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Investigation of Cowpea (*Vigna unguiculata* (L.) Walp.)–Insect Pollinator Interactions Aiming to Increase Cowpea Yield and Define New Breeding Tools

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Abstract: Impact of pollination on the agri-food sector is of paramount importance. Pollinators contribute to the maintenance of ecosystems, the reproduction and survival of many plants, and their presence usually leads to increased yield and quality of agricultural products. Breeding and selecting for plant traits for enhancing pollinator visits could therefore lead to more resilient farming systems. In stating the advantages of enhancing pollinators in agricultural systems, this study was designed aiming to assess six cowpea accessions for their flower traits and their effect on insect-pollinators. Pollinators species abundance and foraging activity was recorded and their impact on yield was investigated. Twenty-five of the twenty-seven flower traits studied differed statistically significantly among cowpea accessions. The main pollinators recorded belonged to the genus *Xylocopa* (Latreille, 1802). Seed and fresh pod yield was not affected by pollinators. The floral traits related to pollinators abundance and foraging activity were flower color, inflorescence position and the hours that the flowers per plant remained open during the day. However, they were not related linearly to pollinators abundance and foraging activity; therefore, they did not constitute safe traits for selection aiming to increase pollinators visitation. The findings suggested that other traits, such as pollen and nectar reward, probably perform a more important role in attracting pollinators compared to flower traits.

Keywords: biodiversity; floral resources; foraging activity; landraces; production; resilient ecosystems

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1. Introduction

Pollination effect in the agri-food sector is considered of utmost importance as it contributes positively to yield and quality of agricultural products [1], and therefore to food security. Without pollinators presence, productivity of many crops that depend considerably on them would be drastically reduced [2]. Pollinators also contribute to ecosystems maintenance, and reproduction and survival of many plants, including domesticated and wild species [3]. Among pollinator species, bees are considered as the main contributors [1], visiting over 90% of staple crops worldwide [4].

Numerous conservation policies have been proposed and enacted to protect pollinators and the agricultural environments that host them [2,5]. Policies also aim to compensate for the rapid decline in pollinator numbers observed [6,7]. Maintaining wild pollinators diversity, which is considered one of the most important factors for successful pollination, composes also one of the main policies' goals [5]. Towards this direction, increased efforts have been made for breeding plant varieties that are highly attractive to pollinators, and their inclusion in breeding programs. Usage of pollinators friendly varieties leads to pollinators diversity enhancement, plant heterozygosity maintenance, increased yield, and more sustainable and resilient ecosystems [8–10].

Floral traits investigation is a prerequisite for introducing a pollinator friendly variety in a breeding program. Assessment on existence and availability of suitable pollinators and their interaction with plant material used should take place separately on the different environments [11,12], as environmental and climatic conditions prevailing can also affect pollinators species presence and pollinators flights and may influence the behavior of plant species in terms of its advertisement to be or not be attractive for pollination [13,14]. Plant intra-population diversity, sub-populations proportions, and, therefore, populations genetic composition are affected by environmental conditions [15].

Legumes are among the plant species that are affected by pollinator visitation rates. Although they usually constitute predominantly self-fertilizing species, their flowers are configured to be receptive to pollinator visits and increase yield when they are present [10]. Cowpea is a mainly self-pollinated legume species [16]. Its flowers mostly are self-fertilizing before opening (cleistogamous species) [17,18]. Its flower morphology encourages this purpose [19] as the anthers are in direct contact with the stigma [16,20]. However, cross-pollination has been reported [21] ranging from 1% to 9.5% in countries near its center of origin in Africa [19,21,22]. In the U.S., very low cross-pollination rates up to 1.4% [18] have been reported, while a rate of about 10% has been recorded for *Vigna* in tropical regions by Rachie et al. [23].

Prevailing climatic conditions drastically affect cross-pollination rates [24,25] by modifying insect flights [26]. Cross-pollination rates vary also according to plant species and pollinators population dynamics [18] and applied sowing distances [17]. In adverse conditions, self-pollination appears to be a mechanism to ensure fertilization and survival, while cross-pollination is not encouraged [27]. Various cowpea floral traits [26,28], such as the intra and extrafloral nectaries that maintain [29] and pollen production [28,30], affect pollinators attractivity. Pollinators visitation therefore varies among genetic material used [29,31].

Bee species from various families are reported worldwide as cowpea pollinators [28], such as the honeybee (*Apis mellifera* Linnaeus, 1758) [16,17], species of the genus *Xylocopa* [17,22,26,31,32], bumblebees (*Bombus* sp.) [17,29], and species of the Megachilidae family [17,22,31]. In addition, hummingbirds (family Trochilidae) [29] and species of the families Noctuidae, Pieridae, and Vespidae [28] are also mentioned as effective pollinators. Contradictory results were obtained for species of the Lepidoptera order and honeybees regarding if they can be positive pollinators of cowpea [17,28,31,33].

Contribution of pollinator visits to cowpea yield and yield-related traits varied among similar studies. Number of seeds per pod, weight of seeds per pod (g), weight of fresh pods (g), and pod length (cm) were reduced when they were covered to prevent insects' visitation [30,34], while total fresh pod weight (g) was not affected [28]. Presence of pollinators influenced seed yield of twenty-three oilseed rape cultivars expressing different flower traits [35]. This differentiation underlines the importance of investigating plant-pollinator relationships separately in each environment.

Taking into consideration the multiple advantages the knowledge of plant-pollinator interplay offers and its implementation in breeding, the low genetic diversity that characterizes cultivated cowpea, and the lack of previous relevant record for cowpea-pollinators interaction around Mediterranean, this study aims to record cowpea floral traits (phenological, visual, and design characteristics), investigate their contribution to the attraction of insect-pollinators and assess possible pollinators effect on seed and fresh pod yield. At the same time a first record of pollinator species effective on cowpea in the area was attempted.

2. Materials and Methods

2.1. Plant Material and Experimental Design

The experiment was conducted at an experimental field of Agricultural University of Athens (N 37°59′10″, E 23°42′29″, altitude 24 m), during spring–summer 2017. Five cowpea

(*Vigna unguiculata* (L.) Walp.) landraces, two of them originated from Greece (VG2, VG21) collected on-farm, one from Portugal (Vg60), kindly provided by the University of Trás-os-Montes e Alto Douro (Vila Real, Portugal), and two from Spain (BGE038478, BGE038479), kindly provided by the Centro de Recursos Fitogenéticos (CRF)-INIA (Madrid, Spain), were assessed. Breeding line IT97K-499-35 with origin from Nigeria was also used. Cowpea accessions varied regarding their seed coat color, color around the hilum (eye color), flower color, and raceme position (Table 1; Figure 1), while they expressed uniformity regarding their seed color and eye color. A Randomized Complete Block Design (RCBD) with four replications was used. Each plot consisted of forty plants. Sowing took place on 29th of May in 2017, by placing one seed per hole, at a depth of 3 cm. Plant spacing between lines was 40 cm, and 30 cm within each line.

Table 1. Cowpea accessions, breeding status, origin, flower color, raceme position, seed coat color, and eye color.

Accession	Breeding Status	Country of Origin	Flower Color	Raceme Position	Seed Coat Color and Eye Color
IT97K-499-35	Breeding line	Nigeria	White with purple pigmentation and a red tip on the back of the standard petal	Mostly above the canopy	White with black eye
VG2	Landrace	Greece	White	On the upper canopy surface	White/cream
VG21	Landrace	Greece	Violet	Mostly above the canopy	Brown
Vg60	Landrace	Portugal	White with purple pigmentation	Mostly above the canopy	Cream with brown eye
BGE038478	Landrace	Spain	Violet	Throughout canopy	Brown/beige
BGE038479	Landrace	Spain	Light Violet	Throughout canopy	Brown/red

