STUDIES FOR A SUSTAINABLE MANAGEMENT OF *SCYPHOPHORUS ACUPUNCTATUS* (COLEOPTERA, DRYOPHTHORIDAE) AT THE HANBURY BOTANIC GARDENS

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ABSTRACT

Scyphophorus acupunctatus Gyllenhal, 1838 Coleoptera: Dryophthoridae, the agave weevil, is an exotic species native to Central America that is now found on five continents. This species is parasite of several species of Asparagaceae (Agavoideae and Nolinoideae) and is the major pest of wild and cultivated Agaves. *Scyphophorus acupunctatus* is a threat to Agave and related genera collections in botanic gardens, as well as ornamental plants in urban areas, in the Mediterranean Basin.
In 2018 *S. acupunctatus* was first detected in Hanbury Botanic Gardens, La Mortola (Ventimiglia, IM - Italy); since then, the parasite has killed several specimens of *Agave* and related genera, causing significant damage to the botanic collection.
The study, here presented, was carried out using pheromone and agave baited traps in order to assess the damage caused to the collection and to evaluate the influence of temperature, relative humidity, and rainfall on the activity of *S. acupunctatus.* The effect of the treatments against the parasite was also evaluated.
The highest level of *S. acupunctatus* activity was recorded in spring (moderate rainfall (51.8 mm), a temperature 22° - 26°C, relative humidity > 60%). Pheromone traps were more effective than baited traps. The monitoring data following the treatments revealed a drop in captures of the species, suggesting a lethal effect of the applied products. The level of damage assigned to host plants revealed the presence of at least one infested plant in 12 of the 14 monitored areas of Hanbury Botanic Gardens' and 1/4 of the individuals in the collection show an infestation symptom.

KEYWORDS: Sustainability, Integrated Pest Management, *Scyphophorus acupunctatus*, *Agave*

INTRODUCTION

The agave weevil *Scyphophorus acupunctatus* Gyllenhal, 1838 (Coleoptera: Dryophthoridae) is a species native to Central America and introduced to many other parts of the world (mainly arid and tropical regions), potentially with the introduction of *Agave sisalana* Perrine as fibres crop (EPPO, reporting Service n. 3-2002). Nowadays the species is recorded in 5 continents (Cuervo-Parra et al., 2019). Agave weevil is a polyphagous species, and it is considered the most important pest of agaves worldwide (Solís-Aguilar et al., 2001; González et al., 2007). Like all holometabolous insects, the development is divided in four stages (egg, larva, pupa, and adult). The species is multivoltine with overlapping generations throughout the year (Solís-Aguilar et al., 2001; Waring & Smith, 1986). Females lay eggs at the base of leaves near the central shoot or in rhizomes, where the tissues are already decomposing (Harris, 1936). *Scyphophorus acupunctatus* is in fact associated with microorganisms, such as fungi, bacteria, and algae, which, penetrating the agave, lead to tissue rot (Waring & Smith, 1986). Several species of bacteria as *Pectobacterium carotovorum* (Jones 1901) Waldee 1945 (Approved Lists 1980) emend. Portier et al. 2019 (=*Erwinia carotovora*), *Pantoea agglomerans* (Beijerinck 1888) Gavini et al. 1989 (=*Erwinia milletiae*), *Pseudomonas* sp. (González et al., 2007), *Pseudomonas fluorescens* Migula 1895 (Ruíz-Montiel et al., 2003), algae as *Prototheca* sp., fungi as *Aspergillus niger* Tiegh., *Kluyveromyces marxianus* (E.C. Hansen) Van der Walt, and various species of yeasts of the genus *Candida* (Velázquez et al., 2006) have been isolated on its body surface and on decaying agave plants.

During her lifetime, a female can lay 30 to 50 eggs (SENASICA-DGSV., 2016). When the egg hatches, the larva feeds on the internal tissues of the plant by excavating tunnels inside the stem and, depending on the host species, it can have a different number of instars. Upon reaching maturity, the larva creates a cocoon with the fibre of the host, where the pupation occurs. Once emerged, the adult can stay inside the plant where it can initiate a second infestation or migrate to a new plant.

The most visible symptom of attack in plant is the presence of a dark brown, viscous, and sticky exudate with a distinct odour (Riba I Flinch & Alonso-Zarazaga, 2007; Cuervo-Parra et al., 2019) emerging from 1 cm diameter hole (Harris, 1936): this symptom indicates that the weevil has entered the plant (Harris, 1936). Small round dots of 2-3 mm on the surface of the leaves (Waring & Smith, 1986) represent the feeding, operated by the rostrum of the adults.

The feeding activity of the larvae determines the rotting or necrosis of the internal tissues, which take on a reddish colour due to a chemical reaction of the plant and/or the development of phytopathogens (SENASICA-DGSV., 2016). Depending on the extent of the infestation and, therefore, on the number of adults and larvae inside the host, the entire stem of the plant can rot with the consequent death of the plant.

Adult weevils can emerge and move from infested hosts to new plants to start a new infestation (Figueroa-Castro et al., 2015). Therefore, control strategies can be directed mainly against immigrating adult weevils.

Although the species was reported devastating important populations of Agave crops (Solís-Aguilar et al., 2001; Camino et al., 2002), around the Mediterranean Basin the species damages are mostly focused on ornamental species, such as the genera *Agave* L., *Beaucarnea* Lem., *Dasylirion* Zucc., *Dracaena* Vand. ex L., *Furcraea* Vent., *Nolina* Michx. and *Yucca* L., which also entail important economic costs (Riba I Flinch & Alonso-Zarazaga, 2007).

In Europe, it was first detected in 1989 in a greenhouse in the Netherlands and it was promptly eradicated. Nowadays the pest is widespread along Mediterranean borders in France - Corsica and Southern France (Germain, 2008) - and Spain - Catalonia, Valencian Community, Murcia, Andalusia (Martín-Taboada et al., 2019) - since 2007; Greece (Kontodimas & Kallinikou, 2010); Cyprus in 2013 (Vassilis & Pavlos, 2015); Croatia (Pernek & Cvetković, 2022); continental Portugal - Algarve (2019) and Setubal (2018) (Naves & Boavida, 2021) and Madeira Archipelago (Andrade, 2022).

In Italy, the species was recorded in 1998 in Lombardia, on *Beaucarnea recurvata* Lem., as a glass-house incursion (Colombo, 2000); in Sicily in 2006 on *A. americana* L. in the parks and gardens of Catania and in the surrounding grounds. Since then, observations of the weevil in natural environments of several Italian regions have been posted on a forum for entomologists (Forum Entomologi Italiani) with the following posts: Basilicata (2013), Apulia (2014), Latium

(2014), Tuscany (2015). In Liguria, it was first detected in 2016, in Bordighera (IM), in a private garden. Since 2018 *S. acupunctatus* was observed in several locations along the Ligurian Riviera, from the French border to Imperia, where *A. americana* colonizes semi-natural coastal environments and slopes along roads and railways. In these habitats, attacked plants may endanger the public by dropping leaves or other plant material onto roadways (Monroy et al., 2021).

