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Influence of insect flower visitors on leaf parameters and nodulation of progenies of cowpea [*Vigna unguiculata* (L.) Walp.]

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ABSTRACT

The study was conducted to determine the differences in leaf area, chlorophyll induction kinetics and level of nodulation of the progenies of cowpea plants whose flowers were not exposed and visited by any organism (control) and progenies of plants whose flowers were visited by *Megachile* sp. (experimental plants). Cowpea seeds obtained from the pods developed from control and experimental plants were sown on a plot of land (6 × 15 m) demarcated into 12 smaller plots (blocks) in a random manner. Seeds were sown 1 m between blocks and 0.4 m between hills; and each block was made up of 12 hills and three seeds sown per hole in a hill about 0.025 m deep. At specific ages of the germinated plants, leaf surface area, chlorophyll fluorescence (ChlF) induction kinetics and number of nodules were then determined and compared. Mean leaf area per plant per block, ChlF decrease ratio (Rfd = Fd/Fs) of the leaves and mean number of nodules per block for the progenies of the control and experimental groups were determined and compared with paired *t*-test. The results obtained show that leaves of the experimental group were smaller, greener and had higher chlorophyll induction kinetics than leaves of progenies of control groups. The experimental plants developed more nodules than the control plants. The findings suggest that *Megachile* sp. might have probably caused pollination of cowpea flowers visited inducing seeds from the plants with some genetic traits that were transferred to progenies and thus causing differential characteristics in leaf area, chlorophyll decrease ratio and nodulation. It is suggested that further researches be conducted on possible cowpea pollination by *Megachile* sp. and its detail effects.

INTRODUCTION

The flowers of many plants are visited by a number of organisms. This kind of flower visitation brings about interactive relationships between plants and animals in the ecosystem. A flower visitor can be any animal that visits the flowers of a plant, many of which end up causing pollination whilst others just end up obtaining food from the flowers and others are predators that feed on pollinators (Zych, 2006; Watts et al., 2012). Flower visitor taxa often vary markedly in their quality as pollinators (Rader et al., 2012). Since not all visiting organisms are pollinators (pollen vectors) because only a small fraction of them are effective pollinators and among the pollinator taxa (Johnson and Steiner, 2000; Zych, 2006; Watts et al., 2012), this work is focused on cowpea insect flower visitors as potential pollinators.

Eardley (2002) considers pollinators as animals that provide pollination services. No animal pollinates flowers deliberately. They visit flowers for food, in the form of nectar, pollen and plant oils. Pollination precedes fertilization and fertilization results directly in the plant

producing seeds and fruits [African Pollinator Initiative (API), 2003].

In the process of visiting a flower and being a potential pollinator an organism can transfer traits that can be very useful to the plant and its progenies. Progeny in this instance is referring to the Mendelian principle where it was considered that if pollination had occurred, then some genetic characters might have been transferred to the plants and as a result such characters would be exhibited in crops that would be obtained from sown seeds of such plants so cross-pollinated. In effect if cross-pollination occurs it can bring about several hybrid effects among which is increase in the vegetative mass and faster growth of plants (Abrol, 1997) and probably high nodulation.

One aspect of vegetative organ of importance to the plant is the leaf area. Leaf area growth determines light interception and is an important parameter in determining plant productivity (Koester et al., 2014). Leaves are of fundamental importance to plants. They constitute the plant's power generation and aerial environmental sensing units. The amount of photosynthetic light harvested depends directly on the leaf-area (LA), which affects plant growth and bio-productivity and hence also the agro-economic return from the crop (Meziane and Shipley, 1999; Vile et al., 2005).

Generally, the vegetative parts including leaves of grain legumes such as cowpea are commonly fed to livestock after their seeds have been harvested. Species which are cultivated only to feed livestock are called fodder or forage legumes, or if they are grown in mixtures with pasture grass they are called pasture legumes (Onwueme and Sinha, 1991). In the tropics, legumes are grown to control weeds, restrict soil erosion and enrich soil nitrogen and are, therefore, known as cover crops. They are often grown to cover the ground in plantations of trees such as rubber and cocoa (Onwueme and Sinha, 1991). According to Singh et al. (1995), the tender leaves of cowpea are eaten as spinach-like vegetable, while it's immature pods and seeds are also consumed as vegetables. Farmers in the dry savanna use cowpea haulms as a nutritious fodder for their livestock (Singh et al., 1995). These suggest that some biological visitations may bring about positive effects in the leaves of progenies.

