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Research Article

How can Blue and White Light Pulses Stimulate *Atta Cephalotes* in Trails Below Fruit Trees?

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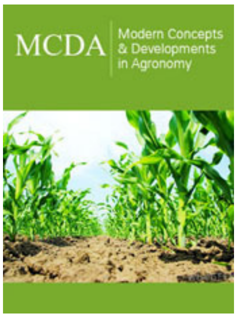

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Abstract

Leaf-cutter ants (*Atta cephalotes*) forage for leaves, buds and flowers on mango trees. Ants transport these food resources to their nest using the trails they have set up. An artificial trail with laser-phototransistor units was used to measure ant activity and speed. An embedded system based on the SAM21 microcontroller-controlled illumination intensity and measured ant speed. Continuous and pulsed light using L300 LED linear arrays were applied to the trail to study ant stimulation. Under continuous nocturnal light changes, ants walking speed remained constant, unless their leader moved quicker. It was found that blue light at different intensities did not affected ant's speed. Ants collided with pulsed illumination (7 on- 7-off) even during the day, when it was scattered through natural illumination. With the help of mirror inserted in the trail to focus light pulses to the ant eyes, ant collisions increased to 23%.

Keywords: Illumination control; *Atta cephalotes*; Ant speed counting; Artificial trail; Ant collisions

Introduction

Worker ants follow trails towards the foraging site where they cut leaves, flowers, and buds into fragments [1]. Trail flow determined by ant speed, collisions, and density, maximizes the rate of food delivery to the nest [2]. A single *Atta cephalotes* forager experiences more than twenty collisions along a 100m path, reducing net speed by 21% for outbound workers [3]. Ants are active at different times of the day, so they require visual adaptation to deal with high variations in light intensity. The low sensitivity eyes of nocturnal ants [4] use larger lenses and wider photoreceptors than diurnal ants to increase their optical sensitivity [5].

Field insects can be monitored with optoelectronic devices, videography, thermal imaging, and radio frequency systems [6]. Researchers analyze ant movement by filming sections of the principal trail with a digital camcorder [4,7]. Several monitoring systems interrupt a LED or laser beam, and the detector signal triggers a time counter [8]. Photoreceptors in the night-active *M. vindex* ant present three spectral sensitivities with peaks at 370, 430 and 550nm [9]. Foragers showed no signs of disturbance from red light applied with headlamps during nocturnal observation [10].

Regardless of the large amount of physiological research that has been conducted to understand how ants guide themselves towards their forage site, this experimental work studies the possibility of using LED lighting to divert ants from fruit trees. We worked on the presumption that ant speed changes under strobe-light LED stimulation. Spectral blue and UV bands tend to be insect repellent, so one of our main objectives was to evaluate how blue lightning affects ant movement. Another objective was to evaluate if light impacts their eyes and develop a system to orient the light.

Material and Methods

Artificial trail

Artificial trails were installed at a mango farm located in Guerrero, Mexico (17° 25' 47" N, -101° 11' 19" W, 17m ASL) during September 2019 and two experiments were carried out to study ant stimulation caused by artificial light. An artificial trail consists of a strip of white plastic, lightly abraded with sandpaper, with two 50mm high walls (Figure 1a). The 1000mm long and 25mm wide plastic trail was placed beneath the mango tree trunk where *Atta cephalotes* ants circulate. Four 300-mm long linear arrays (model L300, Smart Vision Lights, USA) supplied by a 12 VDC motorcycle battery illuminated the trail (Figure 1). Each L300 has 12 high-intensity low-power consumption LEDS and illuminates an elliptic area on the trail surface (Figure 1a). The maximum illumination provided is 19,000lx and can be reduced to 350lx with a potentiometer.