In the Hanbury Botanic Gardens (GBH), Ventimiglia (IM), Italy, four kilometres from the French border, where *Agave* and related genera collections have suffered severe damages, the species was discovered, for the first time, in 2018. In GBH, the weevil was first observed on *Dasylirion longissimum* Lem. and one year later, it was detected on *A. americana* var. *marginata* Trel. and other entities.

Several actions have been tested and implemented for the agave weevil pest management in agave crops in the native distribution range (Rodríguez-Rebollar et al., 2012; Terán-Vargas et al., 2012), including integrated pest management. However, in order to apply any management strategy in areas of new colonization, it is necessary to consider that the condition of agaves and related genera grown in open ground in Mediterranean botanic gardens differs significantly from that of agave crops. Succulent beds in botanic gardens are characterized by environmental heterogeneity, species diversity, variability of density, and the size and age of hosts and potential hosts. Therefore, it is important to understand the population dynamics of *S. acupunctatus* to obtain information on the habitat selection processes of this beetle depending on the heterogeneity of the agronomic management practices, species diversity, and the environmental variables (humidity, temperature, and precipitation), as pointed out for *A. tequilana* plots in Mexico by Rodríguez et al. (2020).

GBH collection of *Agave* and related genera is unique, with plants of various sizes and ages growing in open ground alongside hundreds-old historical specimens of *Beaucarnea* Lem., *Yucca* L., *Nolina* Michx, and *Dasylirion* Zucc. Agaves grow to full size in La Mortola, where they can complete their biological cycle and bloom more or less regularly depending on the species. Agaves grow in ornamental beds, alongside other exotic species groups, or among natural Mediterranean vegetation in these gardens. GBH collection also includes plants of great relevance descending from the original specimens studied by Alwin Berger, author of monographs on the various succulent groups and curator of the garden at the time of Sir Thomas Hanbury (Berger, 1912).

This biological-environmental diversity requires specific control plans. To contain the negative impact caused to the collection of *Agave* and related genera by the weevil, since October 2020 the Hanbury Botanic Gardens (GBH) - University of Genoa and the Council for Agricultural Research and Agricultural Economics (CREA) of Sanremo cooperate to carry out studies for the weevil control using an integrated pest management (IPM) approach (Boero et al., 2021).

GBH, as Regional Protected Area and Special Area of Conservation and public garden, must follow the guidelines of the National Action Plan for the sustainable use of plant protection products (Ministry of Agricultural, Food, and Forestry Policies, 2014), which, in order to limit chemical pollution, biodiversity loss, and health problems, impose some restrictions on the use of synthetic products.

The main purpose of this research is to understand the complex relationship between insect and host and to investigate the biology and ecology of *S. acupunctatus* in GBH. These aspects have not yet been studied outside the natural range of the species, in a protected area such as a Mediterranean Botanic garden and are vital to develop an effective management protocol.

Therefore, our studies was set to achieve the following objectives:

- Update the inventory of all potential guests susceptible to *S. acupunctatus* of the Gardens.
- Define the species phenology - the period/s of activity during the year - of this insect in the Mediterranean through monitoring with use of two types of traps and visual assessment.
- Evaluate the influence of the annual variation of temperature and relative humidity in the monitored flowerbeds on the activity of *S. acupunctatus.*
- Evaluate the efficiency of the two types of traps.
- Evaluate the effect of the treatments performed.
- Evaluate the damage to the plant collections.

STUDY AREA

The study area is located on Capo Mortola, a promontory on the western coast of Liguria, in the municipality of Ventimiglia, a few kilometres from the French border (Fig. 1). It includes a Regional Protected Area of about 20 ha and the western part of the Special Area of Conservation (SAC) “Capo Mortola” of about 50 ha. This SAC extends from sea level up to 260 m a.s.l., and its geographical limits have the following coordinates: 43°46'45.0"N, 7°32'32.7"E, and 43°47'21.2"N, 7°33'30.1"E.

The study area includes the entire surface of Hanbury Botanic Gardens; the compendium covers an area of 18 ha, of which about 9 ha are dominated by natural and semi-natural habitats such as cliffs, mixed woods, pine forests, garrigues, scrubs, arid grasslands, reeds, dry stone walls, and ruins; the remaining part is cultivated as a garden with exotic plant collections. The study area has a Mediterranean pluviseasonal oceanic bioclimate with an upper thermomediterranean thermotype and dry ombrotype (Rivas Martínez et al., 2011; Pesaresi et al., 2017), with a mean annual temperature of 15.8 °C (+/- 0.8°C) and a mean annual rainfall of 770 mm (+/- 227 mm) calculated on 1978-2022 years. The mean temperature in the hottest month of the year (August) is 23.7 °C (+/- 1.2 °C), while in the coldest month (January) is 9.3 °C (+/- 1.2 °C).

The two sides of the syncline of Capo Mortola are very well exposed with bioclastic limestones (Capo Mortola Calcarenites) while the core of the grey blue marls (Olivetta San Michele Silty Marls) has almost completely been obliterated by several terraces. Along the north side of the area, some weakly cemented yellowish sands are detected (Faccini et al., 2015).

The collection of *Agave* and related genera extends over several ornamental beds and includes hundreds of specimens, on a total area of about 3 ha.

