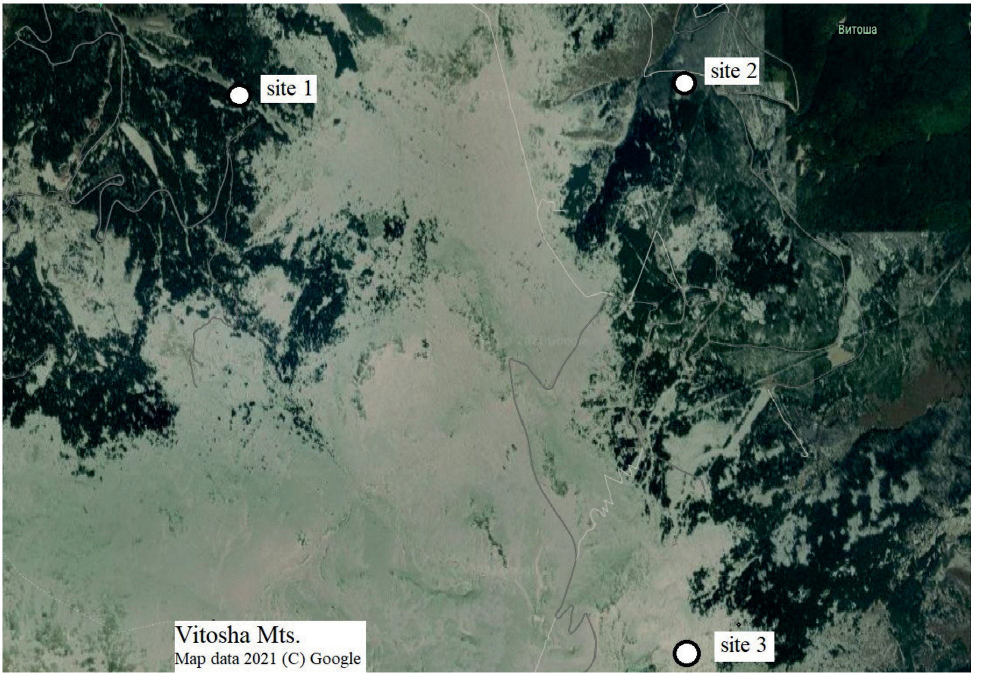

Figure 1. Study sites in Vitosha Mts., SW Bulgaria as follows: site 1 – 42°36'13.2"N, 23°15'04.0"E, site 2 – 42°36'20.5"N, 23°17'39.4"E, site 3 – 42°34'05.1"N, 23°17'52.6"E Study sites 1 and 2 were in open woodlands in the coniferous forest belt. Study site 3 was in the subalpine meadows just above the coniferous forest belt.

B. wurflenii is a montane species) it is hard to distinguish with certainty between these two species in the field as their colours are the same. Also, it is hard to distinguish *B. hortorum* (L., 1758) from *B. subterraneus* ssp. *latreillellus* (Kirby), and *B. lucorum* (L., 1758) from *B. terrestris* (L., 1758) in the field. Therefore, we preferred the approximate approach rather than irrelevant "precision" and in the result and discussion parts they appear as undistinguished pairs *B. wurflenii* and/or *B. lapidarius, B. hortorum* and/or *B. subterraneus, B. lucorum* and/or *B. terrestris*. A pollinator activity index was calculated as the quotient of the number of pollinators recorded and the minutes of observation multiplied by 60 minutes. The most numerous plants flowering simultaneously in the neighbourhood and their bumblebee visitors were recorded.

A Linear regression analysis of the total activity of bumblebees at each study site against the time/years of observation was applied to test the trend in the bumblebees' activity. The sequence of years of observations has a serious interruption (from 1994 to 2017) and thus the model does not allow prediction. It is just informative, rather qualitative than quantitative. Therefore, we refrained from Linear regression analysis of each bumblebee species' activity during the period of observations as well as the fruit and seed set.

The free pollination fruit set was tested by monitoring 100 flowers of *G. asclepiadea* each year for development of fruit capsules. The seed set was tested by counting the matured seeds and non-fertilised ovules of 10 fruit capsules. The damage inflicted by insect predators on the maturing seeds was not calculated for this research (see Kozuharova et al. 2018). Fruits that set were considered successful even if they were damaged subsequently. Seed set was evaluated using undamaged and undehisced opened fruits.