One of the features of the leaf that is of great importance is chlorophyll fluorescence (ChlF). Fluorescence is a short-lived type of luminescence created by electromagnetic excitation. That is, fluorescence is generated when a substance absorbs light energy at a short wavelength (higher energy) and then emits light energy at a longer wavelength (lower energy). The length of time between absorption and emission is usually relatively short, often of the order of 10-9 to 10-8s. For any fluorescent molecule, the wavelength of emission is always longer than the wavelength of absorption (Integrated DNA Technologies, 2011). Before fluorescence is emitted, energy must be absorbed. In the case of photosynthesis and chlorophyll (Chl) fluorescence, the absorbed energy is solar energy in a spectral region almost identical with visible light, in the interval 400 – 700 nm for most photosynthesizing systems. Electromagnetic radiation with wavelength from this interval is the photosynthetically active radiation (PAR). The main photosynthetic pigment, chlorophyll produces absorption spectra (Figure 1).

Chl fluorescence emission spectrum is a dependence of fluorescence intensity on wavelength of emitted fluorescence signal (usually in interval of 600 - 800 nm) upon fixed excitation wavelength (usually a maximum absorption of Chl a about 436 nm) or Chl b (about 470 nm) in the blue spectral region. On the other hand, Chl fluorescence excitation spectrum is a dependence of fluorescence intensity on excitation wavelength (usually in interval of 400 - 500 nm) for fixed wavelength of emitted fluorescence signal which usually corresponds to position of selected peak in the emission spectra in the interval of 600 - 800 nm (Dusan, 2016).

According to Baker and Rosenqvist (2004), there is no doubt that measurements of ChlF, when applied with appropriate care, can provide useful information about leaf photosynthetic performance. Cerovic et al. (1999) also stated that it has been observed in recent times that ultraviolet light induced ChlF is a good method for plant monitoring in agricultural and plant science applications. Based on the ratios of ChlF emission spectra intensities at 683 and 731 nm, F683/F731, and other significant intensity ratios, this technique can discriminate between normal and stress conditions in vegetation (Chappele et al., 1984; Lichtenthaler, 1990; Saito et al., 1998; Subhash and Mohanan, 1995). Therefore, ChlF is a useful tool to probe the probable efficiency of photosynthesis of a leaf, for that matter cowpea leaves.

Directly connected with leaf characteristics is the level of nitrogen available to the plant. Nitrogen is the most commonly used limiting nutrient in plants though plants need them in large quantities (Sørensen and Sessitsch, 2007). However, applying Nitrogen (N_2) chemical fertilizers is largely an inefficient process as 30-50% of applied nitrogen fertilizer is lost to leaching resulting in significant environmental problems such as the eutrophication of water ways. Meanwhile, legumes use nitrogen fixing bacteria, specifically symbiotic rhizobia within their root nodules to counter the limitation. Roberts (1986) intimates that the best known nitrogen fixing organisms are bacteria that live in the roots of leguminous plants such as peas, beans, cowpea and clover. Such plants are able to thrive in soils deficient in nitrates and they owe this ability to nitrogen-fixing bacteria in their roots. The bacteria enter the young plant through its root hairs and they cause the cortical cells of the root to proliferate, forming swellings or root nodules. It