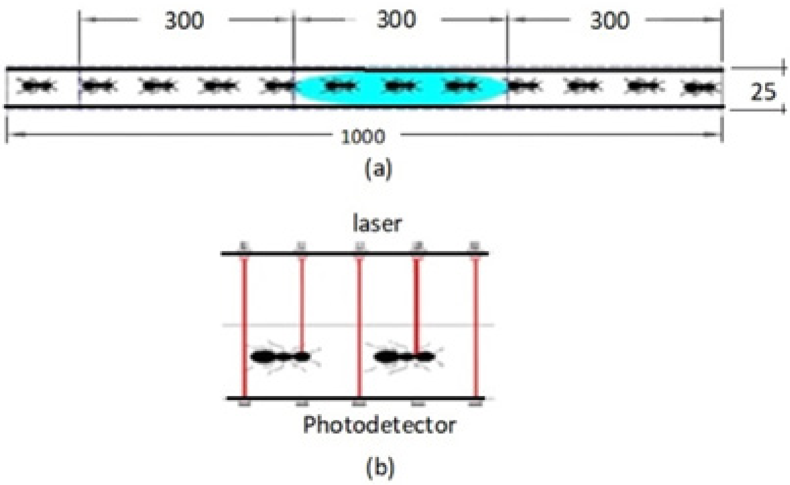

Figure 1: Ant trail showing
(a) top view, and (b) laser-photodetector unit.

Controller

A SAMD21 (Microchip Technology Incorporated, USA) microcontroller with a 48MHz 32-bit ARM Cortex M0+ processor was employed to control trail illumination and measure ant speed (Figure 2). The AS illumination control signal turns-on the L300 LED array at the desired intensity and time. The SAMD21 microcontroller outputs digital voltages (DO) through an 8-bit parallel bus to feed four digital analog converters (DAC) to provide the AS signals. Light impulses are easily programmed by turning on and off the LED arrays.

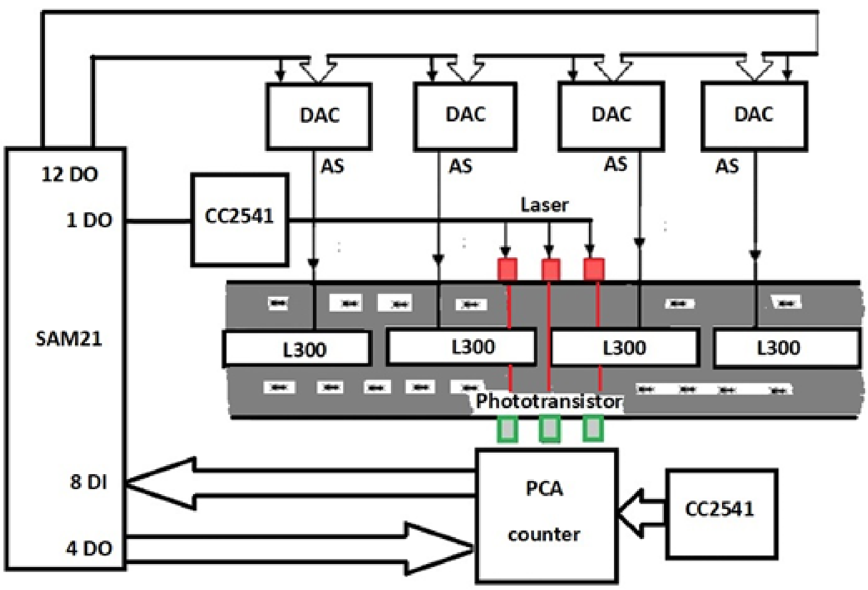

Figure 2: Block diagram of the SAM21 controller showing digital analog circuits (DAC), lasers, photodetectors, L300 LED arrays, PCA counter and CC2541 circuits.

Ant speed was measured with laser photodetector units, and 5mW red lasers (D650-51 US Laser, USA) provided a constant non-dispersive beam. The photodetector strip was fixed to one wall, and the lasers to the opposite wall (Figure 1b). The photodetectors (KDT00030, Fairchild, USA) present a linear current-illumination response. Laser-phototransistor units determine ant speed by counting the number of one ms laser beam pulses. When an ant walks in front of the laser light and blocks it, a variable named TPA (Time-Period Ant) is obtained. The TPA value of two consecutive phototransistors 10mm apart help to calculate ant speed. As a space exists between two ants walking in succession another variable known as TPBA (Time-Period Between Ants) can be obtained. The CC2451 (Texas Instruments, USA) microcontroller provides 1ms pulses that are amplified to drive the lasers (Figure 2). The pulses are software generated with a NOP instruction within a loop to obtain precise timing. Another CC2541 microcontroller was used to control a PCA counter to obtain ant TPA or TPBA storing the values in memory.