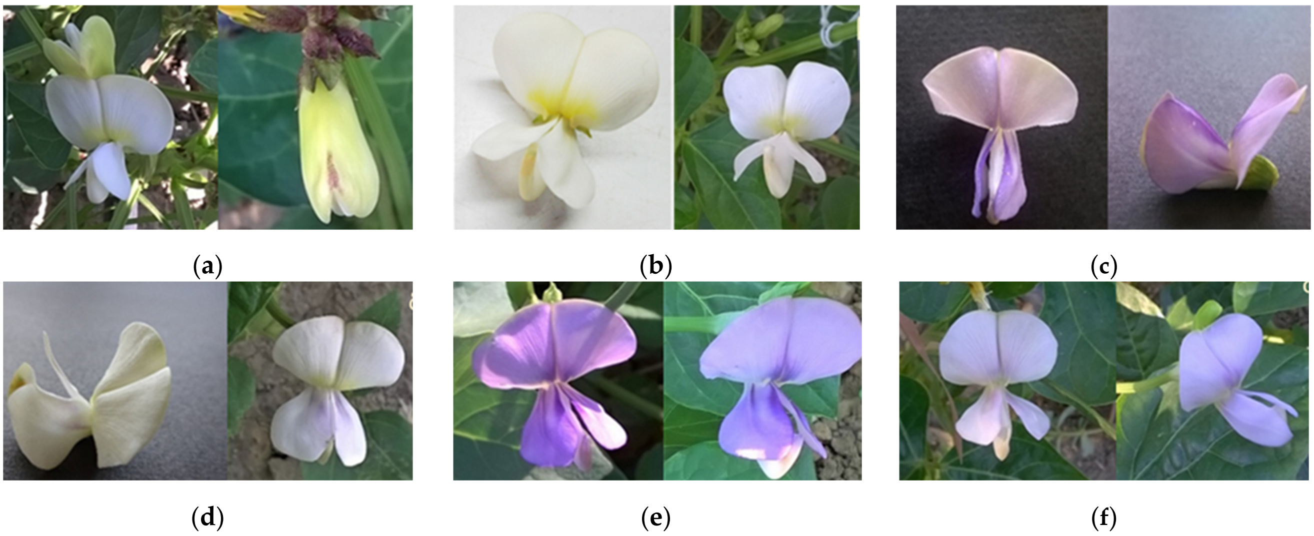


Figure 1. Flower color of the plant material used, where: (**a**) flowers of breeding line IT97K-499-35, (**b**) flowers of local population VG2, (**c**) flowers of local population VG21, (**d**) flowers of local population Vg60, (**e**) flowers of local population BGE038478, and (**f**) flowers of local population BGE038479.

2.2. Growth Conditions

The soil was clay with a loamy texture (pH 8.1). Plants were drip irrigated and supplied with 1200 kg ha^{-1} of a mineral fertilizer (NPK 11-15-15). During the growing season, no chemical control means were used. Weeds were manually controlled inside the plots area, while in the corridors that the plots formed weed growth was allowed. Monthly meteorological data throughout the cultivation period are presented in online Supplementary Material S1.

2.3. Floral Traits

Floral traits were grouped into three categories, namely phenological, visual and design flower traits, based on the protocol of Suso et al. [9], and Suso and Maalouf [36]. Phenological traits recorded were days to flowering from sowing (DFL), days to the last flower opened (DLFL), and duration of flowering (FDUR) defined as the time interval between days to flowering from sowing (DFL) and days to the last flower opened (DLFL). Visual traits recorded, referred to the number of open flowers per plant (NOF), the time that the flowers began to open (FLO) and close (FLC) within a daytime interval (h), flower color (FC), and raceme position (RACP). The hours that the flowers per plant remained open within a day were also calculated as the period from time that flowers opened till flowers closure (DFDUR = FLC-FLO). Record on visual traits performed on eleven observation days during flowering period.

Design floral traits were recorded regarding petal dimensions and included standard petal length (SPL) (cm), standard petal width (SPW) (cm), standard petal area (SPA) (cm^2), standard petal perimeter (SPP) (cm), standard petal angle (SPAN) (°), length of floral tube (FTUBL) (cm), keel length (KL) (cm), keel width (KW) (cm), keel perimeter (KP) (cm), keel opening (KOP) (cm), and keel angle (KAN) (°). The ratio of standard length to its width (SPL/SPW) and the ratio of standard length to keel length (SPL/KL) were also calculated. Five traits were also recorded, referring to flower's sexual dimension and male and vector matching, namely stamen (STL) and ovary length (OL) (cm), style length (SL) (cm), style-ovary angle (SOA) (°), and the number of ovaries (ON).

Measurements of phenological and visual traits were taken on ten central plants per plot. For floral design traits, measures were taken on two completely open flowers of each one of the ten central plants per plot. For flower design traits a scanner and UTHSCSA Image Tool program (http://www.uthsca.edu/dig/itdesc.html, accessed on 8 October 2017) were used. Additional observations on flower traits and flowering behavior of the accessions (i.e., non-opening of the flowers) were recorded.

2.4. Yield Related Traits

Regarding fresh pod production, twenty fresh pods per accession were collected and weighed. Fresh pods collected were on the appropriate harvesting stage [37]. Results are presented as weight of fresh pod (g). Seed weight per plant (g) was also recorded from ten central plants per plot. Seed weight per plant was then used for seed yield (kg ha^{-1}) calculation.

2.5. Pollinators Record and Investigation of Inter-Crossing

Pollinators observations took place only during days with favorable weather conditions for insects, i.e., not rainy, or with strong wind blowing. All observations on pollination carried out by the same researcher. Pollinators observations lasted for five minutes per plot and were recorded in the same ten plants that the floral traits were measured. Positive visits were considered the ones that pollinators were approaching the flower's physical opening and not from the side (Figure 2) when a flower was completely open according to the protocol described by Suso et al. [12]. The flowers that were visited positively by a pollinator species then changed their color and started to close, as they did not need to advertise themselves anymore. Pollinators abundance (number of positive pollinator species recorded per plant) and foraging activity (number of positive pollinators visits recorded per plant) were then determined. The seed color, eye color, flower color, and raceme position characterization was based on cowpea descriptors proposed by the International Board for Plant Genetic Resources [38].

(a) (b)

Figure 2. (**a**) Positive visit of a pollinator on cowpea flower; (**b**) Non-positive visit of a pollinator on cowpea flower.

2.6. Statistical Analysis

Floral traits, yield traits, and pollinators abundance and foraging activity subjected to One-way Analysis of Variance (One-way ANOVA). *Tukey's* mean comparison method ($\alpha = 0.05$) was used to make comparisons among accessions, using the statistical software Statgraphics Centurion XVII [39]. Regression Analysis was performed: (i) between pollinator abundance and foraging behavior, (ii) between pollinators abundance and foraging activity with each one of the flower traits recorded, and (iii) between pollinators abundance and foraging activity with seed weight per plant and fresh pod weight. Cross-tabulation Analysis was also used to investigate possible correlations among flower traits with abundance and foraging activity of pollinators.

3. Results

3.1. Flower Traits

During flower traits recording, some plants were observed to exhibit one flowering flush, while others two flowering flushes, extending their flowering duration. Plants that exhibited two flowering waves were observed in all accessions. At the same time, it was observed that breeding line IT97K-499-35 had many flowers that did not open to become amenable to cross-pollination. Self-fertilization became evident as the unopened flowers turned their colors from white/purple to yellow. Due to high temperatures prevailed during summer period, flower abortion was observed, therefore a lower pod and seed yield was obtained.

Significant statistical differences were observed among the cowpea accessions for the three phenological traits studied ($p \leq 0.05$) (Table 2). Landraces BGE038478 and BGE038479 presented a later beginning of flowering in comparison to the other accessions and needed 85.15 and 82.80 days from sowing to enter flowering stage, respectively. VG2 and Vg60 landraces, on the other hand, were the most early flowering accessions. BGE038478 and BGE038479 along with the breeding line IT97K-499-35 were also the latest matured accessions, with 103.80, 103.85, and 104.62 days from sowing to the last flower that remained open, respectively. Flowering period lasted from 15.20 days (VG2) to 29.68 days (IT97K-499-35) (Table 2).

Table 2. Phenological traits and coefficients of variation (CV%) for each cowpea accession and trait studied. Means ± SE in columns with different letters are statistically significantly different at the 0.05 level, by *Tukey's* HSD means comparison method.

Accessions	Days to Flowering from Sowing (DFL)	Days to the Last Flower Open (DLFL)	Duration of Flowering (FDUR)
IT97K-499-35	74.94 ± 2.29 b	104.62 ± 2.29 a	29.68 ± 1.62 a
CV%	17.81	12.74	31.91
VG2	55.64 ± 0.72 c	71.18 ± 0.92 c	15.20 ± 1.16 c
CV%	7.37	8.00	48.46
VG21	57.62 ± 1.09 c	82.53 ± 1.93 b	22.35 ± 1.86 b
CV%	11.49	14.77	52.54
Vg60	55.23 ± 1.39 c	79.00 ± 1.54 b	22.78 ± 2.16 ab
CV%	15.92	12.37	57.36
BGE038478	85.15 ± 1.34 a	103.80 ± 1.26 a	18.65 ± 0.73 bc
CV%	9.99	7.69	24.90
BGE038479	82.80 ± 1.95 a	103.85 ± 1.68 a	21.05 ± 1.29 bc
CV%	14.93	10.21	38.71
Level of significance	***	***	***

*** Statistically significant difference at a level 0.001.

Cowpea flowers opened only in the morning hours throughout flowering period. More specifically, the flowers remained open during 7 a.m. to 1 p.m. and then were fertilized and turned into yellow. Time of flower opening, and closure of the accessions were finely differentiated among days (Supplementary Material S2; S3) probably due to variability in air temperatures prevailed. VG2 was characterized by a slightly earlier opening of flowers in comparison to the rest of the accessions. Mean number of open flowers per plant did not differ statistically significantly in most observation days. The highest number of open flowers for VG2, VG21, and Vg60 were presented on the 5th of August, while BGE038478 and BGE038479 were on the 29th of August (Supplementary Material S4).