Results

Since the flowering period of the observed foraging plant is late summer and early autumn, we were observing the latter part of bumblebee colony activity. The species composition of the bumblebees visiting the flowers of *G. asclepiadea* remains basically the same over time. It is as follows: *Bombus pascuorum* (Scopoli, 1763), *B. wurflenii /B. lapidarius, B. pratorum* (L., 1758), *B. hortorum /B. subterraneus* ssp. *latrellellus, B. terrestris* and *B. lucorum*. The bumblebee activity varied over the years as well as within the study sites (Figs 2–5). A slight decline of bumblebees' activity is observed on the chart for the period 2017 – 2020 in comparison to 1990–1994 (Fig. 2).

Even though the linear regression model is just informative and not suitable for prediction, decreasing trends are observed at all three study sites (Figs 3–5).

Peculiarities which cannot be detailed in the charts are worth noting. In the first days of September 1991 high activity of *B. hortorum/B. subterraneus* males was recorded in the flowers of *G. asclepiadea*, at site 1, where they were feeding on nectar (Kozuharova and Anchev 2004, Figs 2, 3 and 7 A). The high activity of *B. wurflenii* in 2017 at site 2 was due to frequent visits of nectar robbing workers together with the less active nectar foragers (Figs 2, 4 and 7 B and C).

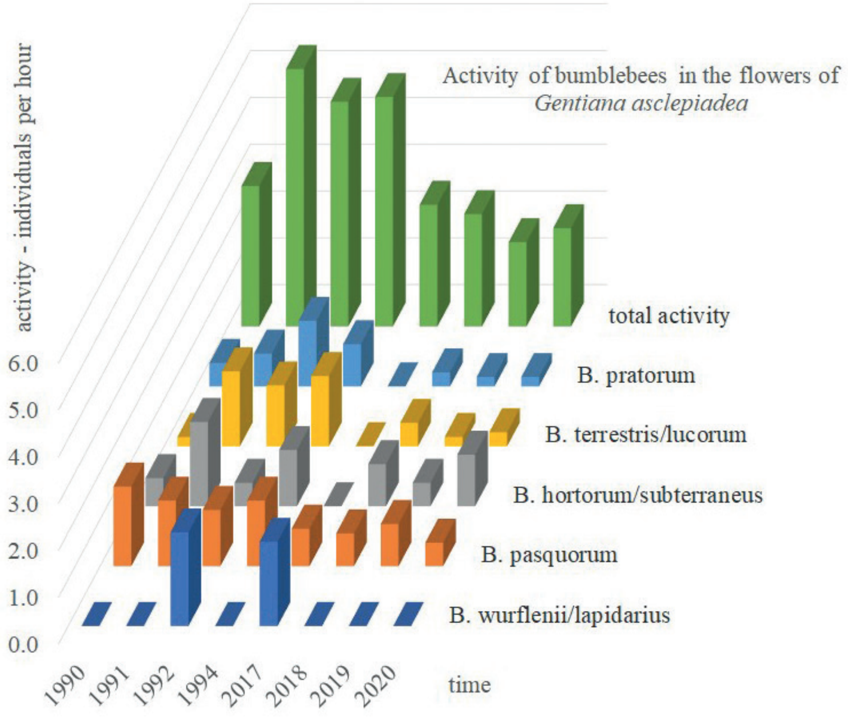

Figure 2. Average annual activity of bumblebees in the flowers of *Gentiana asclepiadea*.