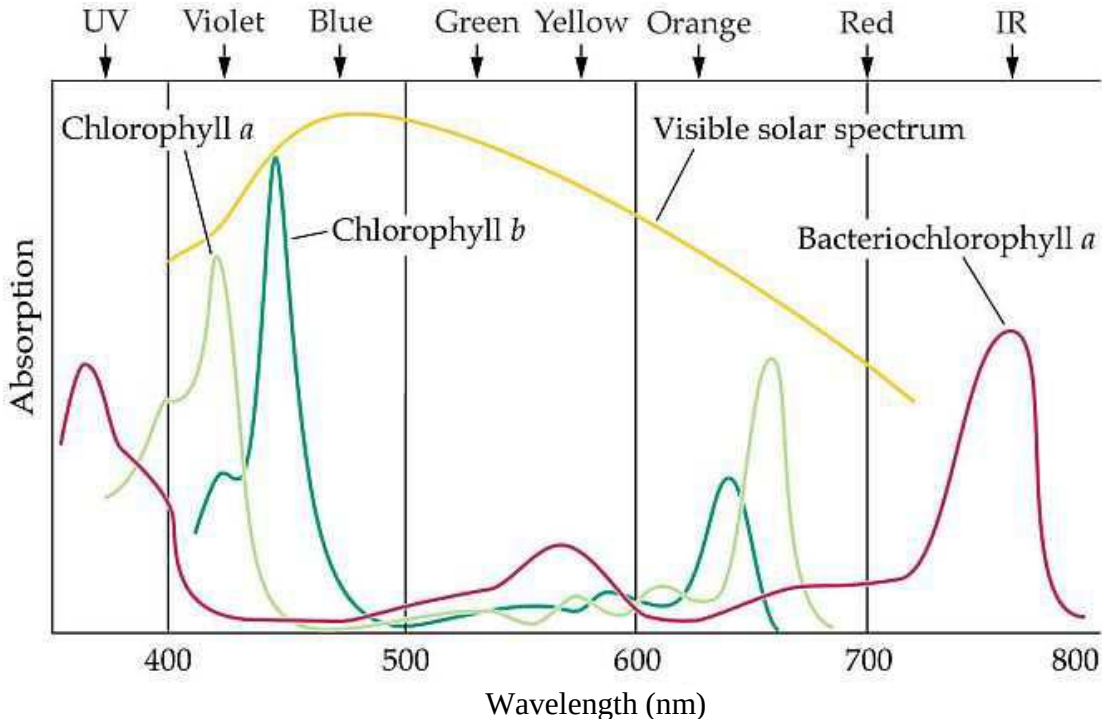

Figure 1: Absorption spectra of chlorophyll.
Source: Dusan L. (2016). http://biofyzika.upol.cz/userfiles/file/Chl_fluorescence_for_PhD_students.pdf

is further explained that when legumes set up a symbiosis with bacteria in the genus *Rhizobia*, the plants develop a new organ on the roots (a nodule) to house the bacteria (Kassaw et al., 2015). In the cells of the nodule the bacteria multiply rapidly, fixing atmospheric nitrogen and building it up into amino acids and proteins (Roberts, 1986). Therefore, there is a strong need to reduce the reliance on chemical nitrogen fertilizers and instead optimize alternative nitrogen inputs. Thus, biological nitrogen fixation (BNF) is one alternative to nitrogen fertilizers. It is known that, with few exceptions, legumes can enter into an intricate symbiotic relationship with specific soil bacteria called rhizobia (Schubert, 1982). These facts point out that nodulation in legumes, for that matter cowpea, is a very useful phenomenon to the plant itself and mankind.

Since cowpea is generally considered a self-pollinated crop (Vaz et al., 1998); many scientists give little, if any consideration at all, to hybrid effects of cross-pollination to plants produced from seeds of the crop (Asiwe, 2009). Thus, the researcher's curiosity was aroused upon sowing seeds of cowpea obtained from plants whose flowers were not exposed to any organism and those exposed to and visited by bee species known as *Megachile* sp. The plants whose flowers were not exposed to any organism were treated as the control plants while those exposed and visited by *Megachile* sp. were taken as the experimental plants.

The researcher observed that leaves of the progenies of the experimental plants were smaller and greener than those of the progenies of control plants. Therefore, the researcher decided to find out the differences in leaf area, chlorophyll induction kinetics and level of nodulation of the two groups of plants. Such determination would suggest whether the visitation of *Megachile* sp. to the experimental group brought any difference as a result of them being probable pollinators transferring some traits to the offspring (progenies) of the visited plants. The specific objectives of the study were to find out the differences between the:

- Leaf area of the progenies of the control and experimental plants
- Chlorophyll induction kinetics of the progenies of the control and experimental plants
- Nodulation of the progenies of the control and experimental plants

The study was driven by the following research question:

- What difference is there between the leaf area, chlorophyll induction kinetics and number of nodules of the progenies of the control and experimental plants?

The following null hypotheses guided the study:

- There is no significant difference between the leaf area of the progenies of the control and experimental plants
- There is no significant difference between the number of nodules of the progenies of the control and experimental plants.

METHODOLOGY

This study was undertaken at Ekwamkrom in the Gomoa Central District of the Central Region of Ghana. The seeds obtained from the pods developed from plants whose flowers were visited by *Megachile* sp. (experimental plants) and plants whose flowers were not exposed and visited by any organism (control plants) in an experimental farm during the minor rainy season of 2006 were sown again in May, 2007. Seeds were sown on a plot of land 6 m by 15 m in dimension. The plot of land was demarcated into 12 smaller plots and for the purpose of this study termed as blocks (six for control group and six for experimental group). Seeds from the control group and those from experimental groups were sown on alternating blocks (completely randomized blocks). For the sake of this study cowpea plants that developed from the control group were termed as progenies of control and those developed from seeds obtained from the experimental group were termed as progenies of experimental group.

Seeds were sown 1 m between blocks and 0.4 m between hills (stands). Each block was made up of 12 hills and three seeds were sown per hole in a hill about 0.025 m deep. After germination, plants were thinned to two seedlings per hill in cases where all the three seeds germinated. Leaf surface area, ChlF induction kinetics and number of nodules were then determined and compared.