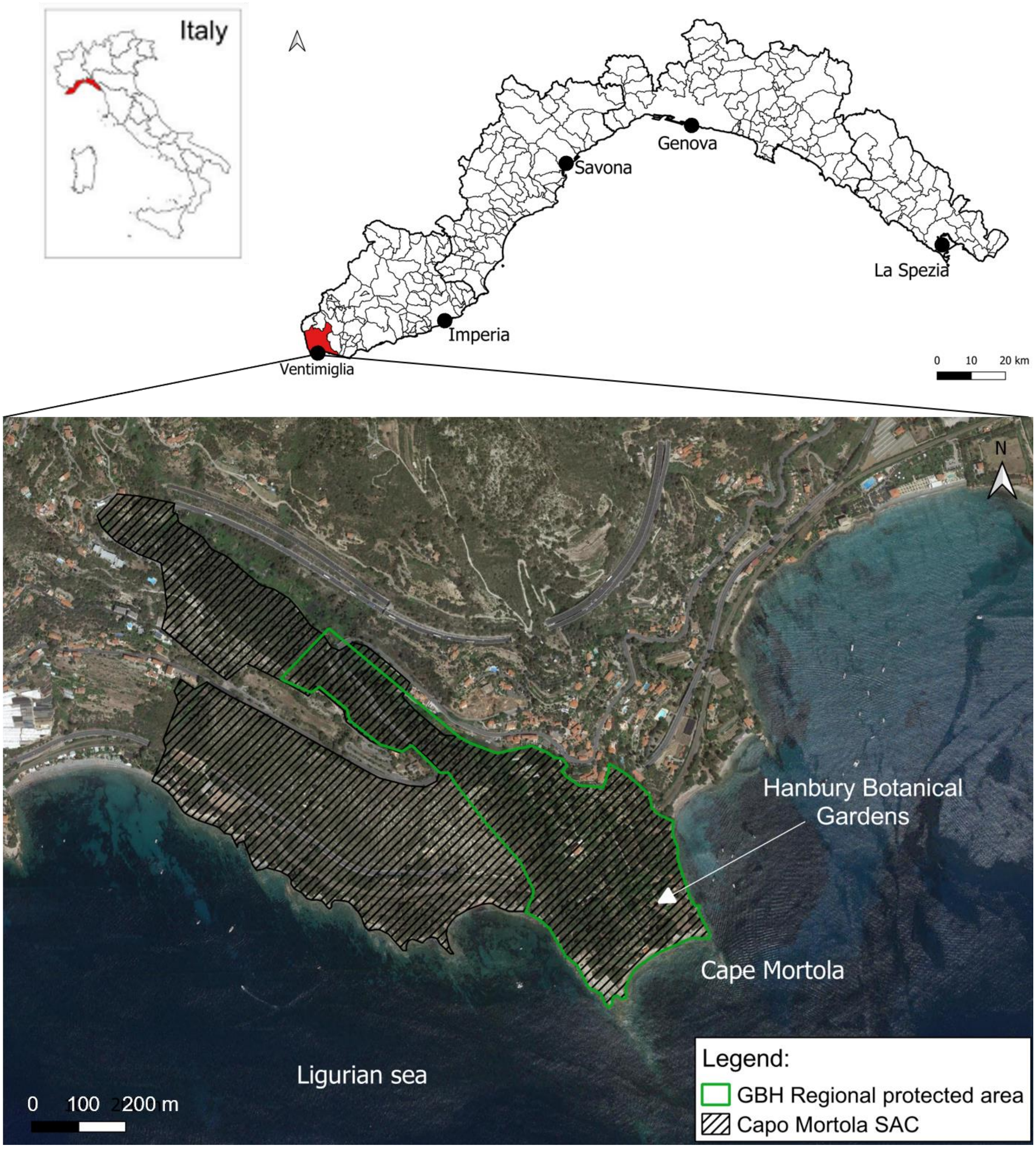

Figure 1. Study area: GBH Regional protected area, Capo Mortola, Ventimiglia (IM).

Materials and Methods

Plant material

One of the first steps of the study was the updating of the inventory of potential hosts of *S. acupunctatus* at GBH. The list of species and specimens of the collection of *Agave* and related genera was revised: the number of taxa and specimens of the genera *Agave*, *Yucca*, *Dasylirion*, *Beaucarnea*, *Furcraea*, *Dracaena*, and *Nolina* was recorded for each flowerbed. For some plants the identification was not possible as within the GBH there are specimens of agave spontaneously born from seeds that could have a hybrid origin. The taxonomy of the genus *Agave* is difficult due both to the complex nomenclature, with many ancient names of uncertain application and many poorly or even undocumented names of horticultural origin, and because of the high variability and plasticity of many taxa, including the possibility of hybridization and introgression (Thiede, 2020).

Nomenclature is accorded to *Plants of the World Online* (POWO, 2023), except in some critical cases i.e. *A. americana* var. *marginata*.

Monitoring with traps

To monitor *S. acupunctatus*, two types of pitfall traps, buried in 14 areas characterized by the greater presence of host plants were installed. The first type consists of a white plastic bucket with a volume of 5 liters and two rectangular openings (4.0 x 2.0 cm) at ground level to allow the weevils to enter. The bottom is perforated with four holes of 0.5 cm diameter to avoid water stagnation (Fig. 2 A). Each trap contained pieces of agave in a 10% sugar solution as bait, placed in a tray inside the bucket. The bait was replaced every 15 days. We installed 20 traps; they have been placed close to the agaves, according to Figueroa-Castro et al. (2016); they were checked once a week; they were maintained from June 2021 to June 2022. The number of captured adults in each trap was recorded.

In November 2021, 3 additional Arysta LifeScience “Rhynchotrak” commercial traps, which are used in France for monitoring the red palm weevil were installed. These traps are yellow containers constituted by two parts with an opening along the circumference of the trap; the upper part is equipped with a small grid to which a pheromone dispenser is attached on the lower part. The trap is buried so the opening is at the with ground level (Fig. 2 B).

The traps were triggered with the pheromone “ECONEX SCYPHOPHORUS ACUPUNCTATUS®”, which releases pheromones for 40 days in the field. In order to increase the catching capacity of the traps, pieces of agave have been added as bait to increase the attractivity as suggested by Figueroa-Castro et al. (2016).

The monitoring was performed once a week from November 2021 to October 2022.

Summarizing, monitoring data of *S. acupunctatus* were recorded through the two types of traps for a total duration of 1 year and 5 months: in the first period, only through handcraft traps with bait; subsequently, from November 2021 to June 2022, also with pheromone traps; and from July 2022, only with the latter.

The position of the traps is shown in Figure 3.

Recording of temperature and relative humidity in the monitored areas

Eleven data-loggers (Tinytag plus 2, Gemini Data Loggers Ltd, Chichester, West Sussex, UK) (Fig. 2 C) were installed to measure temperature and relative humidity in flowerbeds. The data-loggers were placed in some monitored flowerbeds and recorded values of temperature and relative humidity (from 0 to 100% RH) every hour of the day. Every six months, data were downloaded using "Tinytag Explorer 6.0" software. Data recording began in June 2021 and ended in October 2022. Their position is shown in Figure 3. A weather station located in GBH (43°46'58'' N, 7°33'14'' E) provides rainfall data for the garden.

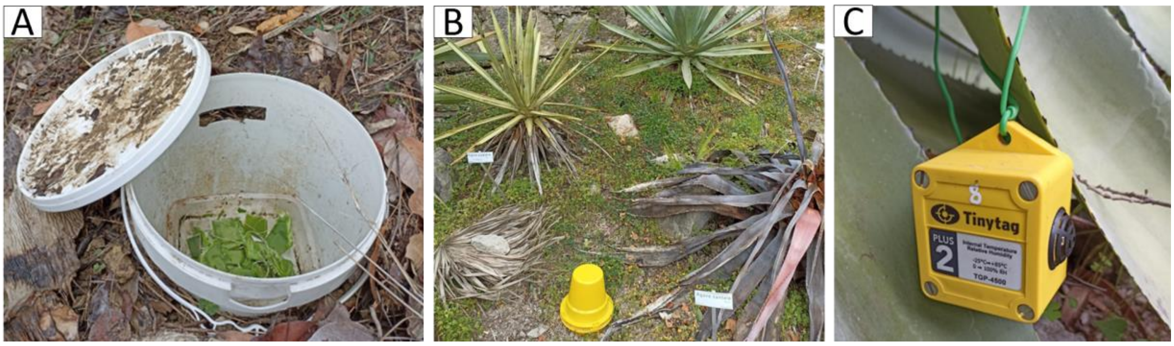

Figure 2. Materials used in monitoring. A) Handcrafted pitfall trap, with bait represented by pieces of agave leaves; B) Commercial pitfall trap baited with pheromone; C) Data-logger for measuring temperature and relative humidity.