Regarding petal floral design recorded traits, statistically significant differences were observed among cowpea accessions, except for keel opening (KOP) and keel angle (KAN) (Supplementary Material S5). Landrace BGE038478 presented the longest standard petal length (2.35 cm), standard petal width (1.22 cm), and floral tube length (1.27 cm), while VG2 presented the shortest standard petal length (2.11 cm) and the shortest floral tube length (1.10 cm). VG2 presented also one of the shortest standard petal widths (1.04 cm) and the smallest standard petal area (515.99 cm^2). Vg60 presented the largest standard petal area (603.31 cm^2) and standard petal angle (64.60°). VG21 presented the largest standard petal perimeter (6.90 cm) while BGE038479 the smallest one (6.51 cm). VG21 was the landrace that presented the largest keel length (2.12 cm), keel width (2.21 cm), keel perimeter (6.25 cm), and standard petal length to standard petal width ratio (2.18). On the other hand, BGE038478 presented the shortest keel length (2.04 cm), shortest keel width (1.16 cm), and the lowest standard petal length to standard petal width ratio (1.93) (Supplementary Material S5).

Accessions exhibited also statistically significant differences regarding sexual dimension and male and vector matching traits studied (Supplementary Material S6). VG21 presented the highest stamens length (0.819 cm), ovary length (2.029 cm), style length (0.790 cm), and the number of ovaries (11.44). BGE038478 had the largest style-ovary angle (114.31°), and IT97K-499-35 had the smallest one (104.59°) (Supplementary Material S6).

3.2. Yield Related Traits

Breeding line IT97K-499-35 was the only one that differed statistically significantly among the accessions regarding fresh pod weight (g) and seed weight per plant (g) (Table 3). Among landraces, VG2 and VG21 were the ones that produced the lowest fresh pod weight and seed pod weight per plant.

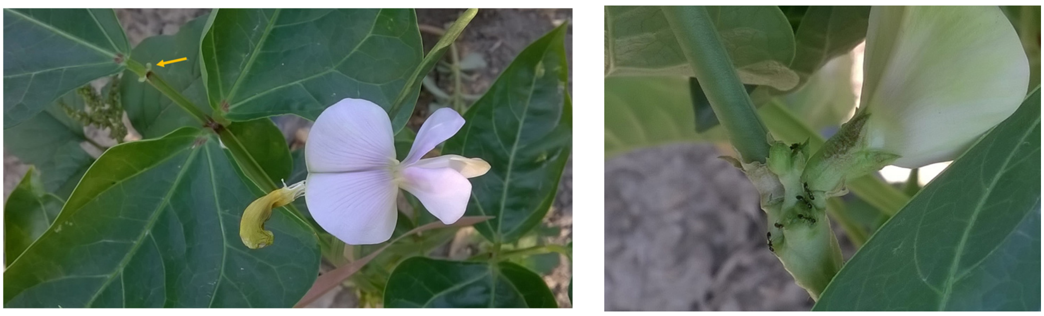
Table 3. Yield and yield related trait studied for each accession and coefficients of variation (CV%) for each accession and trait. Means ± SE in columns with different letters are significantly different at the 0.05 level, by *Tukey's* HSD means comparison method.

Accessions	Fresh Pod Weight (g)	Yield (kg ha^{-1})
IT97K-499-35	3.98 ± 0.16 a	887.43 ± 82.23 a
CV%	18.12	79.99
VG2	2.41 ± 0.13 b	598.76 ± 62.30 b
CV%	24.11	65.81
VG21	2.50 ± 0.19 b	560.47 ± 57.94 b
CV%	33.78	65.39
Vg60	2.19 ± 0.17 b	552.37 ± 62.98 b
CV%	34.72	72.11
BGE038478	1.96 ± 0.18 b	543.24 ± 45.29 b
CV%	40.83	52.74
BGE038479	2.24 ± 0.13 b	525.49 ± 53.01 b
CV%	25.28	63.79
Level of significance	***	***

*** Statistically significant difference at a level 0.001.

3.3. Pollinators Record

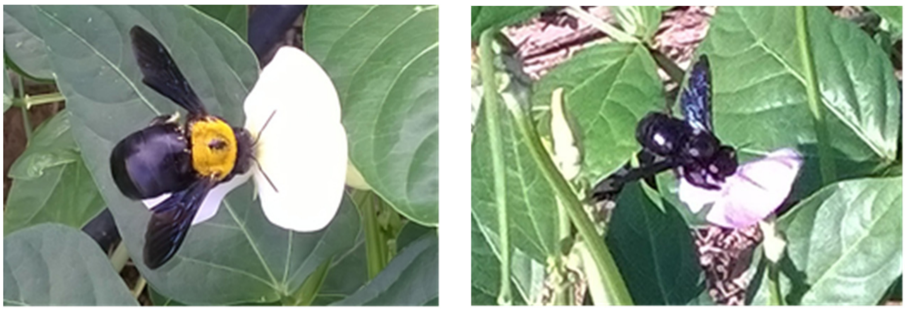
During cultivation season, insects that positively visited *Vigna* flowers and insects with dual behavior, hence either positively visited flowers or stole nectar, were observed. In the case of insects with dual behavior, their visits were characterized as positive or negative, respectively. Flowers from which nectar was stolen became evident from an opening formed in the flower side. However, there were also quite a few insects that exclusively visited the extrafloral nectaries located on the stipels of trifoliolate leaves and on the inflorescences' stalks, such as ants and wasps (*Polistes* sp., *Vespa* sp.) (Figure 3).



(**a**) (**b**)

Figure 3. (**a**) A pair of nectaries on the stipels of a trifoliate leaf; (**b**) Nectaries on a cowpea inflorescence stalk.

Two species of butterflies, *Carcharodus alceae* (Esper, 1780) and *Lampides boeticus* (Linnaeus, 1767) were also recorded visiting cowpea flowers either for nectar or foraging. However, they were not characterized as positive pollinators as they had no power to trigger cowpea flowers and their long proboscis was capable of serving their purpose when receiving the nectar without disturbing the flower. Positive visits were observed on cowpea flowers by species belonging to the Apidae family, named *Xylocopa pubescens* (Spinola, 1838), and *Xylocopa valga* (Gerstäcker, 1872) (Figure 4). These species assisted as pollinators and consumed nectar from the flowers.



(**a**) (**b**)

Figure 4. (**a**) *Xylocopa pubescens* foraging in cowpea flower of VG2; (**b**) *Xylocopa valga* foraging in cowpea flower of BGE038478.

Honeybees were also observed, whereas they rarely succeeded in positively visiting cowpea flowers, as they did not have the necessary strength. However, they were visiting flowers which had their stigma and anthers already exposed because they had already received positive visit from the species *Xylocopa pubescens* (Spinola) and *Xylocopa valga* (Gerstäcker) (Figure 5).

(**a**) (**b**)

Figure 5. (**a**) Flower with exposed anthers and stigma; (**b**) *Apis mellifera* bee foraging in a previously exposed flower.

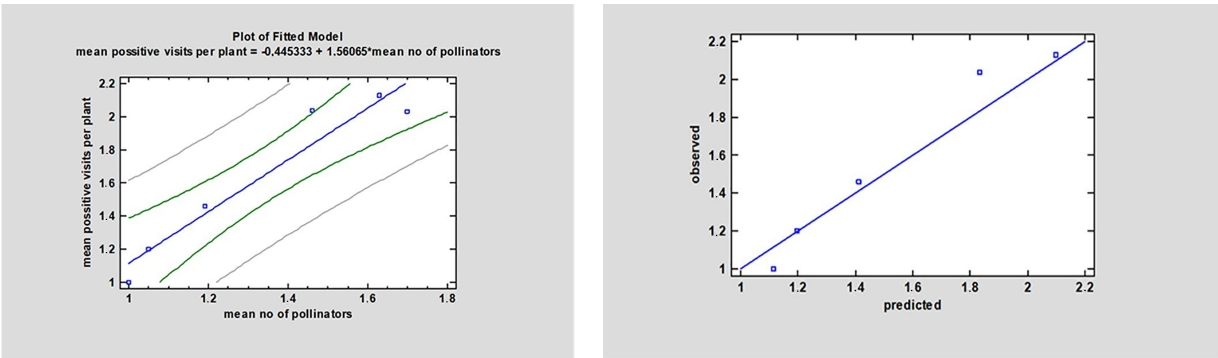
Honeybees were in that way considered as secondary positive pollinators. *A. mellifera* also appeared to be attracted to native flora species that flowered at the same time with cowpea, such as *Portulaca oleracea* L. and *Tribulus terrestris* L. Unlike honeybee, the larger pollinator species, that were recorded to visit cowpea flowers, were not observed visiting any other flora species in the field. Visits by species *X. pubescens, X. valga* and *A. mellifera* were therefore counted as positive visits. As positive visits by honeybee, along with normal visits, the secondary visits that they made on already exposed stamens and stigmas from other insects were included, because in this way there was also possibility of transferring pollen from one flower to another.