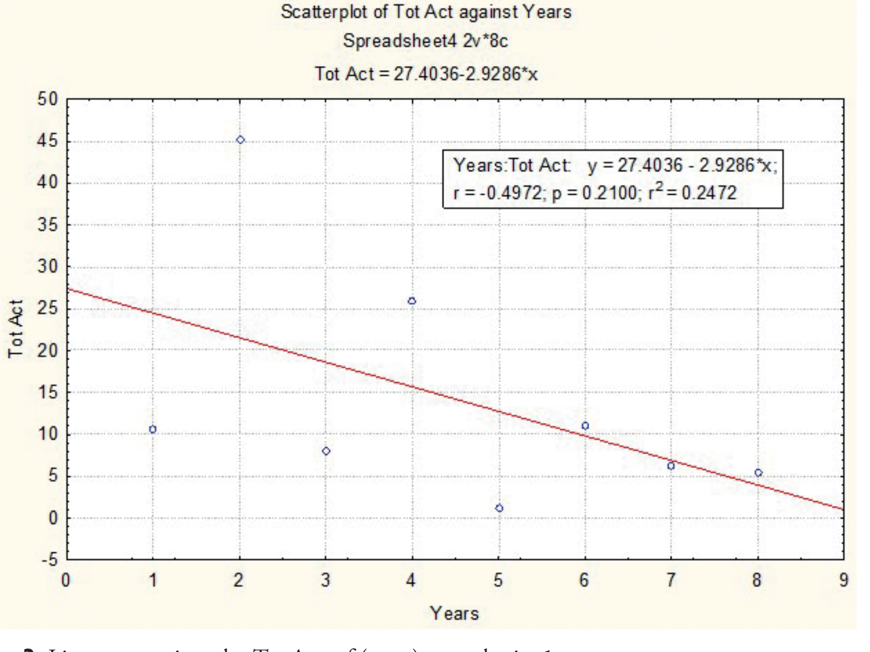

Figure 3. Linear regression plot Tot Act = f (years) at study site 1.

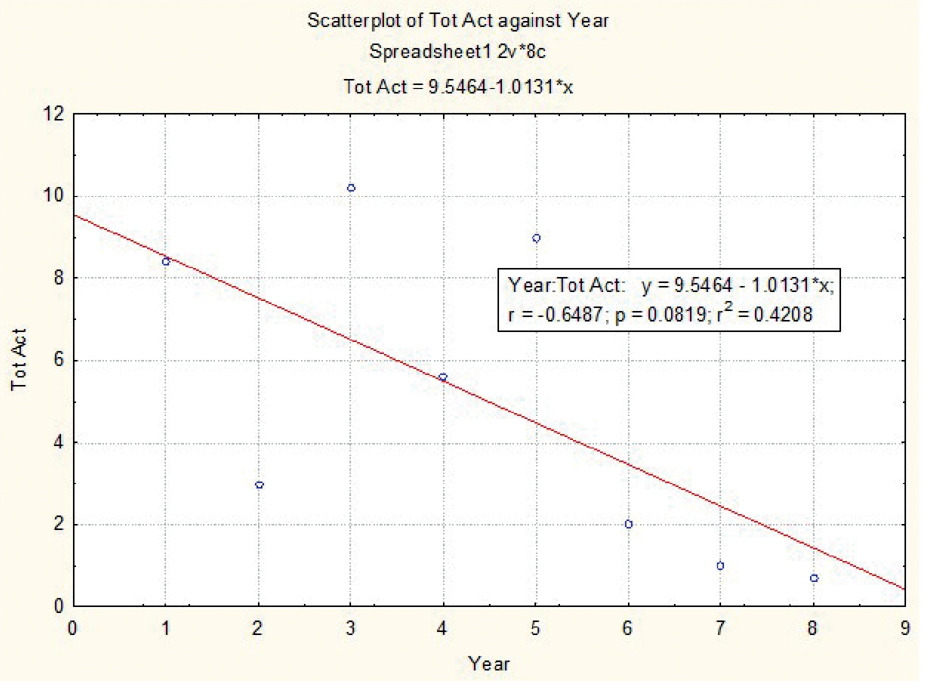

Figure 4. Linear regression plot Tot Act = f (years) at study site 2.