Visual monitoring and damage detection

Adult weevils, when outdoors, tend to locate in the basal parts of the younger leaves of a rosette (Waring & Smith, 1986). So, since May 2022, monitoring of the traps has been integrated through the visual control of the presence of *S. acupunctatus* on the host plants once a week, between 9 and 12 a.m.

Consequently, to ascertain its presence on the hosts, it was necessary to carefully observe the central portions of the individual plants (Fig. 4). This procedure has been implemented for hosts of the *Agave* genus and only for some *Yucca* species, such as *Y. gloriosa* L. and *Y. aloifolia* L., which do not grow very tall; for species with an arborescent habit and higher than 1.5 m, it was not possible to carry out this type of monitoring, as well as for agaves placed in impervious positions. The observed weevils, when possible, were captured and taken to the laboratory.

Periodical inspection of plants made it possible to identify the attacked plants, define the levels of damage, and identify severely attached or dead plants to be removed and destroyed. The damage scale proposed by the Consejo Regulador del Tequila (1999) used to determine the damage level of bole rot disease in blue agave (*A. tequilana*) was apply in this study with few modifications – to allow the monitoring of all *Agave* species and to the genus *Furcraea* - as reported in Table 1. The Consejo Regulador del Tequila scale amendments also include some intermediate symptoms, such as:

- feeding marks, which represent the feeding activity carried out with the rostrum of the weevils. They are defined by Waring & Smith (1986) as "chewing marks" and are useful for estimating unsuitable hosts for weevil reproduction, as not all species with this symptom are subsequently infested;
- wrinkled leaves at the base, which is a symptom of plant dehydration. Many American gardening and dissemination sites report that this type of symptomatology develops when the weevil damages the root system, which begins to lose its functionality. The damage can in fact be due to the feeding of adults and larvae at the base of the roots, and consequently, the leaves, receiving a smaller quantity of water, begin to wrinkle. According to Velázquez et al. (2006), the damage to the hosts is primarily caused by the larvae’s activity, which cause wilting and rotting by feeding inside the plants; wrinkled leaves are considered a symptom of wilting in this case;

- presence of holes of about 1 cm in diameter, from which a viscous exudate comes out; sign of the presence of weevils inside the plant (Harris, 1936); useful, therefore, to indicate the infestation event.

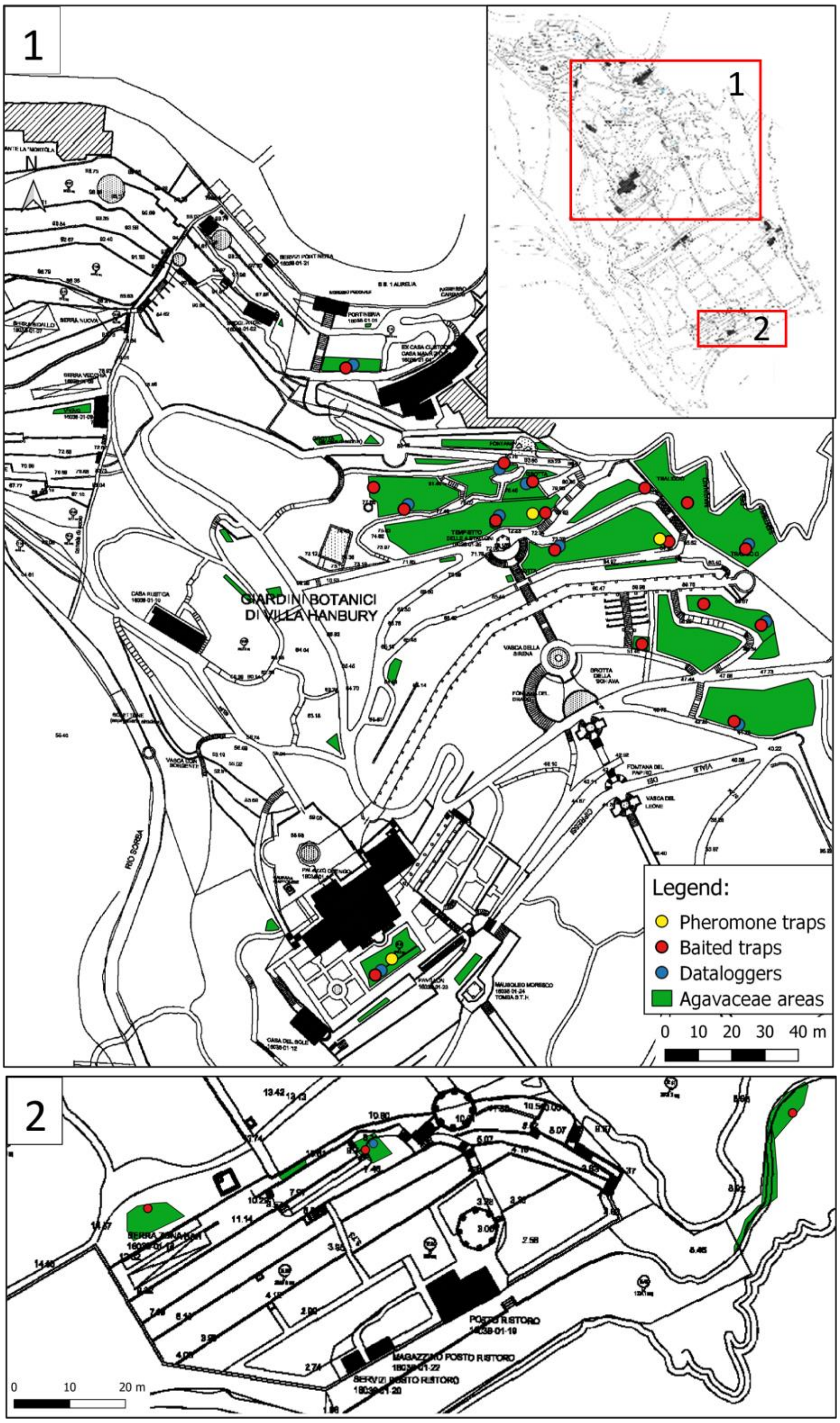

Figure 3. Map relating to the location of two types of traps and data-loggers in GBH. 1) Northern portion of the GBH characterized by the greater presence of host plants. 2) Southern portion of the GBHs.