Mean number of pollinator species per plant and mean number of positive visits per plant differed statistically significantly among the accessions (Table 4). VG21 had the highest mean number of pollinators species per plant (1.70 pollinators species per plant) and VG2 the highest mean number of positive visits per plant (2.13 positive visits per plant). Pollinators abundance was also positively related to the foraging activity of pollinators (p = 0.002, R^2 = 92.34) (Figure 6).

Table 4. Pollinators abundance, foraging activity and coefficients of variation (CV%) of each accession and trait. Means ± SE in columns with different letters are statistically significantly different at the 0.05 level, by *Tukey's* HSD means comparison method.

Accessions	Pollinators Abundance	Pollinators Foraging Activity
IT97K-499-35	1.00 ± 0.01 c	1.00 ± 0.01 b
CV%	10.00	10.00
VG2	1.63 ± 0.17 ab	2.13 ± 0.29 a
CV%	50.72	67.00
VG21	1.70 ± 0.10 a	2.03 ± 0.20 ab
CV%	34.50	55.73
Vg60	1.19 ± 0.08 bc	1.46 ± 0.14 ab
CV%	33.71	48.31
BGE038478	1.05 ± 0.05 c	1.20 ± 0.09 b
CV%	21.30	34.20
BGE038479	1.46 ± 0.12 abc	2.04 ± 0.23 ab
CV%	40.34	54.96
Level of significance	***	**

** Statistically significant difference at a level 0.01; *** Statistically significant difference at a level 0.001.



(**a**) (**b**)

Figure 6. (**a**) Regression model of foraging behavior and pollinator abundance; (**b**) Predicted and observed values for the regression of foraging behavior on pollinator abundance.

3.4. Floral Traits Relation with Pollinators Abundance and Foraging Activity

Regression analysis did not reveal relation between pollinators abundance and foraging activity with the design floral traits studied, and therefore indicated no predictability of pollinator abundance and foraging behavior by them. Among phenological traits, only non-linear relations of pollinators abundance (Pollinators abundance = 1/(−0.2594 + 0.0008 × DUR^2, p = 0.025, R^2 = 75.43) and foraging activity (Pollinators foraging activity = 1/(−0.2693 + 0.0008 × FDUR^2, p = 0.024, R^2 = 75.64) were recorded with the duration of flowering (FDUR).

Cross-tabulation Analysis performed showed a significant relation of pollinators foraging activity (p = 0.002, Lambda = 0.000) with flower color, but not with their abundance (p = 0.541, Lambda = 0.000). Among different flower colors, increased preference recorded for the violet ones, expressed by accessions VG21 and BGE038478. A statistically significant relation was also observed between raceme position with foraging activity (p = 0.019, Lambda = 0.029) but not with pollinators abundance (p = 0.721, Lambda = 0.000). More visits were recorded on accessions exhibited racemes above the canopy, namely VG21 and Vg60. The duration that the flowers remained open during the day (DFDUR) was positively related to pollinators abundance (p = 0.044, R^2 = 67.98) and foraging activity (p = 0.048, R^2 = 66.56) on 5th of August. On 5th of September there was also a positive relation observed between the hours that the flowers per plant remained open during a day (DFDUR) with pollinators abundance (p = 0.013, R^2 = 82.34), and with their foraging activity (p = 0.019, R^2 = 78.31).

Regression Analysis also revealed a linear correlation, on the 5^{th} of August, between the mean number of open flowers and the abundance of pollinators (p = 0.049, R^2 = 65.87), but also a non-linear correlation with the foraging behavior of pollinators (p = 0.023, R^2 = 76.09). There was not a significant relation, either between the time interval that the flowers remained open within a day with pollinators abundance and foraging activity, neither to the mean number of open flowers in any other observation day.

3.5. Pollinators Abundance and Foraging Activity Relation to Yield

Regression Analysis did not show a linear correlation of pollinator abundance (p = 0.526, R^2 = 10.76) and foraging behavior (p = 0.366, R^2 = 20.63) with fresh pod yield, neither with seed yield (p = 0.322, R^2 = 24.13) and (p = 0.279, R^2 = 28.13), for pollinator abundance and foraging behavior, respectively.

4. Discussion

Legumes include a large number of predominantly self-pollinated species, which, maintain the possibility of cross-pollination as a mechanism for sustaining their diversity [40]. Cowpea is a typical example of such a plant species because while it is a mainly self-pollinated species, it also maintains certain floral traits, like extrafloral nectaries which attract insect pollinators [41].

4.1. Plant Traits

Plant material used in our experiment presented statistically significant differences in terms of phenological, visual and most flower design characteristics studied to investigate their effect on attracting insect-pollinators. Therefore, the diverse genetic material used in our study was considered as suitable for flower traits identification that could be used in breeding for enhanced pollinators visitations.

Cowpea flowers opened during the morning hours (7:00 a.m.) and remained open until noon (1:30 p.m.), being in that way, throughout this time available for possible pollination by insects. Corresponding times for opening (6 a.m.) and closing (12 p.m.) of cowpea flowers are reported by Ige et al. [16]. Among the accessions, VG2 presented a slightly earlier flower opening in the morning, which allowed its flowers to be visited by insects earlier than the other accessions, and resulted in a more extended flower opening during the day. For some plants, from all accessions, two flowering flushes were observed that also lengthened insects' visitation period. Two flushes were also reported for cowpea plants by Hall [42] with a one-week period time interval.

Compared to the other accessions of our study, BGE038478 was characterized by larger standard petal dimensions (length, width, and area) that could be possibly able to attract insect pollinators more easily. In comparison to the rest of the accessions VG21 presented greater keel dimensions, such as keel length and width that could allow largest insects landing. Floral display size has been found to modify pollinators visitation, plant-pollinator patterns [31,43] and even flower size to covariate with pollinators size [44].

Regarding yield traits, high air temperatures and drought that prevail in the area during late spring and summer, a period during which cowpea plants flower and set pods, have hindered pollen viability and caused increased amount of flower and pod losses [45–47]. Breeding line IT97K-499-35 was the only one that resulted in statistically significant higher fresh pod weight and seed yield production compared to the other accessions. It had also many flowers that did not open to become amenable to cross-pollination. However, the flowers were self-pollinated forming pods and seeds. Breeding line IT97K-499-35 is a drought tolerant line [48], so it was bred to be productive under stress conditions. Plants are used to adjust their cross- and self-pollination levels to safeguard their production and survival [49]. Promotion of self-pollination of the breeding line possibly was the reason of the increased seed production observed.

4.2. Pollinators Observations

Several insect species appeared to be attracted by the extrafloral nectaries of cowpea, among those species are ants, but they were unable to expose the stamens and stigma of the flowers to allow cross-pollination. Honeybees were attracted by cowpea flowers, but only on rare occasions were able to trigger large cowpea flowers. They usually visited flowers that already had the stamens and stigma exposed after a larger insect had visited them. The inability of honeybees to cross-pollinate cowpea flowers has been previously reported by Wousla et al. [31] and Purseglove [41]. Fohouo et al. [34] also reported that the visits of the bee *A. mellifera adansonii* (Latreille, 1804) to cowpea flowers were only positive in a 4.70%. This observation contradicts the reports of Ige et al. [16], Asiwe [17] and Musa et al. [30] where the honeybee is encountered as a positive insect-pollinator of cowpea and even considered as the main pollinator [33]. Different honeybee subspecies abundant in each region may contribute to this differentiation as they differ morphologically [50]. Honeybees observed to visit other species that were abundant in the corridors that the plots formed where the weed growth was allowed. The foraging behavior therefore depended also on the availability of other plant species present. Foraging of honeybees has been previously reported to range among different plant species [51] and to prefer pollen sources with increased protein content [52].

Main positive cowpea visitors in our experimental area, were species of the genus *Xylocopa*. Species of the genus *Xylocopa* along with species of the genus *Megachile* (Latreille, 1802), are worldwide among the most effective pollinators of cowpea [17,22,28,31,53] and of legumes that exhibit large flowers [54,55]. Species of these genera have the size and therefore the ability to trigger cowpea flowers as reported by Wousla et al. [31]. Cultivation of cowpea in the present experimental area therefore appears to contribute to the provision of food to these non-domestic pollinator species, and thereby encourage the maintenance and growth of their population.