Experimental design

Once the top-illuminated trail was installed two experiments were carried out. Experiments A and B analyze TPBA (time-period between ants) and collision number under different lighting schemes.

A. Experiment A: Ant TPBA and TPA as they travel through the trail under blue and white LED illumination

Eight experiments were carried out using four different continuous light intensities and LED color arrays (blue or white). Each experiment was repeated 5 times with 100 ants each. Night, overcast, cloudy and midday illuminations were of 10, 355, 1000 and 9000lx, respectively. These lighting intensities using $\log_{10}$ values correspond to 1, 2.55, 3 and 3.95lx, respectively. Trail entrance was closed and opened when the leader light-adapted ant was introduced and followed by 99 ants. Leader ant light adaptation period was of one hour and took place in a parallel trail.

B. Experiment B: Ant TPBA and number of impacts within the trail under different white light pulses

The experiment was carried out with 10 repetitions using 50 ants each. Top light-pulses were applied vertically towards the ant body. In the first experiment, seven and fourteen 5-ms light pulses were applied to ants walking at night and at dawn. Five repetitions were used per each light pulse quantity. TPBA and collisions were counted and stored. Statistical analyses were performed using SAS 9.4 (SAS Institute, Cary, North Carolina, United States of America). Regression analyses obtained the mean value, the standard error and the F value.

Result

The length of ants was measured in 100 specimens, resulting in mean and standard deviations of 2.52 and 0.5mm, respectively. The controller provided AS signals of high resolution with increments of 30lx and the laser-phototransistor units worked continuously for 10 days without heating. Thermal heating that could be caused by the lasers was limited and did not affect ant traffic. Power savings were obtained by using a 1mW laser and the spot size at the other side of the wall was identical. Ant TPBA and speed measurements were very accurate as the built-in 8051 microcontroller within the CC2541 provided precise 1ms pulses.

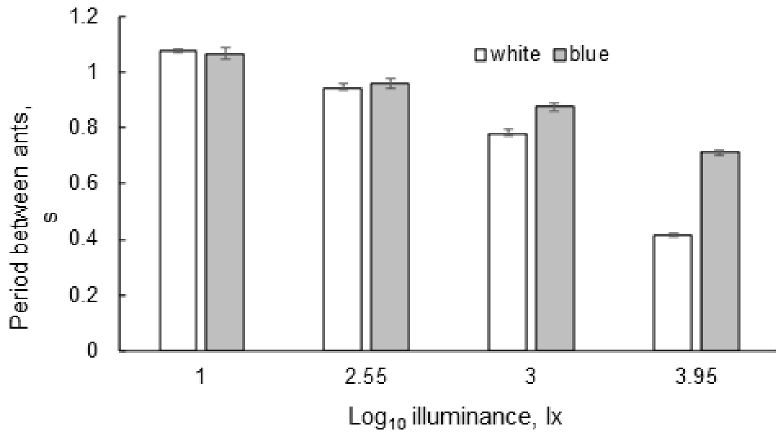

Figure 3: Period in-between two ants (TPBA) under different light intensities provided by blue and white linear arrays.

In experiment A constant ant movement was observed at night along the 25-mm wide trail before light intensity increased to intense midday illumination (10,000lx). Ants were not disturbed and continued walking at the same speed. *A. cephalotes* foragers moved along the night trails near the mango tree trunk with speeds varying between 16.1 and 19.2mms^{-1}, corresponding to TPBAs of 0.95 and 0.83 seconds, respectively. The light-adapted leader changed its speed when the light intensity was varied, and the worker ants followed suit (Figure 3). The TPBA of the ants walking along the trail at a faster speed decreased as illumination intensity increased. Both white and blue LED arrays affected ants TPBA at night. A linear 10-lx illumination intensity can be expressed as $\log_{10}$