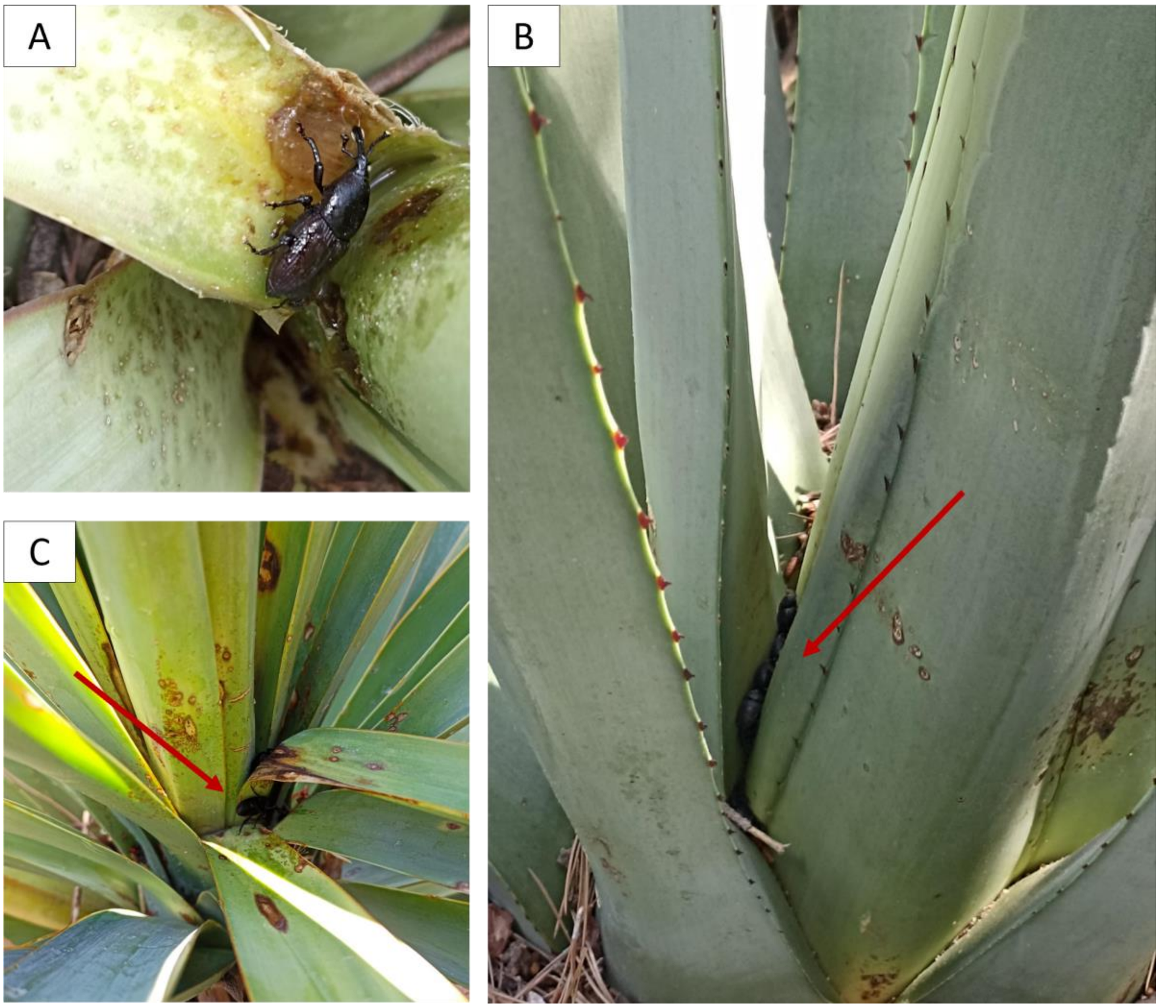

Figure 4. Weevils observed on host plants. A) Weevil observed on *Agave mitis*; B) Aggregation of weevils observed on *Agave angustifolia*; C) Weevils observed on *Yucca gloriosa*.

The damage level was assigned to the individuals in the flowerbeds monitored, and a mean value was assigned for the stoloniferous or rhizomatous species that grow in groups. The latest damage assessments were conducted in October 2022.

During the observation of the host plants, it was decided to carry out the eradication of the plants showing severe symptoms of attack, corresponding to the damage level 5 and 6, as a phytosanitary measure useful for the containment and the contrast to the dispersion of the insect. Consequently, no plants belonging to category 7 (dead plant) were reported, a condition that can easily be found in semi-natural areas where there is no host plant management.

Treatment

In order to contrast the damage caused to the *Agave* collection by *S. acupunctatus*, treatments with commercially available and authorized products based on deltamethrin were carried out. A total, of three treatments was carried out in May 2021, December 2021 and mid-

July 2022. All treatments were performed by spraying the leaves of the host plants according to the doses indicated by the manufacturer.

Table 1 - Damage levels assigned to host plants.

Damage level	Symptoms description
0	Apparently healthy plant
1	Plants with pierces on the leaves - feeding marks - surrounded or not by a brown necrotic ring
2	Leaves wrinkled at the base: plant with a suffering appearance.
3	Presence of gummy/crystalline secretions and presence of characteristic oval-shaped holes 1 cm diameter : white or dark brown viscous and sticky exudate near perforations
4	From 1 to 5 necrotic injuries over 1/2 the length of the leaves
5	One or more necrotic injuries over the leaves, or more than 6 injuries over 1/2 the length of the leaves
6	Necrotic injuries in the head (crown), but healthy bole or piña, the disease (decomposition and fermentation diseases: rot symptoms with a with a characteristic odor of rotting) has not yet reached the piña
7	Head completely damaged; the rot disease had invaded the plant: collapsed agave plants.