Determination of leaf area

Many methods have been used for determining the area of leaves. Examples include mechanical planimeter, photoplanimeter (Dovan et al., 1958) weight of image (Carcton and Foote, 1965), length by width measurements (Donald and Black, 1958); resistance to air flow (Jenkins, 1959; Mayland, 1969), and an electronic instrument for the nondestructive measurement of leaf area, leaf width, and leaf length. Each researcher develops a method that works best with a given crop and facility available. In this study the length by width measurements as used by Donald and Black (1958) was adapted. Therefore, the leaf area was determined by using simple tracing of leaves on graph sheets. Twenty five leaves from five plants from each block for the progenies of the control as well as experimental group were traced on graph sheets. In order to select the leaves, four opened leaves were counted just below the terminal or apical bud downwards each branch. Five leaves were then selected starting from the fourth counted leaf. The five selected leaves were then traced on graph sheets. The surface area of each leaf was then determined. Using Microsoft Office Excel 2007, means, variances, standard error and paired *t*-test (at 0.05 probability level) were calculated and presented in Tables. The means were compared using the paired *t*-test to determine the differences in the leaf area for the control and experimental plants.

Determination of chlorophyll fluorescence induction kinetics

ChlF induction kinetics was used to assess the photosynthetic performance of the leaves of the progenies of the control and experimental groups. Fifteen completely developed cowpea leaves were obtained from five different plants forming the progenies of experimental as well as control groups for the measurements. To select a leaf, three opened leaves were counted just below the terminal or apical bud downwards each branch. The third, fourth and fifth leaves from the apex of each branch were then picked for the measurements in each case for the progenies. Thus, three leaves were picked from each branch making it fifteen leaves for each type. Complete spectra using a compact continuous violet laser diode fluorosensor (Gustafsson et al., 2000; Anderson et al., 2004) emitting at 396 nm were used as an initial test for ChlF wavelength selection. The peak wavelengths of the ChlF were selected for ChlF induction kinetics (Kautsky effect). In the Kautsky effect, the leaves were illuminated for 5 min using the same compact continuous violet laser diode fluorosensor (Gustafsson et al., 2000; Anderson et al., 2004) after the leaves had been pre-darkened for 20 min. The leaves were placed on a non-fluorescence aluminum plate to reduce possible spectra noise.

During the ChlF induction kinetics the fluorescence intensities of two peaks and their ratios were recorded within the period of observation. The slow part of the Kautsky's effect from a maximum fluorescence (Fmax) intensity level followed by a slow fluorescence decay until a steady-state fluorescence (Fs) at 5 min were recorded. The same processes were used for the other leaves. In all, thirty measurements were taken for the two groups. The whole process was presented graphically, then from the slow fluorescence decrease (Fd = Fmax-Fs), the fluorescence decrease ratio (Rfd = Fd/Fs) of the leaf was calculated. At the same time, the changes in the F685/F740 during the different phases of the induction

Table 1. Average leaf area (in cm^2) of progenies.

Block	Control group		Experimental group		df	t-value
	Mean ± SE	Variance	Mean ± SE	Variance		
1	17.08±0.02	0.006508333	8.42±0.02	0.008066667	24	372.18***
2	16.66±0.02	0.01035	6.95±0.01	0.002983333	24	386.37***
3	17.93±0.04	0.041020667	5.40±0.02	0.013292667	24	271.11***
4	17.41±0.01	0.003077333	4.95±0.01	0.004925	24	731.92***
5	21.13±0.02	0.006131	4.42±0.01	0.002514333	24	907.71***
6	18.30±0.02	0.013741	4.12±0.02	0.005897667	24	559.77***

*significant level, P≤0.05; **significant level, P≤0.01; ***significant level, P≤0.001.
Source: Researcher's field work, 2007.

Table 2. Over all statistical values computed for leaf area of progenies.

Type of statistics	Control group	Experimental group
Mean	18.09113	5.711933
Variance	2.162646	2.307743
Observations	150	150
Df	149	
t Stat	56.42345***	
P(T<=t) two-tail	1.9E-102	

*significant level, P≤0.05; **significant level, P≤0.01; ***significant level, P≤0.001.

kinetics were followed.

Assessment of nodulation of roots

Six hills (holes) per block were randomly selected but making sure each hill had two plants. Therefore, there were 12 selected plants per block. After harvesting the pods, the 12 selected plants on each block were uprooted. The number of nodules on the roots of each plant was then counted. The average number of nodules per block for the progenies of the control as well as progenies of experimental group was calculated. Using Microsoft Office Excel 2007, means, variances, standard error and paired *t*-test (at 0.05 probability level) were calculated and presented in Tables. The means were compared using the paired *t*-test to determine the differences in the number of nodules for the control and experimental plants.