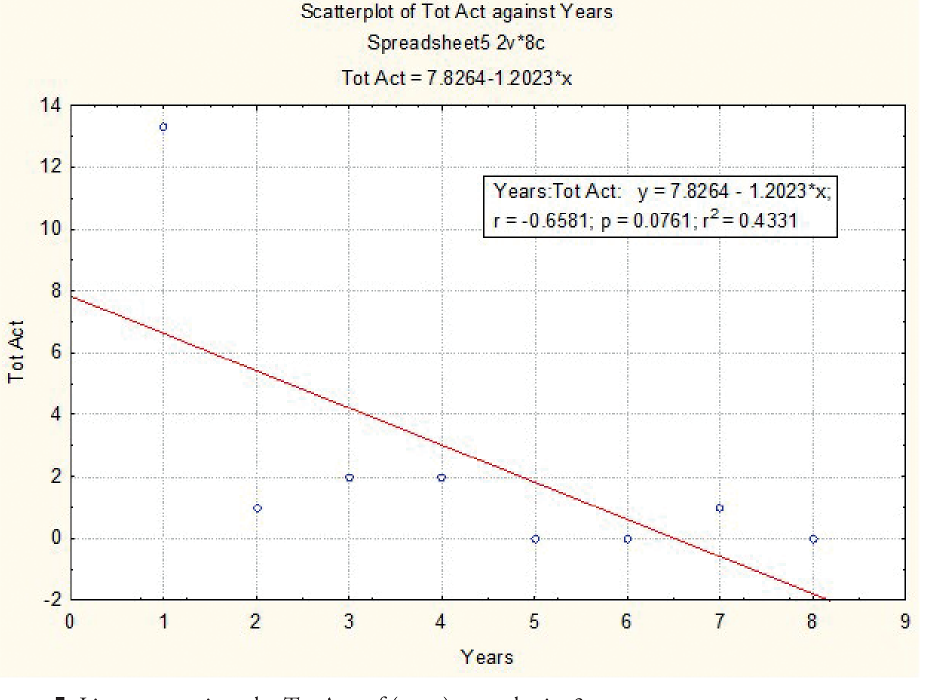

Figure 5. Linear regression plot Tot Act = f (years) at study site 3.

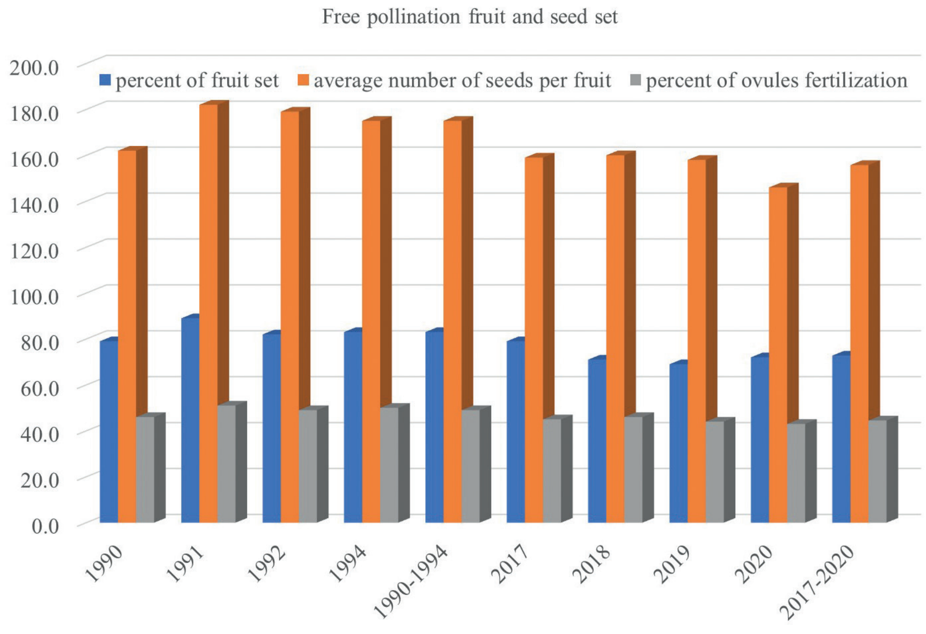

Figure 6. Free pollination fruit and seed set of *Gentiana asclepiadea*.

The free pollination fruit set tested by counting fertilised fruits versus flowers that failed to set fruit, as well as the seed set tested by counting the matured seeds and non-fertilised ovules, are presented on Fig. 6. A slight decrease of the fruit and seed set was observed during the final four years.

Discussion

A fluctuation in bumblebees' activity within the years and the study sites was observed. Such fluctuation in bumblebees' activity seems to be a normal process depending on several factors including colony initiation, pathogens, parasitoids, predators, food resources and landscape etc. (Bowers 1985; Goulson 2010; Persson and Smith 2013). The fluctuation in the activity of bumblebees in the flowers of *G. asclepiadea* can be explained by the dynamics of colony formation and foraging range. According to Goulson (2010) it is a matter of chance whether a bumblebee colony will be established in the vicinity of the observed plant population and, if so, of which bumblebee species. However, a relatively small proportion of bumblebees seem to forage close to the nest and the foraging range of bumblebees can reach up to 10 km. Thus, they reduce intracolonial competition as well as probability for predation to their nests. Also, according to Goulson (2010), the failure rate of colonies seems to be very high, although data is sparse. Fewer than 30% of *B. pasquorum* colonies produce any new queens. And the success of *B. lucorum* colonies is even less – about 14% and all this depends on various factors (Goulson 2010).