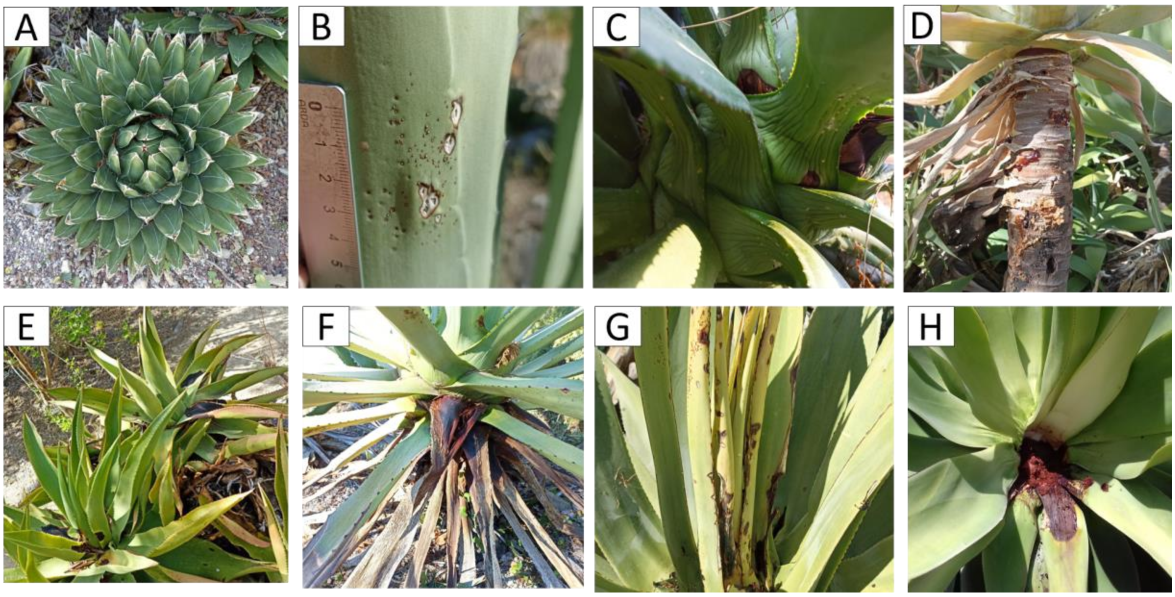

Figure 5. Damage levels assigned to host plants. A) *A. victoria-reginae*, healthy plant (Lev.0); B) Feeding marks (Lev.1) healed on *A. angustifolia*; C) Leaves wrinkled at the base (Lev.2) and beginning of necrosis on *A. decipiens*; D) Presence of brownish resinous secretions and flicker hole (Lev.3), on *A. attenuata* stem; E) Necrotic lesions affecting less than half the length of the leaves (Lev.4) on *A. obscura* x *mitis*; F) Necrotic lesions over half the length of the leaves (Lev.5), necrotized leaves of *A. angustifolia*; G) Necrotic lesions that only partially affect the apex of the rosette, axis not yet affected (Lev.6), *A.* hyb. *obscura*; H) The rot has invaded the whole heart of the rosette (Lev.7), plant of *A. polyacantha* doomed to die.

RESULTS

The update of the list of Agavaceae and related genera revealed 89 taxa as potential hosts of *S. acupunctatus*. Taxa include several species, hybrids and varieties, some of which are widespread over several sites with many specimens (*A. salmiana* var. *ferox* (K. Koch) Gentry, *A. titanota* Gentry, *Dasylirion serratifolium* (Karw. Ex Schult. & Schult.f.) Zucc., *Yucca gigantea* Lem.). Only a few species are represented by single plant (*A. karwinskii* Zucc., *A. sebastiana* Greene, *D. wheeleri* S. Watson ex Rothr, *Y. carnerosana* (Trel.) McKelvey). Furthermore, there are groups of rosettes connected by rhizomatous stems that are part of a single individual (*A. attenuata* Salm-Dyck, *A. mitis* Mart., *A. ghiesbreghtii* K. Koch). Most of the plants are in open ground, but some are grown in nursery greenhouses. *Scyphophorus acupunctatus*, since its arrival, has affected a total of 28 taxa in this collection; some of these get more infested than others; for example, before starting this study, all individuals of *A. americana* subsp. *americana* were infested to death.

The number of adult weevils caught varied over the study and depended on the trap. Only 45% of the baited traps and all three of the pheromone traps recorded captures. Pheromone traps captured a higher number of weevils than baited traps, but the two types of traps showed similar temporal dynamics of insect activity. The captures showed the onset of weevil activity in the spring of 2021, a decline in activity in the summer and winter months, and a recovery in the following spring of 2022. Activity was recorded throughout the monitored period, and the largest number of adult weevils captured was recorded in the spring of 2022 (Fig. 6). The cumulative number of weevils caught shows an increase in the last monitored year.

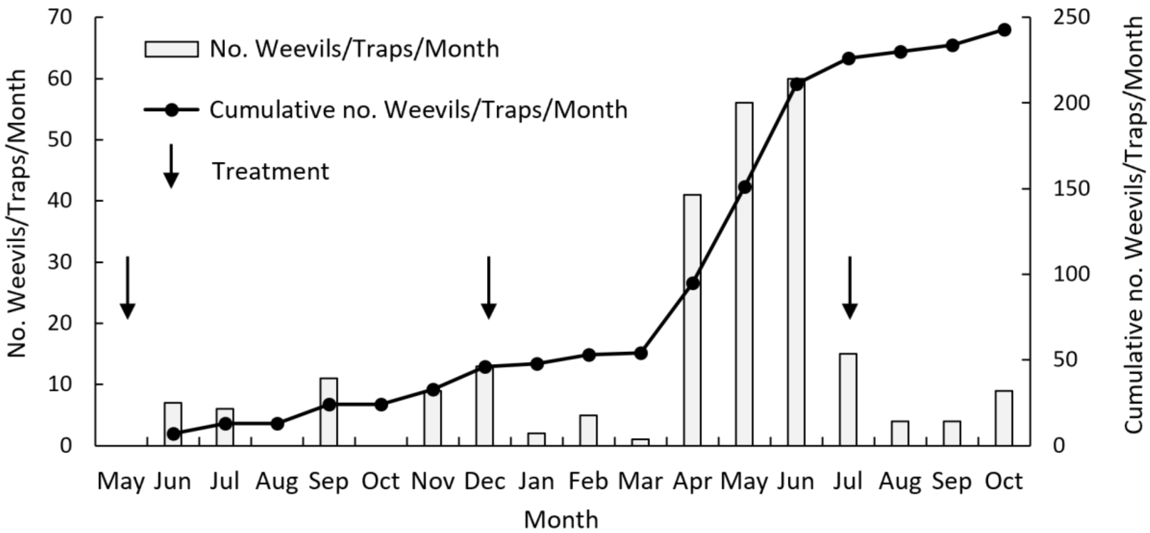

Figure 6. Total numbers and cumulative numbers of *Scyphophorus acupunctatus* captured by the two types of traps per months. The monitoring was performed from June 2021 to October 2022. Treatments were conducted in May 2021, December 2021 and mid-July 2022.

Similar to monitoring with traps, the results of visual monitoring (Fig. 7 A) show an elevated number of weevils observed on plants in the spring months, such as May 2022 and June 2022, and a decrease in August 2022 and September 2022. No weevils were observed in July 2022, while the maximum peak was recorded in October 2022. A different scenario was observed for the weevils found inside the eradicated plants (Fig. 7 B): July 2022 and October 2022 represent the months in which the greatest number of weevils was found, but a high number of adult weevils were also found in August 2022 and September 2022.