RESULTS AND DISCUSSION

Leaf area

The least average leaf area of 4.12±0.02 cm^2 was recorded on block six for the progenies of experimental plants whilst the highest mean value of 8.42±0.02 cm^2 was recorded on first block. In the case of the progenies of control the least average leaf area was 16.66±0.02 cm^2 and was recorded on the second block whilst the largest leaf area of 21.13±0.02 cm^2 was recorded on block five. Generally, the leaves of plants for the experimental group were smaller than that of the control group. In each case, the *t*-values show that the differences between the mean leaf areas of the control and experimental groups were significant for each block (df = 24; P≤0.001) as presented in Table 1. Furthermore, the overall statistical values computed for leaf area of progenies show that there were significant differences between the means for the progenies of control and that of the experimental group (*t* = 56.42345; P≤0.001; df = 149) as shown in Table 2. Thus, the null hypothesis was rejected implying that the differences were not due to chance but must be induced by certain traits.

The findings in this study are somehow opposite to the findings of Gonzalez et al. (2010) in a different experiment. In an experiment to gain more insight into the genetic control of leaf size in Arabidopsis (*Arabidopsis thaliana*) by performing a comparative analysis of transgenic lines that produce enlarged leaves under standardized environmental conditions, Gonzalez et al.

(2010) grew plants in semi-hydroponic conditions such that the plants were grown in rock wool, an inert porous substrate that transports water and fertilizers to the roots by capillary action. It was found out that the plants produced larger leaves than those of the control plants. When the five lines were grown under *in-vitro* conditions Gonzalez et al. (2010) found out that an increase in leaf area was observed as well, although the extent of the increase differed from that under rock wool conditions. In the current study the larger leaves were produced by the control groups.

Gonzalez et al. (2010) again observed that the final size of plant organs, such as leaves is tightly controlled by environmental and genetic factors that must spatially and temporally coordinate cell expansion and cell cycle activity. An increase of the total leaf area can be due to a change of the length and/or the width of the leaf (Gonzalez et al., 2010). They attributed the differences in sizes of the leaves to different genes, that the genes had contrasting effects on leaf shape and area, indicating that they affect different aspects of leaf growth. In this study also the cause of different leaf sizes might be due to an internal factor which is probably genetic. The probable trait bringing the differences in leaf area might have come from cross pollination by *Megachile* sp. transferred from the seeds to the progenies of the experimental plants. Thus, it is suggestive that the drastic reduction in leaf area for progenies of the experimental plants was likely due to cross-pollination of flowers that formed the seeds from the experimental farm in the minor rainy season. Therefore, it will not be out of place to suggest that cross-pollination of cowpea by *Megachile* sp. is likely to lead to the reduction in leaf area of progenies. This may be of an advantage since cowpea is a dry season crop. The small leaf areas may prevent excessive loss of water from the surfaces and thereby conserving moisture in the plant to prevent wilting. However, for these claims to be confirmed there is the need for further studies on them to provide empirical proof.

Since the blocks were randomly arranged to take care of soil conditions it was expected that there would be a trend for both control and experimental groups where the least and the biggest leaf areas should be recorded on the same blocks for the two groups. However, this is not the case. Here, it is difficult to explain but may be attributed to zonal soil nutrient differences which were not determined. This is considered as a weakness of the study.

Chlorophyll fluorescence induction kinetics

Observation of the leaves showed that those from experimental plants looked greener than leaves from the control group. This was suggestive that leaves from plants in the experimental group might have more chlorophyll than those from the control group. In that case, it was expected that leaves from experimental plants would be more photosynthetic than those from the control plants. Meanwhile, Walker (1987) asserts that time-course of Chl fluorescence yield (or fluorescence intensity) termed fluorescence induction kinetics (FIK) provides a direct insight into the utilization of the excitation energy by PS II and indirectly also by other complexes within thylakoid membranes. Together with other spectroscopic and biochemical methods, recording of Chl fluorescence helps elucidate many important mechanisms of photosynthesis. At present, Chl fluorescence is widely used as a nondestructive diagnostic tool in photosynthesis research (Krause and Weis, 1991; Schreiber, 2004). Karlson (1992) emphatically states that, from these measurements, an increase of the ratio F690/F740 is caused by a lower chlorophyll content and or decline in photosynthesis. Based on the aforementioned premises the researcher decided to ascertain the likely photosynthetic efficiency of the leaves of the two groups of plants by undertaking ChlF induction kinetics. The results are presented in Figure 2 and Table 3.