Given that the main pollinator visitors observed were wild bees, the preservation and enhancement of their natural habitats and nesting sites near cowpea cultivated areas are considered essential for their conservation [29], but also useful for promoting environmental stability [3]. Maintaining natural habitats near cultivated areas helps to enrich the number of pollinator-insect species [4]. Establishing honeybees near the crop would probably not help in the present environment, unlike in Nigeria where common bee is considered an efficient pollinator for cowpea [30].

The presence and effectiveness of each pollinator species, including the possibility of contributing to cross-pollination and its rate, are not equivalent measures as cross-pollination is also affected by environmental factors [12]. Pollinator species recorded have been found to be different for legume crops even in different countries of Europe. Plant-pollinator interactions and inter-crossing levels vary among genetic material used and the experimental location, as different pollinator species and native flora are present. Several different pollinator species were recorded to visit Andean lupin flowers between two locations, one in Athens, Greece where *Anthophora* and *Megachile* bees were recorded and one in Cordoba, Spain where *Andrena* and *Anthophora* genera were recorded among visitors [56]. Pollinator species of faba beans recorded in Spain and France, and their foraging behavior were also differentiated [57].

4.3. Floral Traits Relation with Pollinators Abundance and Foraging Activity

Among the traits studied, flower color, raceme position, and flowering duration (h) during a daytime period were related to pollinators abundance, while flower design features and number of open flowers per plant were not able to predict the foraging behavior of the recorded pollinator species, despite the differences observed among the accessions. Thus, cowpea flowers design traits did not perform such a significant role in attracting pollinators in comparison to faba bean [9], and therefore, they do not constitute a primary breeding target aiming to encourage insect visitation and pollination.

Violet flowers attracted greater number of all pollinator species recorded and received more positive visits compared to the other flower color categories. Nevertheless, visits from all insect-pollinator species recorded also in accessions with white or white with purple pigmentation flowers (Figure 7). Visits varied among the observation dates and sometimes visits on white flowers were in correspondence with the number of visits recorded in violet flowers.

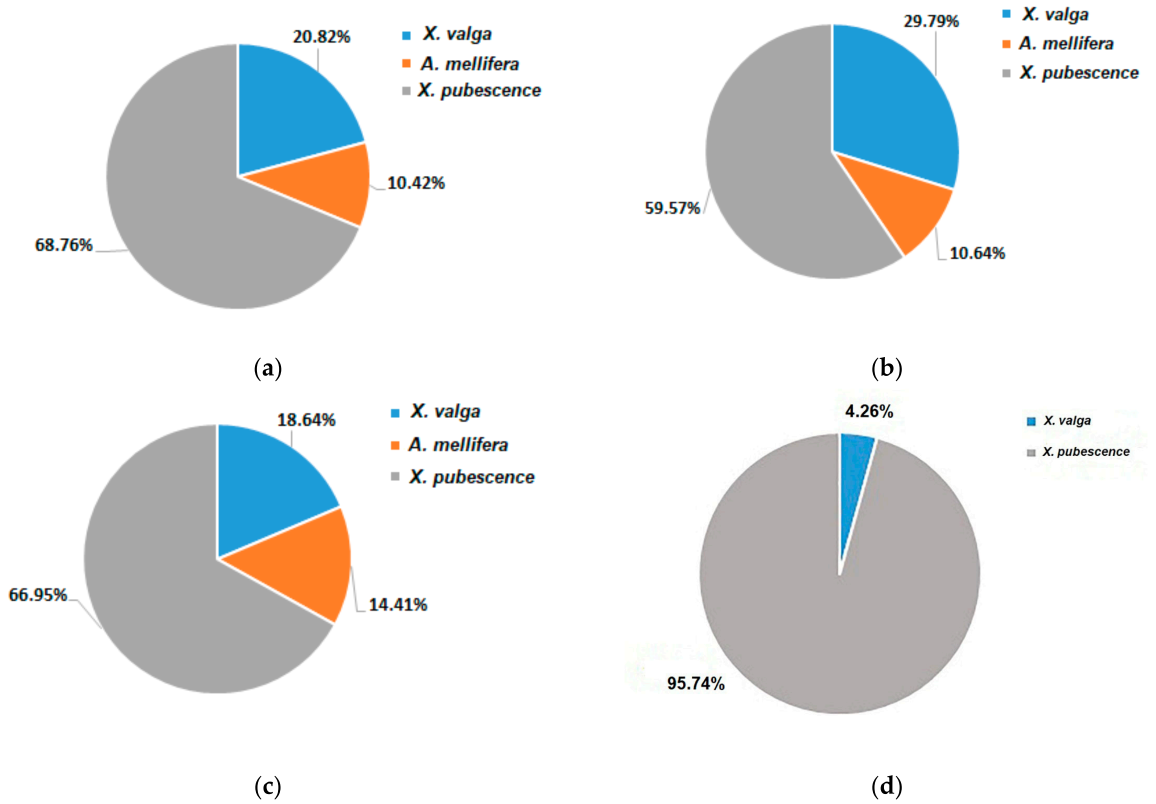


Figure 7. Number of positive visits per pollinator species on: (**a**) white-colored flowers; (**b**) white/purple-colored flowers; (**c**) purple-colored flowers; and (**d**) light purple-colored flowers.

This fact shows that color may influence pollinator visitation, but it is not the primary factor of enhancing their foraging [28]. Similarly, while most positive visits were recorded on accessions which exhibited their racemes above the canopy, visitation was not only increased to these accessions (Figure 8). This observation suggests that raceme position performs a role in pollinator attraction and visitation as in other plant species [58], but likely interacts with other factors and is not sufficient to predict accurately cowpea visitation preferences.

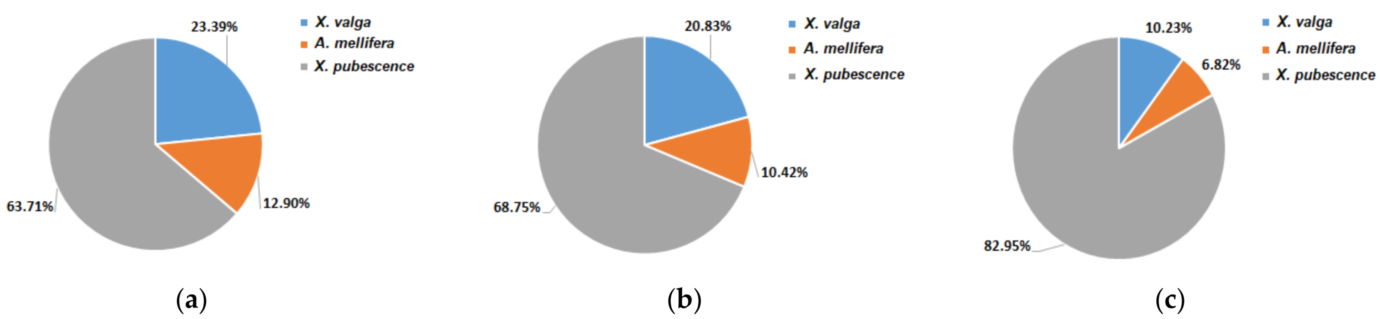


Figure 8. Number of positive visits on plants with racemes: (**a**) mostly above the canopy; (**b**) on the upper canopy surface; and (**c**) throughout canopy.

Significant correlation of foraging behavior with the hours that the flowers remained open during the day (h) (DFDUR) were recorded only on two observation days, and that was not of a linear relation. Flowering beginning and closure time during a day varied per accession and observation date in other species as well, possibly due to environmental factors, such as temperature [59]. In general, climatic conditions appear to influence pollinator visitation rates [60,61]. Relying therefore solely on this trait, aiming to enhance pollinators visits, would be risky and likely ineffective. Other traits, such as nectar quality and quantity, may influence increased visitation and pollinators attraction in cowpea [28] and not flower traits exhibited. In aiming to enhance pollinators visitation rates, cowpea breeders should investigate other traits besides floral ones.

However, pollinators could promote either or both cross-pollination and self-pollination of cowpea [29]. Therefore, the number of insect pollinators recorded is inconsistent with cross-pollination rate between flowering cowpea accessions. Insect size and the distances used between different genotypes appear to perform a key role in cross-pollination ability of cowpea [31,62]. Out-crossing rates have also been found to vary according to the planting pattern used. In the study of Asiwe [17], higher out-crossing rates were observed when cowpea types with different flower color were planted in alternate rows than in concentric cycles, reaching up to 0.85%. Out-crossing of this study [17] was also studied through the assessment of F_2 seeds. Sowing separated plots of each accession used in our experiment might have not enhanced pollinators visitation and activity. Therefore, other planting patterns should be tested. Genetic material used is also an important factor that affects cross-pollination rates. Cross-pollination seems to be encouraged in countries around the African cowpea center of origin, where wild types exist, and their flower morphology favors cross-pollination [18].