Figure 7. A *Bombus hortorum/subterraneus collecting* nectar in 1991 at site 1 **B** *B. wurflenii/lapidarius* worker robbing nectar of *Gentiana asclepiadea* in 2017 at site 2 **C** *B. wurflenii/lapidarius* worker taking nectar from a male stage flower in 2017 at site 2 **D** *B. wurflenii/lapidarius* and pollinating *Epilobium angustifolium* at site 3.

The observed fluctuations in pollinator activity are related to the study sites where observations were performed. It is quite interesting that, in general, fewer bumblebees were recorded at study site 3 – subalpine meadows. At this site *Epilobium angustifolium* L. and *Solidago virgaurea* L. had large populations and these possibly over-competed for bumblebee pollinators. Bumblebees are known to favour *S. virgaurea* (Teräs, 1976). And *E. angustifolium* was observed to attract a lot *B. wurflenii/B. lapidarius* and *B.*

pratorum (Fig. 7D). However, the pollen of *E. angustifolium* was poorly represented in the pollen loads of *G. asclepiadea* (Kozuharova and Anchev 2004). At study sites 1 and 2, *Cirsium appendiculatum* Griseb. and *Prenantes purpurea* L. were competitors for bumblebees such as *B. pratorum*, *B. wuflenii*/*B. lapidarius*, *B. hortorum*/*B. subterraneus* ssp. *latreillellus* but these plants never made large patches like *S. virgaurea* and *E. angustifolium.* Unfortunately, our fruit and seed test materials were taken randomly, so we cannot confirm pollen limitation in relation to the study sites.

The high activity of *B. hortorum*/*B. subterraneus* ssp. *latreillellus* males feeding on nectar in the flowers of *G. asclepiadea* during the first days of September can be explained by their phenology (Goulson 2010). *Bombus pratorum* and *B. hortorum* nests last for about 14 weeks from founding, compared to about 25 weeks for the sympatric *B. pascuorum,* which in general means that no more workers are reared once the colony switches to producing reproductive individuals (Goulson 2010). The high activity of *B. wurflenii* in 2017 was due to frequent visits of nectar robbing workers (Fig. 7B.). *Bombus wurflenii* is known as a nectar robber (Utelli and Roy 2001, Goulson et al. 2013). Obviously, it has difficulties to reach the nectar of *G. asclepiadea* hidden in deep pockets. Nectar robbery – extracting nectar through holes made at the base of the corolla tube – has a wide spectrum of consequences for the plant, that ranges from negative, neutral, to positive according to life history traits of the interacting organisms and the ecological mechanisms involved (Rojas-Nossa et al. 2021). In the case of *G. asclepiadea* it can be regarded as neutral to slightly positive at the stage when the workers pollinate while trying to reach the nectar the normal way Fig. 7C). In any case these were not frequent visitors compared to *B. pasquorum, B. hortorum*/*B. subterraneus* subsp. *latreillellus* and *B. lucorum*/*B. terrestris.*

The slight decline of bumblebees' activity recorded in 2017–2020 in comparison to 1990–1994 reflected on the fruit set and the seed set. Our data demonstrate that even in a mountain habitat with fewer direct hazards for bumblebees a negative effect on pollinator activity over time is still detectable. Our results correspond to a research study which provides evidence that insect biomass fell by 76% on German nature reserves between 1989 and 2016 (Hallmann et al. 2017). There is no obvious explanation for the recorded decline of bumblebees in the flowers of *G. asclepiadea.* Some speculations could be offered. A large amount of the land in the foothills of the mountain which used to be meadows was integrated into Sofia's suburbs and urbanized with all the telecommunication infrastructure and car traffic. Also, in the last few years, adjacent agricultural land around Sofia is actively used for sunflower, oilseed rape and corn production. However, at this research stage we cannot say if these factors affect the bumblebees' colonizing habitats at higher altitude and about 15 km away from the urbanized area and 20 km away from the agricultural activity. Global warming is known to be a serious hazard for particular bumblebee species such as *Bombus monticola* (Smith, 1849) and *B. mucidus* (Gerstaecker, 1869) (Manino et al. 2007). Some bumblebees react to the climate change by relocation to higher altitudes (Marshall et al. 2020), however we do not have enough data for altitudinal assessment and thus no climate change conclusions can be done.

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