In this study, Fmax intensity of progenies of experimental plants was higher (128±0.03) than that of progenies of control plants (48±0.02). Similarly, the Fs for leaves of the progenies of experimental plants is higher (70±0.02) than that of leaves of the control plants (29±0.04). Eventually, the Rfd of the leaves of experimental plants was higher (0.83±0.01) than that of the leaves of the progenies of the control group (0.66±0.02), as shown in Table 3. Therefore, in both cases, the ratio F690/F730 is higher at Fmax intensity than at Fs. This agrees with the findings of Hak et al. (1990) that the ratio F690/F730 is somewhat higher at Fmax than at Fs, but there is a very good correlation between both values. Hak et al. (1990) also intimate that the ratio F690/F730 is a good indicator of the chlorophyll content and can be used as a non-destructive measure of the chlorophyll content of leaves. As such they concluded that the ratio F690/F730 decreases with increasing chlorophyll content of developing leaves. In this study, the Rfd is higher for the experimental group (0.83±0.01) than the control group (0.66±0.02) suggesting that there was a decrease in the ratio for the control group. This implies that the control group had more chlorophyll in the leaves compared to the experimental group (Karlson, 1992). This was the opposite of what was expected because on the field the leaves of the experimental group looked greener suggesting that they might have contained more chlorophyll than the leaves of the control group. The explanation here is that, since the leaves of the control plants were larger than that of the experimental plants, their chlorophyll content would have been spread in the broader leaves making them to look less greener than the leaves of experimental plants. On

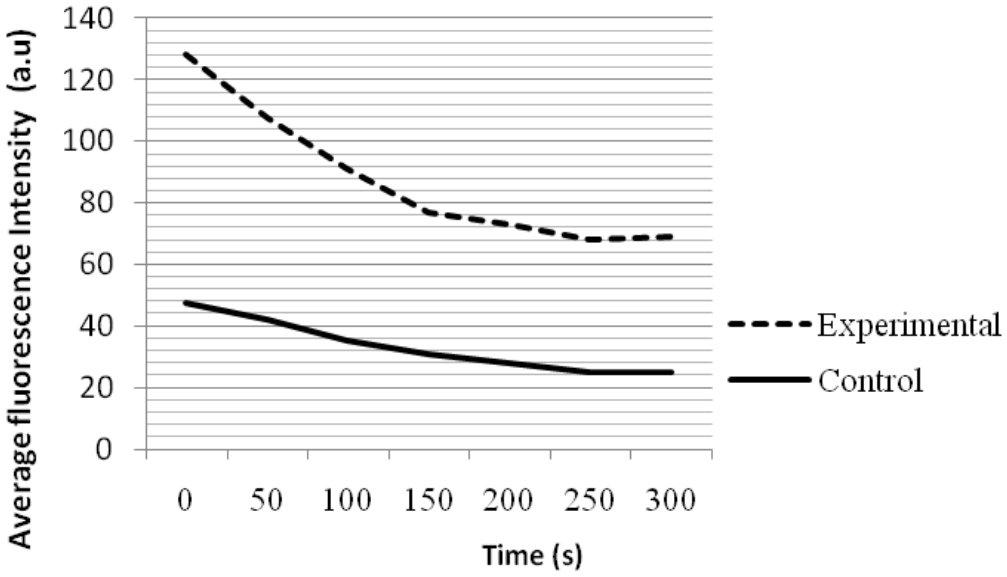

Figure 2. ChlF induction kinetics of progenies.

Table 3. Average ChlF induction kinetics.

ChlF induction kinetics parameter	Progenies of control plants	Progenies of experimental plants
Fmax intensity	48±0.02	128±0.03
Fs	29±0.04	70±0.02
Fd	19±0.03	58±0.04
Rfd	0.66±0.02	0.83±0.01

Source: Researcher's field work, 2007.

the other hand, the limited chlorophyll content of the leaves of the experimental plants would have been condensed in the very small leaves making them look greener than the control.

Stefanov and Terashima (2008) stated that decreases in Rfd could be associated with an increased non-photochemical loss. This suggests that the leaves of the experimental plants may be less efficient in terms of photochemical processes for that matter photosynthesis. This difference may be attributed to differences in the basal fluorescence of leaves of the two types of progenies. The probable cause in the difference may be attributed to traits transferred from plants from which seeds sown were obtained. Thus, it can be argued that plants whose flowers were visited by *Megachile* sp. might have acquired some special traits from cross-pollination caused by the *Megachile* sp. thereby causing leaves of the progenies of the experimental plants to exhibit higher fluorescence ratio but probably less efficient in carrying out photosynthesis.