During seed harvesting, there was no change of seed coat color and eye color observed in comparison to the beginning seed samples, since the seed coat color and eye color are maternal traits [63–65], while epistatic effects are also reported [66]. Ajayi et al. [66], while assessing crossability of cowpea genotypes, found that maternal effects did not influence the seed coat color of F_1 generation, and were therefore able to define if the genotypes were inter-crossed in an early generation. Nwofia [67], while crossing black and brown seeded genotypes with a white seeded genotype in the F_1 seed generation, found that all seeds obtained were of black or brown color regardless with which genotype was used as maternal material, indicating dominance of the black and brown seed coat color to the white seed coat color. According to Herniter et al. [68], a possible cross of a genotype with white color and black eye (e.g., IT97K-499-35) with a genotype with brown red seed color (e.g., BGE038479) would give a F_1 generation with black colored seeds, while a cross of a genotype with white color and black eye (e.g., IT97K-499-35) with a cream color and brown eye genotype (e.g., Vg60) would give seeds with gray mottled coat and black eye. Moreover, a crossing of a cream seed colored with brown eye genotype (e.g., Vg60) with a brown-seeded genotype (e.g., BGE038479) would give a F_1 generation with light brownish-beige colored seeds.

In the present study, that the plants were subjected to open pollination, different seed morphotypes were included, such as brown seeded and white seed-colored accessions with black eye. The seeds produced could be further used to indicate a successful or not successful inter-crossing among plant material used. As maternal effects prevail, seeds should be sown and F_1 generation should be assessed regarding their seed traits so that we could have indisputable results regarding inter-crossing possibility by the pollinator species foraging on cowpea flowers.

Genetic diversity recorded in cowpea in the European area is lower than that in African countries [69,70]. Limited diversity observed for the species in European countries compared to Africa combined with the fact that there was no apparent inter-crossing in the present study suggests the possibility of lower inter-crossing rates around Mediterranean in comparison to cowpea center of origin. However, the method does not provide information regarding intra-crossing and therefore more research is needed.

4.4. Pollinators Abundance and Foraging Activity in Relation to Yield

In the present study, pollinators abundance and foraging behavior did not affect fresh pod and seed yield. Fohouo et al. [34] observed no correlation of honeybees' visitation with seed yield, whereas there was a positive correlation with yield-related traits, such as pod length and number of seeds per pod. Dingha et al. [28] also did not observe correlation of pollinators visitation with fresh pod yield (fresh pod weight), while Vaz et al. [29] observed increased fruit set, but not an increase in the number of seeds per pod following an increased pollinator visitation rate. Musa et al. [30] also reported a positive effect of honeybees on the number of pods per plant and the number of well-developed seeds per plant. This differentiation is probably due to the types of pollinators recorded in each case and their match to cowpea flowers. Characteristically, Wousla et al. [31] reported that insects of the family Megachilidae are more capable of increasing fruit set in cowpea compared to insects of the genus *Xylocopa* as they transfer pollen in a different way. Therefore, bee visitation may not be able to increase cowpea yield, but helps to ensure its production and increase its quality characteristics as it happens with other plant species [71,72]. Other traits, like number of seeds per pod and the number of empty pods or poorly filled pods should also be addressed in relation to the abundance and the foraging behavior of insect pollinators recorded in this study, as pollinators presence have been found to result in a greater seed set per pod [30].

5. Conclusions

Through this study an attempt was made to understand the patterns of plant-pollinator interactions in cowpea, aiming to enhance farming ecosystems resilience via breeding. To our knowledge, insect-pollinators of cowpea in a Mediterranean country, were recorded for the first time. The main pollinators belonged to the genus *Xylocopa*, as reported for cowpea worldwide. The floral traits that attracted them were flower color, raceme position, and duration of flowering, but they were not directly and linearly related to their abundance and foraging activity. This fact does not render these traits solely suitable traits for breeding to increase pollinators visitation. It is likely that other reward traits, such as pollen and nectar, perform an important role in attracting pollinators of cowpea that should be further investigated. Finally, pollinators presence did not affect fresh pod and seed yield of accessions; however, cowpea cultivation found to provide these wild pollinator species remarkable food resources.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ecologies4010010/s1, Supplementary Material S1. Mean, max, and min monthly air temperature (°C), monthly total precipitation (mm), and average monthly wind speed (km h^{-1}) during cultivation (May-October 2017); Supplementary Material S2. Time of flowers opening per accession for each observation date; Supplementary Material S3. Time of flowers closure per accession for each observation date; Supplementary Material S4. Mean number of open flowers per plant for each accession and observation date. Means ± SE in columns with different letters are significantly different at the 0.05 level, by *Tukey's* HSD means comparison method; Supplementary Material S5. Design flower petal traits studied for each accession and coefficients of variation (CV%) for each accession and trait. Means ± SE in columns with different letters are significantly different at the 0.05 level, by *Tukey's* HSD means comparison method; Supplementary Material S6. Design flower's sexual dimension and male and vector matching traits studied for each accession and coefficients of variation (CV%) for each accession and trait. Means ± SE in columns with different letters are significantly different at the 0.05 level, by *Tukey's* HSD means comparison method. All the necessary Supplementary Material (S1, S2, S3, S4, S5, and S6) mentioned in the text of the manuscript is attached separately with the manuscript.

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References

1. Khalifa, S.A.M.; Elshafiey, E.H.; Shetaia, A.A.; El-Wahed, A.A.A.; Algethami, A.F.; Musharraf, S.G.; AlAjmi, M.F.; Zhao, C.; Masry, S.H.D.; Abdel-Daim, M.M.; et al. Overview of Bee Pollination and Its Economic Value for Crop Production. *Insects* **2021**, *12*, 688. [CrossRef] [PubMed]
2. Christmann, S.; Bencharki, Y.; Anougmar, S.; Rasmont, P.; Smaili, M.C.; Tsivelikas, A.; Aw-Hassan, A. Farming with Alternative Pollinators benefits pollinators, natural enemies, and yields, and offers transformative change to agriculture. *Sci. Rep.* **2021**, *11*, 18206. [CrossRef] [PubMed]
3. Potts, S.; Imperatriz-Fonseca, V.; Ngo, H.T.; Aizen, M.A.; Biesmeijer, J.C.; Breeze, T.D.; Dicks, L.V.; Garibaldi, L.A.; Hill, R.; Settele, J.; et al. Safeguarding pollinators and their values to human well-being. *Nature* **2016**, *540*, 220–229. [CrossRef] [PubMed]
4. Klein, A.-M.; Vaissière, B.E.; Cane, J.H.; Steffan-Dewenter, I.; Cunningham, S.A.; Kremen, C.; Tscharntke, T. Importance of pollinators in changing landscapes for world crops. *Proc. R. Soc. B.* **2007**, *274*, 303–313. [CrossRef]
5. Gemmill-Herren, B.; Garibaldi, L.A.; Kremen, C.; Ngo, H.T. Building effective policies to conserve pollinators: Translating knowledge into policy. *Curr. Opin. Insect Sci.* **2021**, *46*, 64–71. [CrossRef]
6. Majewska, A.A.; Altizer, S. Planting gardens to support insect pollinators. *Conserv. Biol.* **2018**, *34*, 15–25. [CrossRef] [PubMed]
7. Potts, S.G.; Roberts, S.P.M.; Dean, R.; Marris, G.; Brown, M.A.; Jones, R.; Neumann, P.; Settele, J. Declines of managed honeybees and beekeepers in Europe. *J. Apic. Res.* **2010**, *49*, 15–22. [CrossRef]
8. Palmer, R.G.; Perez, P.T.; Ortiz-Perez, E.; Maalouf, F.; Suso, M.J. The role of crop-pollinator relationships in breeding for pollinator-friendly legumes: From a breeding perspective. *Euphytica* **2009**, *170*, 35–52. [CrossRef]
9. Suso, M.J.; Harder, L.; Moreno, M.T.; Maalouf, F. New strategies for increasing heterozygosity in crops: *Vicia faba* mating system as a study case. *Eyphytica* **2005**, *143*, 51–65. [CrossRef]
10. Suso, M.J.; Bebeli, P.J.; Christmann, S.; Mateus, C.; Negri, V.; Pinheiro de Carvalho, M.A.A.; Torricelli, R.; Veloso, M.M. Enhancing Legume Ecosystem Services through an Understanding of Plant–Pollinator Interplay. *Front. Plant Sci.* **2016**, *7*, 333. [CrossRef]
11. Descamps, C.; Quinet, M.; Jacquemart, A.-L. The effects of drought on plant–pollinator interactions: What to expect? *Env. Exp. Bot.* **2021**, *182*, 104297. [CrossRef]
12. Suso, M.J.; Pierre, J.; Moreno, M.; Esnault, R.; Le Guen, J. Variation in outcrossing levels in faba bean cultivars: Role of ecological factors. *J. Agric. Sci.* **2001**, *136*, 399–405. [CrossRef]
13. Arista, M.; Talavera, M.; Berjano, R.; Ortiz, P.L. Abiotic factors may explain the geographical distribution of flower colour morphs and the maintenance of colour polymorphism in the scarlet pimpernel. *J. Ecol.* **2013**, *101*, 1613–1622. [CrossRef]
14. Jiménez-López, F.J.; Ortiz, P.L.; Talavera, M.; Arista, M. Reproductive Assurance Maintains Red-Flowered Plants of *Lysimachia arvensis* in Mediterranean Populations Despite Inbreeding Depression. *Front. Plant Sci.* **2020**, *11*, 563110. [CrossRef] [PubMed]
15. Gigord, L.D.B.; Macnair, M.R.; Smithson, A. Negative frequency-dependent selection maintains a dramatic flower color polymorphism in the rewardless orchid *Dactylorhiza sambucina* (L.) Soò. *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 6253–6255. [CrossRef]
16. Ige, O.E.; Olotuah, O.F.; Akerele, V. Floral Biology and Pollination Ecology of Cowpea (*Vigna Unguiculata* L. Walp). *Mod. Appl. Sci.* **2011**, *5*, 74–82. [CrossRef]
17. Asiwe, J.A.N. Insect mediated outcrossing and geneflow in cowpea (*Vigna unguiculata* (L.) Walp): Implication for seed production and provision of containment structures for genetically transformed cowpea. *Afr. J. Biotech.* **2009**, *8*, 226–230.
18. Safety Assessment of Transgenic Organisms in the Environment: OECD Consensus Documents Vol. 6, Chapter 5, 2016. Available online: www.oecd-ilibrary.org/docserver/9789264253421-8-en.pdf?expires=1568975050&id=id&accname=guest&checksum=D1AF6F1763B8F7F34E1EA7F71F5038EC (accessed on 29 November 2022).
19. Fatokun, C.A.; Ng, Q. Outcrossing in cowpea. *J. Food Agric. Environ.* **2007**, *5*, 334–338.
20. Lush, W.M. Floral morphology of wild and cultivated cowpeas. *Econ. Bot.* **1979**, *33*, 442–447. [CrossRef]
21. deMooy, B.E.; deMooy, C.J.; Burke, D.W. Estimation of Percentage Natural Outcrossing in Cowpea (*Vigna unguiculata* (L.) Walp. in Botswana. USDA Publications, Reports of Bean Improvement Cooperative and National Dry Bean Council Research Conference, 1990; p. 122. Available online: https://naldc.nal.usda.gov/download/IND92013799/PDF (accessed on 25 November 2022).