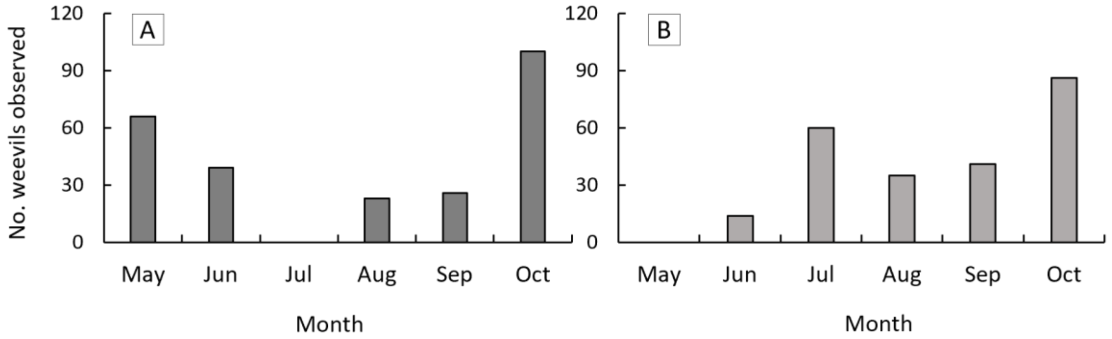

Figure 7. Results of visual monitoring. A) Numbers of weevils observed on host plants from May 2022 to October 2022. B) Numbers of weevils observed inside the eradicated plants from May 2022 to October 2022.

Based on data from data-loggers, the mean temperature and mean relative humidity of the months monitored revealed variability according to the period considered (Fig. 8). The mean maximum temperature was recorded in July 2022 (29.6°C), while the mean minimum temperature was recorded in December 2021 (11°C). The mean maximum relative humidity was recorded in October 2022 (76.3%), while the mean minimum humidity was recorded in March 2022 (58.7%). Based on records from the weather station, the maximum rainfall was recorded in November 2021 (84.4 mm), while the minimum was in July 2022 (0.6 mm); most of the months monitored, with the exception of November 2021 and April 2022, showed drought conditions.

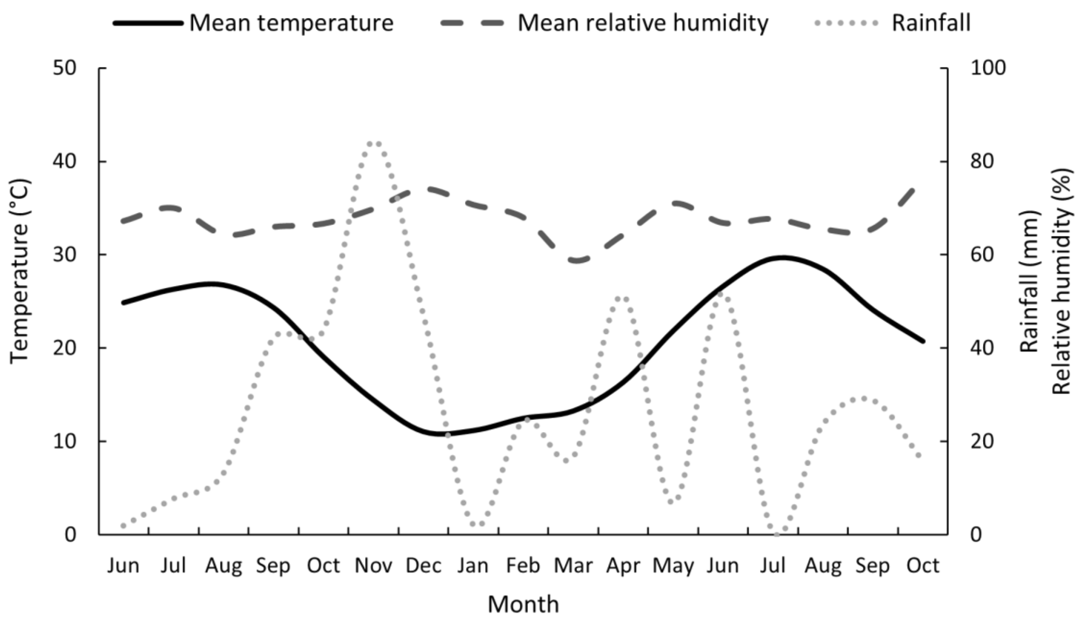

Figure 8. Trend of mean temperature (°C) and mean relative humidity (%) obtained from the values recorded by data-loggers. Trend of cumulative rainfall (mm) obtained from the values recorded by the weather station. The monitoring was performed from June 2021 to October 2022.

Analysing the mean temperature data as a function of the number of weevils captured by the two types of traps, a statistically significant positive correlation was found between the individuals captured by the pheromone traps and the mean monthly temperatures for the period November 2021-June 2022 (Pearson' r = 0.96; p = 0.0001). No correlation was found between captures from baited traps and humidity values.

The damage level was assigned to a total of 273 host plant individuals. The results of the damage levels were reported both as damage levels assigned to the total individuals of the monitored areas (Fig. 9 A) and as mean damage percentages assigned to individuals in each bed (Fig. 9 B). Figure 9-A shows 164 individuals of level 0, i.e., apparently healthy plants, and a decreasing numbers of plants as the severity of the damage increases. Feeding marks were the most common symptom (49 individuals). Figure 9-B highlights a high mean damage percentage of levels 2 and 3: these data indicate an infestation in most of the flowerbeds. In only 2 flowerbeds out of 14 there were no damages and no signs of the presence of the weevils. The data of the damage categories in percentage were analysed using the Kruskal-Wallis test, and statistically significant differences between the different categories ($H(6)$= 37.5, p < 0.001) were observed. These differences were then analysed using the Dunn test that indicated the presence of about three groups of similar level, the first represented by healthy plants, the second by an initial phase of infestation, and the third, less frequent, by an infestation more advanced.

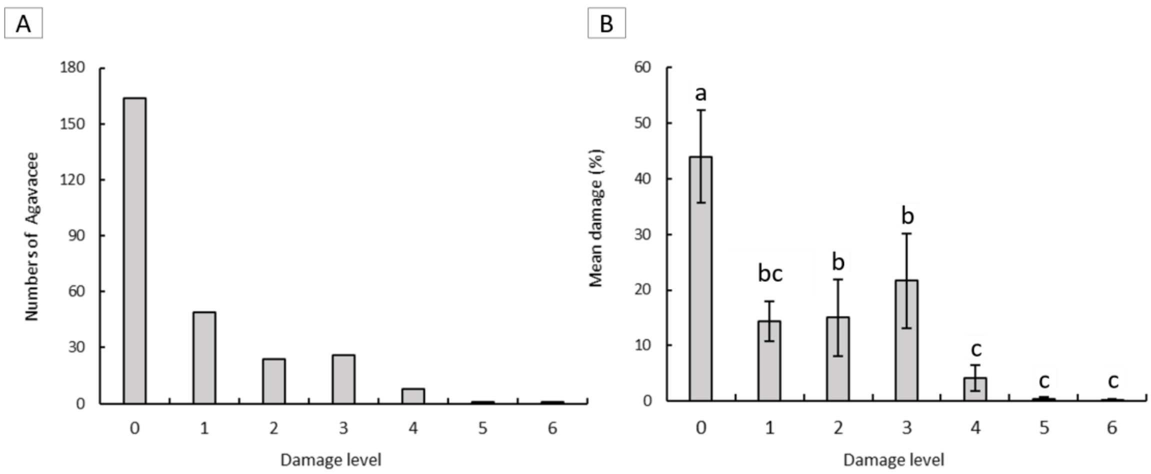

Figure 9. Damage level. A) Results of the damage level assigned to each individual in the monitored areas; B) Mean damage percentage assigned to each flowerbed monitored. The black bars and small bars indicate the mean and standard error of the mean, respectively; means that do not share the same letters are significantly different (Dunn's test, p < 0.05, n = 14).