Notwithstanding all above, it must be noted that several factors can lead to a decrease (quenching) of Chl fluorescence. For example, the excessive irradiance, low/high temperature, drought, toxic chemicals, heavy metals and the interpretation of the fluorescence signal depend on the ability to resolve contributions made by each of the mechanisms. To this purpose, the very useful quantitative information on photosynthetic processes can be decoded from Chl fluorescence kinetic curves using a set of Chl fluorescence parameters (Roháček et al., 2008). Though in this study, plants were in randomized blocks and all the setups were exposed to the same environmental conditions; it would not be out of place if soil conditions were determined to find out if they would have differential effects on chlorophyll fluoresce induction kinetics. This would have taken the study to a higher level. It is suggested that soil conditions and any other probable factors should be taken care of in subsequent studies. Also, determination of chlorophyll levels in the leaves would have been a useful source of information

Table 4. Mean number of nodules per plant for the progenies.

Block	Control group		Experimental group		df	*t*-value
	Mean ± SE	Variance	Mean ± SE	Variance		
1	3.33±03	0.011960606	26±0.02	0.003018182	11	-709.44***
2	3.33±0.01	0.001147727	61.67±0.01	0.001345455	11	-3907.63***
3	11.67±0.03	0.008433333	57.00±0.02	0.003093182	11	-1861.33***
4	2.50±0.01	0.001815152	23.17±0.02	0.006093182	11	-922.39***
5	9.67±0.02	0.003226515	47.67±0.01	0.001763636	11	-2734.52***
6	3.33±0.01	0.00099697	24.00±0.02	0.005917424	11	-890.64***

*significant level, P≤0.05; **significant level, P≤0.01; ***significant level, P≤0.001.
Source: Researcher's field work, 2007.

Table 5. Over all statistical values computed for nodulation of progenies.

Type of statistics	Control group	Experimental group
Mean	5.638888889	39.91930556
Variance	13.26660156	262.4148657
Observations	72	72
df	71	
t Stat	-20.25433109***	
P(T<=t) two-tail	3.19387E-31	

*significant level, P≤0.05; **significant level, P≤0.01; ***significant level, P≤0.001.

which needs to be done in subsequent studies.

Nodulation

In this study, the roots of the progenies of the experimental plants developed higher number of nodules than the roots of the progenies of control plants (Table 4). For the experimental plants, the highest mean number of nodules (61.67±0.01 nodules) was recorded on block two followed by block three (57.0±0.01 nodules) and block five (47.67±0.01). In the case of the control plants, the highest mean number of nodules (11.67±0.01 nodules) was counted for plants in block three followed by block five (9.67±0.01 nodules). In blocks one, two and six, the same mean number of nodules (3.33±0.01 nodules) was counted for the control plants. Generally, higher number of nodules was counted from experimental plants compared to the control plants. From Table 4, the *t*-values show that the differences between the mean number of nodules for control and experimental groups were significant in each block (df = 11; P≤0.001). Also, the overall statistical values show that the differences between the mean number of nodules for the experimental and control groups were statistically significant (*t* = -20.25; P≤0.001; df = 71) as indicated in Table 5. Here also, the null hypothesis was rejected implying that the differences were not due to chance but real and may be due to a factor. What must be this factor?

A number of theories come to play. For example, rhizobia bacteria are known to be free living, that is, they are already living in the soil even when forage legumes are not present. Thus when an appropriate host forage crop is planted in the soil, the rhizobia may be present and come into contact with root hairs of the host plant. Most Rhizobia and host plants are highly specific and legumes can either attract rhizobia to root hairs directly by excretory compounds or by induction of nod gene activity in the bacteria (Sørensen and Sessitsch, 2007). Once the correct *Rhizobia* spp. are present, forage roots apparently stimulate the bacteria to reproduce itself and thus larger and larger numbers of bacteria are produced. The bacteria gradually form an infection thread which allows the bacteria to enter root cells of the plant through root hairs. Bacteria in the root cells gradually grow and develop into structures called bacteroids. During the infection process, the bacteria stimulate cell division in the root cells resulting in the eventual formation of extra organs attached to the roots called nodules (Sørensen and Sessitsch, 2007).

Considering the facts alluded to by Sørensen and Sessitsch (2007) and the findings in this study concerning nodulation, it can be argued that the differential levels of

nodulation among the progenies of the control and experimental plants is due to a factor. According to Caetano-Anolles and Gresshoff (1991) and Mengel (1994) at the whole-root system level of a plant, the number of nodules depends on both internal and environmental factors. The known environmental factors that affect the number of nodules are nitrate concentration in the growing medium (Macduff et al., 1996), soil compaction (Katoch et al., 1983), air and soil temperature (Munevar and Wollum, 1981; Rawsthorne et al., 1985), air carbon dioxide concentration (Murphy, 1986), and light intensity (Kosslak and Bohlool, 1984). However in the current study, all the cowpea plants were exposed to the same environmental factors. Therefore, the factor that brought about the differences in nodulation may be internal rather than environmental. The probable internal factor may be a genetic trait transferred when *Megachile* sp. visited flowers of the original plants from which the progenies were obtained.