(10)=1lx. The experiments provided a negative correlation between white illumination and TPBA (*r*=-0.934, *P*<0.0001, N=800). Ants irradiated with 1100-lx white light present a TPBA with minimum standard error (779±0.68 ms; mean±SE), at 17.6mms^{-1}. With a white illumination of 9000lx (3.95 in log10), TPBA decreases to 413.8±0.77ms (mean± SE). This TPBA corresponds to an ant speed of 37mms^{-1}. Blue LED illumination was well correlated against ant speed (r=-0.968, p<0.001, N=800). Maximum ant speed of 21mms^{-1} was achieved when blue LED arrays supplied 9000lx. TPBA of 713.4±0.38ms was monitored with the controller.

In experiment B, ants 25mm long and 12.5mm apart, followed each other along the 1000mm trail. Ants walking during the day at 23mms^{-1} crossed the 1000-mm trail in 43.48s. At night, ants crossed the trail in 62.5s at a speed of 16mms^{-1}. TPBA values obtained during the night were similar for both number of light pulses (7 or 14). It was found that during the day, ants presented an average TPBA of 751.74±0.62ms (Avg ±SE; N= 250), while ants stunned by light-pulses decreased their average TPBA to 284.29±3.3ms. A TPBA of less than 30ms is recorded when two ants following the same path collide. The quantity of collisions doubled at night for both number of light pulses, 7 and 14 (Table 1). The number of collisions showed that it was easier for ants to adapt to dark pulses in the day, than to intense light pulses at night. White-light pulses in the day scatter through natural illumination. During the day, TPBA decreased to 284ms when we turned off the illumination after 7 light pulses. Similar average TPBA was encountered at night, being TPBA standard error higher in the dark. Similar results were found as we turned off the illumination after 14 light pulses.

Table 1: Average TPBA and SE of normal and shocked ants, number of collisions at night (16mms-1) and day after applying 7 and 14 light-pulses.

Collision Number and TPBA in Milliseconds for						
	Day Speed (23mms^{-1})			Night Speed (16mms^{-1})		
Illumination ON/OFF	Collision number	TPBA, Avg	TPBA, SE	Collision number	TPBA, Avg	TPBA, SE
ON: 7 light pulses	3	751.74	0.62	6	952.84	0.73
OFF: after 7 pulses		284.29	3.3		287.1	2.39
ON: 14 light pulses	3	754.74	0.53	6	953.36	0.72
OFF: after 14 pulses		278.27	2.5		283.7	2.52

Light applied vertically from the L300 array, reflects on the ant head but never reaches their eyes (Figure 4a). Under UV radiation it can cause ant body burning. The linear LED array was rotated, and a mirror glued to the top section of the lateral wall (Figure 4b), so that the illumination comes into the ant eye. Maximum ant collisions caused by mirror-reflection (22±1; Avg±SE) occurs at an inclination of 45° and increased during the night (Figure 4c). At this inclination, the maximum probability that a pulsed light reached the eyes was found (Figure 4c). The curve plotted shows a Gaussian behavior as the ant collision number decreased to 5% when the mirror inclination decreased to 40° or increased to 50°.