22. Kouam, E.B.; Pasquet, R.S.; Campagne, P.; Tignegre, J.-B.; Thoen, K.; Gaudin, R.; Quedraogo, J.T.; Salifu, A.B.; Muluvi, G.M.; Gepts, P. Genetic structure and mating system of wild cowpea populations in West Africa. *BMC Plant Biol.* **2012**, *12*, 113. [CrossRef]
23. Rachie, K.O.; Silvestre, P. Grain legumes. In *Food Crops of the Lowland Tropics*; Lealey, C.L.A., Wills, J.B., Eds.; Oxford Univ. Press: Oxford, UK, 1977; pp. 41–74.
24. Liu, Y.; Li, J.; Zhu, Y.; Jones, A.; Rose, R.J.; Song, Y. Heat Stress in Legume Seed Setting: Effects, Causes, and Future Prospects. *Front. Plant Sci.* **2019**, *10*, 938. [CrossRef] [PubMed]
25. Phillips, B.B.; Shaw, R.F.; Holland, M.J.; Fry, E.L.; Bardgett, R.D.; Bullock, J.M.; Osborne, J.L. Drought reduces floral resources for pollinators. *Glob. Change Biol.* **2018**, *24*, 3226–3235. [CrossRef] [PubMed]
26. Pasquet, R.S.; Peltier, A.; Hufford, M.B.; Oudin, E.; Saulnier, J.; Paul, L.; Knudsen, J.T.; Herren, H.R.; Gepts, P. Long-distance pollen flow assessment through evaluation of pollinator foraging range suggests transgene escape distances. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 13456–13461. [CrossRef] [PubMed]
27. Woodcock, T.S. *Pollination in the Agricultural Landscape: Best Management Practices for Crop Pollination*; University of Guelph: Guelph, ON, USA, 2012.
28. Dingha, B.N.; Jackai, L.E.; Amoah, B.A.; Akotsen-Mensah, C. Pollinators on Cowpea *Vigna unguiculata*: Implications for Intercropping to Enhance Biodiversity. *Insects* **2021**, *12*, 54. [CrossRef] [PubMed]
29. Vaz, C.G.; de Oliveira, D.; Ohashi, O.S. Pollinator contribution to the production of cowpea in the Amazon. *HortScience* **1998**, *33*, 1157–1159. [CrossRef]
30. Musa, A.K.; Liadi, M.T.; Adegbite, O.R. Impact of honeybees (*Apis mellifera adansonii*) (Hymenoptera: Apidae) pollination on pod and seed set of cowpea (*Vigna unguiculata* L. Walp) in Ilorin, Southern Guinea Savanna of Nigeria. *WJAR* **2013**, *1*, 83–87.
31. Wousla, E.N.; Andargie, M.; Pasquet, R.S.; Mondon, M.; Menez, V.; Cochin, C.; Paul, L.; Pardon, L.; Roubaud, M. Is bigger better? *Apidae (Xylocopinae)*, *megachilidae* and cowpea (*Vigna unguiculata*) pollination. *Plant Breed.* **2019**, *139*, 156–166. [CrossRef]
32. Stefanie, K.B.; Albert, N.; Fernand-Nestor, T.F. Pollination and yield attributes of (cowpea) *Vigna unguiculata* L. Walp. (Fabaceae) as influenced by the foraging activity of *Xylocopa olivacea* Fabricius (Hymenoptera: Apidae) and inoculation with *Rhizobium* in Ngaoundere, Cameroon. *IJAAR* **2015**, *6*, 62–76.
33. Susan, D.; Murungi, L.K.; Kioko, E. Diversity and abundance of insect pollinators and their effect on yield and quality of cowpea and cucumber in Makueni, Kenya. *Afr. J. Hort. Sci.* **2019**, *16*, 41–52.
34. Fohouo, F.-N.T.; Ngakou, A.; Kengni, S. Pollination and yield responses of cowpea (*Vigna unguiculata* L. Walp.) to the foraging activity of *Apis mellifera adansonii* (Hymenoptera: Apidae) at Ngaoundéré (Cameroon). *Afr. J. Biotech.* **2009**, *8*, 1988–1996.
35. Adamidis, G.C.; Cartar, R.V.; Melathopoulos, A.P.; Pernal, S.F.; Hoover, S.E. Pollinators enhance crop yield and shorten the growing season by modulating plant functional characteristics: A comparison of 23 canola varieties. *Sci Rep.* **2019**, *9*, 14208. [CrossRef]
36. Suso, M.J.; Maalouf, F. Direct and correlated responses to upward and downward selection for outcrossing in *Vicia faba*. *Field Crop Res.* **2010**, *116*, 116–126. [CrossRef]
37. Karapanos, I.; Papandreou, A.; Skouloudi, M.; Makrogianni, D.; Fernández, J.A.; Rosa, E.; Ntatsi, G.; Bebeli, P.J.; Savvas, D. Cowpea fresh pods—A new legume for the market: Assessment of their quality and dietary characteristics of 37 cowpea accessions grown in southern Europe. *J. Sci. Food Agric.* **2017**, *97*, 4343–4352. [CrossRef] [PubMed]
38. *IBPGR Descriptors for Cowpea*; IBPGR Secretariat: Rome, Italy, 1983.
39. *Statgraphics Centurion XVII Version 17.2.0.0. 1982–2016*; StatPoint, Inc.: Herndon, VA, USA, 2016.
40. Papa, R.; Gepts, P. Gene Flow Between Crops and Their Wild Progenitors. In *Encyclopedia of Plant and Crop Science*, 1st ed.; Goodman, R.M., Ed.; CRC Press, Taylor & Francis Group LLC: New York, U.S.A, 2004; pp. 488–791.
41. Purseglove, J.W. *Tropical Crops*; Dicotyledons: London, UK, 1968.
42. Hall, A.E. Phenotyping cowpeas for adaptation to drought. *Front Physiol.* **2012**, *3*, 155. [CrossRef] [PubMed]
43. Grindeland, J.M.; Sletvold, N.; Ims, R.A. Effects of floral display size and plant density on pollinator visitation rate in a natural population of *Digitalis purpurea*. *Funct. Ecol.* **2005**, *19*, 383–390. [CrossRef]
44. Toji, T.; Ishimoto, N.; Egawa, S.; Nakase, Y.; Hattori, M.; Itino, T. Intraspecific convergence of floral size correlates with pollinator size on different mountains: A case study of a bumblebee-pollinated *Lamium* (Lamiaceae) flowers in Japan. *BMC Ecol. Evo.* **2021**, *21*, 64. [CrossRef] [PubMed]
45. Lazaridi, E.; Ntatsi, G.; Fernández, J.A.; Karapanos, I.; Carnide, V.; Savvas, D.; Bebeli, P.J. Phenotypic diversity and evaluation of fresh pods of cowpea landraces from Southern Europe. *J. Sci. Food Agric.* **2017**, *97*, 4326–4333. [CrossRef]
46. Warrag, M.O.A.; Hall, A.E. Reproductive responses of cowpea (*Vigna unguiculata* (L.) Walp) to heat stress. II. Responses to night air temperature. *Field Crops Res.* **1984**, *8*, 17–33. [CrossRef]
47. Farooq, M.; Gogoi, N.; Barthakur, S.; Baroowa, B.; Bharadwaj, N.; Algahamdi, S.S.; Siddique, K.H.M. Drought Stress in Grain Legumes during Reproduction and Grain Filling. *J. Agro. Crop Sci.* **2017**, *203*, 81–102. [CrossRef]
48. Olaji, A.A.; Ilori, C.O. Effects of Drought on Morphological Traits in Some Cowpea Genotypes by Evaluating Their Combining Abilities. *Adv. Agric.* **2017**, *2017*, 7265726. [CrossRef]
49. Van Ginkel, M.; Flipphi, R.C.H. Why Self-fertilizing Plants Still Exist in Wild Populations: Diversity Assurance through Stress-Induced Male Sterility May Promote Selective Outcrossing and Recombination. *Agronomy* **2020**, *10*, 349. [CrossRef]
50. Kandemir, İ.; Özkan, A.; Fuchs, S. Reevaluation of honeybee (*Apis mellifera*) microtaxonomy: A geometric morphometric approach. *Apidologie* **2001**, *42*, 618. [CrossRef]