It is also possible that the roots of the progenies of the experimental plants might have been able to attract more nitrogen fixing bacteria than progenies of the control. This is because the association between bacteria and roots of a host plant via the nodules causes some of the products of the bacteria's nitrogen fixation to pass into the host plant and utilized by it (Roberts, 1986). Therefore, since the roots of the progenies of the experimental plants developed higher number of nodules than the roots of the progenies of the control plants, it is possible that the plants formed from the seeds of the experimental plants might have acquired some useful traits as a result of probable pollination by *Megachile* sp. leading to the formation of higher number of nodules. It is asserted that successful nodule formation and subsequent nitrogen fixation occur only under nitrogen limiting conditions. In the presence of high concentrations of biologically available nitrogen, plants cease nitrogen fixation and nodule formation is suppressed (Schultze and Kondorosi, 1998). Thus, it is possible that probable pollination by *Megachile* sp. might have induced seeds with such genetic traits that were transferred to their progenies bringing about nitrogen limiting conditions that enhanced high production of nodules in the root regions.

Clark et al. (2005) established that BNF must have an important role in the nutritional status of cowpea in Brazil. Since bacteria in nodules fix nitrogen for the plant (Roberts, 1986), it is possible that the numerous nodules on the roots of experimental plants compared to control plants might have led to high levels of nitrogen fixation for the crops. This is suggestive that visitation of *Megachile* sp. to flowers of cowpea plants can possibly cause cross-pollination leading to transfer of genetic traits that will induce high levels of nodulation in progenies to fix much more nitrogen in the soil thereby improving soil fertility. However, these claims also demand further studies to prove or disprove. Therefore, it is worth conducting further research into effects of probable insect cross pollination, especially *Megachile* sp. of cowpea and nodulation to confirm the findings of this study.

Conclusion

There were significant differences between the leaf area of the progenies of the control and experimental plants suggesting that cross-pollination of cowpea by *Megachile* sp. might have taken place leading to reduction in leaf area of progenies of experimental plants. Therefore, if cross-pollination of cowpea by *Megachile* sp. occurs it is likely to lead to reduction in leaf area of the progenies.

Greener leaves from progenies of experimental plants revealed higher ChlF parameters than leaves of the progenies of control plants. This is indicative of the fact that the differences may be attributable to differences in the basal fluorescence of leaves of the two types of progenies. It further suggests that leaves of the progenies of experimental plants might be less effective in carrying out photosynthesis compared to that of the control probably due to cross-pollination effects by *Megachile* sp. Therefore, it is possible for progenies of plants whose flowers are visited by *Megachile* sp. to have acquired some special traits from cross-pollination thereby causing leaves of the progenies to exhibit high fluorescence ratios.

There were significant differences between the mean number of nodules for the progenies of experimental and control plants where progenies of experimental plants developed higher number of nodules compared to progenies of the control plants. Such differences cannot be due to chance but real and may be due to a factor which is probably from cross-pollination brought about by *Megachile* sp. of experimental plants from which the effects have been transferred to the progenies. Hence, it can be said that the roots of the progenies of the experimental group attracted more nitrogen fixing bacteria than progenies of the control leading to the formation of many more nodules which are useful for nitrogen fixation. Therefore, visitation of *Megachile* sp. to flowers of cowpea plants may possibly cause cross-pollination leading to transfer of genetic traits that will induce high levels of nodulation in progenies to fix much more nitrogen in the soil thereby improving soil fertility.

RECOMMENDATIONS

- Since visitation of *Megachile* sp. to flowers of cowpea plants appear to have transferred traits that promoted smaller leaf area, higher leaf fluorescence ratios and higher nodulation in progenies, which are useful to the plants, research scientists should collaborate with Agricultural Extension Officers to disseminate the

usefulness of probable insects pollinators, especially *Megachile* sp. on cowpea flowers.

- Research scientists and agricultural extension officers should educate cowpea farmers to study the characteristics of *Megachile* sp. on the crop so that they can easily notice their presence on cowpea plants on the field.
- Cowpea farmers should notice the time of the day that *Megachil*e sp. is abundant on the crop in their farms and avoids spraying synthetic insecticides that can destroy them during such time periods.
- Agricultural officers should educate cowpea farmers to use plant based insecticides that are friendly to *Megachile* sp. if they are found on the crop during times of spray.

Suggestion for further studies

It is hereby suggested that further research should be conducted to confirm or disprove the findings of the current research.

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