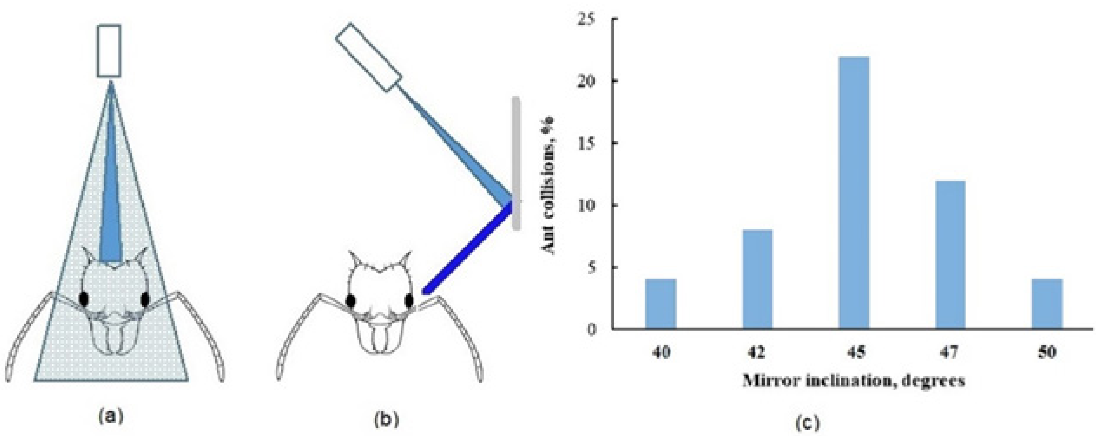

Figure 4: Ant illuminated
(a) with a L300 array fixed at the top
(b) after mirror reflection, and (c) showing collisions (%) for different mirror inclinations.

Discussion

At the University groups of 100 ants were introduced to plastic containers where they had food. Lethal effect on *Atta cephalotes* adults was observed inside the closed plastic containers after being irradiated by 10,000lx white and blue LED strobe light pulses. With white strobe illumination, an average of 33±0.97 (Avg±SE) ants died during the night as the impulses paralyzed ants and stopped their main activities. Most of them moved the head down and formed circular clusters to protect themselves against the light. Mortality decreased during the day and after the second night, ants show some adaptation to strobes, decreasing the mortality to 21.17±0.65 (Avg±SE).

Researchers analyze ant traffic flow with camcorders, but the L300 arrays block the camera's field of view, so we didn't use them. Instead, laser-photodetector units fixed to the trail walls counted ant speed. Red lasers did not bother the ants as they are insensitive to this waveband [9,10]. The L300 was continuously cleaned to reduce lighting loss but only 20% of the light sent reached the ants' eyes after being absorbed by the plastic trail base or the organic soil matter. Although the SAMD21 microcontroller can perform these counting measurements, it cannot perform a time delay routine for illumination and counting at the same time. Continuous lightning can be provided by a potentiometer making the controller less complex, but during precise pulse application it is necessary.

We learnt from our experiments that ants rarely modify their speed with continuous light changes. Major changes were only obtained when light pulses hit the eyes of the ants. Under natural light conditions, walking speed of *Myrmecia* ants decreases from 79.7mm s^{-1} to 20.1mm s^{-1} as light levels drop [4]. These changes occur slowly in time but cannot be perceived within a 1000 mm transit period. At night, ants paused longer as visual landmark guidance became less reliable, so their walking speed decreased by 55% [4].

Lighting performance at the top of the trail is poor. The L300 incident illumination impacted ant's head and body and only a small percentage of light was reflected from the soil to the ants' eyes. Minimal disturbances were observed within the traffic lane when continuous illumination was applied at the top of the trail. As we suspected, only when the light-adapted leader increased its speed, the other ants followed behind closely with no collisions. As trail light intensity became brighter, average TPBA decreased, and walking speed increased. Average continuous illumination is generated by pulse width modulation and depends on source illumination (SI). For example, an average illumination of 900lx can be obtained by using a source illumination of 4500 lx with a 20% duty cycle. It can also be achieved from a SI of 2500lx together with a duty cycle of 36%.

Conclusion

It can be concluded that an illuminated artificial trail installed beneath the mango tree trunk can be used to analyze stimulation of leaf cutting ants. Blue and white linear L300 arrays illuminated the trail as ants walked over. TPBA was obtained with laser-phototransistor units to obtain ant speed. The TPBA of the ants walking along the trail at a faster speed decreased as illumination intensity increased. The number of collisions showed that it was easier for ants to adapt to dark pulses in the day, than to intense light pulses at night.

It can also be concluded that neither blue nor white light applied by the linear arrays disturbed the ants that continued walking at the same speed as the leader. When light pulses were applied at night, ant collisions doubled. As light pulses coming from the top of the trail never reached the ants' eyes, a system with mirrors reflected the flashed light onto the ants' eyes. It requires of an exact mirror inclination and protection against dust in field conditions in order to reflect precisely the light.

Acknowledgement

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