DISCUSSION

The Collection consists of hundreds of specimens belonging to over 80 taxa and represents a scientific and cultural heritage for the GBH. The study here presented clearly shows that *S. acupunctatus* is causing a loss of diversity.

Monitoring results show that the weevil activity occurs throughout the year, as in Mexico (Téran-Vargas et al., 2013), with a peak in spring. The decrease in activity in the winter months is related to the low temperature, while in the summer months (July and August) to the high temperature. In these conditions, the weevils tend to remain inside the hosts, as evidenced by the adult weevils found inside the eradicated plants. In fact, the agave weevil has cryptic habits, and its life cycle take place inside host tissues (Terán-Vargas et al., 2012) making its detection and control difficult (Martín-Taboada, 2019). The presence of larvae of different ages was observed in the eradicated plants in the majority of the cases and on different periods of the year. These results underline that *S. acupunctatus* at the GBH is active during the whole year, with overlapping populations, in agreement with the observations of Waring & Smith (1986), who consider *S. acupunctatus* a multivoltine species on wild and cultivated agaves (Solìs-Aguilar et al., 2001). The maximum peak of activity found in the spring of 2022 may indicate a population increase, even if it may be related to the greater functionality of the pheromone traps. The increase in population may also be related to visual monitoring data, in which the maximum peak of weevils is observed in the month of October 2022, suggesting that new individuals have emerged over time and especially in the summer.

Pheromone traps were more efficient than baited traps in terms of the number of weevils captured, so according to Figueroa- Castro et al. (2013) these results suggest of using pheromone traps for monitoring the populations of this pest. In addition, according to Scheepers et al. (2020) the use of the pheromone can be employed in IPM programs.

No correlation between temperatures and capture records of the baited traps was observed, potentially due to the low number of captured individuals. In contrast when pheromones are used the results show a correlation, in fact, from November 2021 to June 2022, the correlation (r = 0.96) between the number of individuals captured by pheromone traps and the mean monthly temperature indicates that an increase in temperature corresponds to an increase in weevil activity, which instead decreases when the temperature is too high (July/August). These results indicate that there is an optimal temperature range for the external activity of the weevil. In the warmer months (July/August), many adults were, in fact, found inside the eradicated plants.

In the monitored months of 2022, the maximum peak of captured weevils, recorded in June, coincided with the maximum rainfall (51.8 mm), as it occurs in some areas of Mexico, where the attacks are more frequent in the rainy season (Terán-Vargas & Azuara-Domínguez, 2013). However, in Mexico, its activity varies among the cultivation of host plants and geographic location (Cuervo-Parra et al., 2019). In the monitored months of 2021, the maximum peak of captured weevils, recorded in December, was after the maximum rainfall, recorded in November (84.4 mm).

The monitoring data following the pesticide interventions revealed a drop in the captures of individuals of *S. acupunctatus*, indicating a mortality effect of the products used, also considering the discovery of several dead individuals and no adult weevils observed in July 2022. The treatments seem to have been effective for short periods, as the drop in captures in the months following the treatments may also be due to a variation in temperature, relative humidity, and rainfall. Sustainable management methods involve the use of entomopathogenic products, such as different strains of the fungus *Beauveria bassiana* (Bals.-Criv.) Vuill. and the nematode *Steinernema carpocapsae* Weiser, 1955, for which favourable pathogenicity results are being observed in Mexico (González-Hernández et. al, 2011; Aquino-Bolanos et al., 2006). GBH's future goal will be to adopt these biocontrol methods.

The assignment of the damage levels to plants, based on the total individual count, showed a high number of potential host plants with no attack symptoms and few plants with at least one symptom. Instead, considering damage data (%) per flowerbed more than 50% of guests are infested, indicating that most of the monitored flowerbeds present at least one infestation event. In only 2/14 flowerbeds, no damage caused by weevils was found. Damage level 1 (= feeding marks), the most common in the total count, does not necessarily indicate an infestation event: it probably means that the host is used only for feeding and not for reproduction. According to Waring & Smith (1986) the presence of feeding marks on leaves suggests that weevils regularly test plants and find some unsuitable as reproductive hosts. Currently, based on statistical analyses, three groups of damage levels are present in the GBH collection: the first represented by healthy plants (damage level = 0); the second by an initial stage of infestation (damage level = 1-2-3), in which the weevils penetrating inside the plants transmit the microorganisms that create the favourable conditions for the deposition of the eggs; and the third by an advanced stage (damage level = 4-5-6), indicating the development of the larvae that together with the adults produce mechanical (tunnels) and physiological damages to the plants (Cuervo-Parra et al., 2019).

Summarizing, the second group can be interpreted as the result of a recent infestation event; the third group, characterized by the presence of all development stages of weevil (early instar to adult), suggests that plants are being reinfested by residents or immigrants (Waring & Smith, 1986). The maximum peak of weevils observed in October 2022, may indicate the presence of new individuals that have recently emerged and are looking for hosts favourable to reproduction. From the observations made, it is not easy to predict the evolution of the infestation, as the intensity of the damage depends on the number of larvae and adults present inside and on the size of the plant itself.

CONCLUSIONS

Monitoring data through traps and visual observations have highlighted weevil activity throughout the year, with peaks in the spring months; the optimal temperature range for the activity of the weevils is between 22°C and 26°C. Results show that, the use of pheromone traps is recommended, and that because of the Mediterranean climate *S. acupunctatus* can have up to four generations a year.

The monitoring data following the treatments revealed a drop in captures of weevils, indicating a mortality effect of the products used.

The level of damage assigned to host plants revealed the presence of at least one infested plant in 12 of the 14 monitored areas. In total, 18% of the individuals in the collection have feeding marks, and 22% of the individuals in the collection show an infestation symptom (damage level 2 to 6).

The results and the analyses presented in this article highlight the easy adaptation of *S. acupunctatus* to environments different from those of its origin and to new guests, as well as the need to draw up a specific control plan aimed at preserving the historical Agavaceae collection from attack by this invasive alien species.

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