51. Bänsch, S.; Tscharntke, T.; Ratnieks, F.L.W.; Härtel, S.; Westphal, C. Foraging of honey bees in agricultural landscapes with changing patterns of flower resources. *Agric. Ecosyst. Environ.* **2020**, *291*, 106792. [CrossRef]
52. Ghosh, S.; Jeon, H.; Jung, C. Foraging behaviour and preference of pollen sources by honey bee (*Apis mellifera*) relative to protein contents. *J. Ecol. Environ.* **2020**, *44*, 4. [CrossRef]
53. Trianto, M.; Marisa, F. Diversity of Bees and Wasp (Hymenoptera) in Cowpea (*Vigna sinensis* L.) in Agricultural Area at Martapura District, Banjar Regency, South Kalimantan. *J. Sci. Technol.* **2020**, *9*, 29–33. [CrossRef]
54. Westerkamp, C. The co-operation between the asymmetric flower of *Lathyrus latifolius* (Fabaceae-Vicieae) and its visitors. *Phyton* **1993**, *33*, 121–137.
55. Watmough, R. The significance of alfalfa, *Medicago sativa* L. (Fabaceae), as a foodplant for *Xylocopa caffra* (Linnaeus) and *Megachile gratiosa* Cameron (Hymenoptera: Anthophoridae and Megachilidae) near Oudtshoorn, South Africa. *Afr. Entomol.* **1999**, *7*, 307–311.
56. Bebeli, P.J.; Lazaridi, E.; Chatzigeorgiou, T.; Suso, M.-J.; Hein, W.; Alexopoulos, A.A.; Canha, G.; van Haren, R.J.F.; Jóhannsson, M.H.; Mateos, C.; et al. State and Progress of Andean Lupin Cultivation in Europe: A Review. *Agronomy* **2020**, *10*, 1038. [CrossRef]
57. Pierre, J.; Suso, M.-J.; Moreno, M.T.; Esnault, R.; Le Guen, J. Diversité et efficacité de l' entomofaune pollinisatrice (Hymenoptera: Apidae) de la féverole (*Vicia faba* L.) sur deux sites, en France et an Espagne. *Ann. Soc. Entomol. Fr.* **1999**, *35*, 312–318.
58. Klecka, J.; Hadrava, J.; Koloušková, P. Vertical stratification of plant–pollinator interactions in a temperate grassland. *Peer J.* **2018**, *6*, e4998. [CrossRef]
59. Ichimura, K.; Suto, K. Environmental Factors Controlling Flower Opening and Closing in a *Portulaca* Hybrid. *Ann. Bot.* **1998**, *82*, 67–70. [CrossRef]
60. McCall, C.; Primack, R.B. Influence of flower characteristics, weather, time of day, and season on insect visitation rates in three plant communities. *Am. J. Bot.* **1992**, *79*, 434–442. [CrossRef]
61. Gallagher, K.M.; Campbell, D.R. Shifts in water availability mediate plant-pollinator interactions. *New Phytol.* **2017**, *215*, 792–802. [CrossRef] [PubMed]
62. Solís-Montero, L.; Vallejo-Marín, M. Does the morphological fit between flowers and pollinators affect pollen deposition? An experimental test in a buzz-pollinated species with anther dimorphism. *Ecol. Evol.* **2017**, *7*, 2706–2715. [CrossRef] [PubMed]
63. Nzaramba, M.N.; Hale, A.L.; Scheuring, D.C.; Miller, J.C., Jr. Inheritance of antioxidant activity and its association with seedcoat color in Cowpea. *J. Amer. Soc. Sci.* **2005**, *130*, 386–391. [CrossRef]
64. Xu, P.; Hu, T.; Yang, Y.; Wu, X.; Wang, B.; Liu, Y.; Qin, D.; Ehlers, J.; Close, T.; Lu, Z.; et al. Mapping Genes Governing Flower and Seedcoat Color in Asparagus Bean (*Vigna unguiculata* ssp. sesquipedalis) Based on Single Nucleotide Polymorphism and Simple Sequence Repeat Markers. *HortSci* **2011**, *46*, 1102–1104. [CrossRef]
65. Amusa, O.D.; Ogunkanmi, A.L.; Adetumbi, J.A.; Akinyosoye, S.T.; Ogundipe, O.T. Morpho-genetic variability in F_2 progeny cowpea genotypes tolerant to bruchid (*Callosobruchus maculatus*). *J. Agric. Sci.* **2015**, *64*, 53–68. [CrossRef]
66. Ajayi, A.T.; Gbadamosi, A.E.; Olotuah, O.F.; David, E.A. Crossability and inheritance of seed coat colour in cowpea at F_1 generation. *Front. Life Sci. RT.* **2020**, *1*, 58–62.
67. Nwofia, G.E. Inheritance of Leaf Shape, Pod Shape, Pod Colour and Seed Coat Colour in Cowpea (*Vigna unguiculata* L. Walp). *World J. Agric. Sci.* **2014**, *10*, 178–184.
68. Herniter, I.A.; Lo, R.; Muñoz-Amatriaín, M.; Lo, S.; Guo, Y.-N.; Huynh, B.-L.; Lucas, M.; Jia, Z.; Roberts, P.A.; Lonardi, S.; et al. Seed Coat Pattern QTL and Development in Cowpea (*Vigna unguiculata* [L.] Walp.). *Front. Plant Sci.* **2019**, *10*, 1346. [CrossRef]
69. Xiong, H.; Shi, A.; Mou, B.; Qin, J.; Motes, D.; Lu, W.; Ma, J.; Weng, Y.; Yang, W.; Wu, D. Genetic Diversity and Population Structure of Cowpea (*Vigna unguiculata* L. Walp). *PLoS ONE* **2016**, *11*, e0160941. [CrossRef] [PubMed]
70. Sita, K.; Sehgal, A.; Kumar, J.; Kumar, S.; Singh, S.; Siddique, K.H.; Nayyar, H. Identification of high-temperature tolerant lentil (*Lens culinaris* Medik.) genotypes through leaf and pollen traits. *Front. Plant Sci.* **2017**, *8*, 744. [CrossRef] [PubMed]
71. Bishop, J.; Jones, H.E.; Lukac, M.; Potts, S.G. Insect pollination reduces yield loss following heat stress in faba bean (*Vicia faba* L.). *Agric. Ecosyst. Environ.* **2016**, *220*, 89–96. [CrossRef]
72. Gasim, S.M.; Abdelmula, A.A. Impact of Bee Pollination on Yield of Faba Bean (*Vicia faba* L.) Grown under Semi-Arid Conditions. *Agric. Sci.* **2018**, *9*, 729–740. [CrossRef]