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Green Light Synergistally Enhances Male Sweetpotato Weevil Response to Sex Pheromone

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Sweetpotato, commercially grown in over 100 countries, is one of the ten most important staple crops in the world. Sweetpotato weevil is a major pest of sweetpotato in most areas of cultivation, the feeding of which induces production in the sweetpotato root of extremely bitter tasting and toxic sesquiterpenes which can render the sweetpotato unfit for consumption. A significant step towards improved management of this weevil species was the identification of a female-produced sex pheromone [(*Z*)-3-dodecenyl (*E*)-2-butenoate] to which males are highly attracted. Reported here are results of research that documents a nearly 5-fold increase in male sweetpotato weevil catch in traps baited with this pheromone and a green light provided by a solar-powered, light-emitting diode (LED). The combination of olfactory and night-visible visual cues significantly enhanced trap effectiveness for this nighttime-active insect species. These results provide promise for improved sweetpotato weevil detection and suppression in mass trapping programs.

Sweetpotato, *Ipomoea batatas* (L.) Lamarck, based on total production, is one of the ten most important staple crops in the world[1], behind corn, wheat, rice, potatoes, cassava, and soybeans and barley[2], and is commercially grown in over 100 countries[3]. Sweetpotatoes are good sources of vitamins C, B2 (riboflavin), B6, beta-carotene (the vitamin A precursor; abundant in orange-fleshed varieties), as well as dietary fiber, potassium, copper, manganese and iron and have been relied on, in many countries, to provide food security[4]. Around the world, 270 insect and 17 mite species have been listed as pests of sweetpotato either in the field or in storage. Of these pests, weevils in the genus *Cylas* are typically most damaging[5], as pre- or post-harvest feeding of weevils induces production by the sweetpotato root of extremely bitter tasting and toxic sesquiterpenes which can render the sweetpotato root unfit for consumption[6]. Among *Cylas* spp., the sweetpotato weevil, *C. formicarius* (Fabricius) (Figure 1), is the most common pest species, with circumglobal distribution[7]. A significant step towards improved management of this weevil species was the identification of a female-produced sex pheromone [(*Z*)-3-dodecenyl (*E*)-2-butenoate] to which males are highly attracted, potentially being caught in high numbers in traps baited with low dosages of pheromone (10 μg)[6,8]. This pheromone has been used (at low doses) for population monitoring, but there have been several studies where the use of higher doses in a mass trapping program showed potential for its use (at higher doses) in population reduction through mass trapping, leading to reduced damage to roots. An IPM program in India, using mass trapping for sweetpotato weevil (1.0 mg lure per trap and one trap/100 m^2), reported reduction of weevil damage in sweet potato roots from 33% to 9.7% (1st season) and from 39% to 9.5% (2^{nd} season)[9]. Similarly, a mass trapping program in Taiwan, using 1.0 mg pheromone-baited traps for sweetpotato weevil, deployed at a density of 4 traps per 0.1 ha, reported a 57–65% reduction in damage to roots[10].

In the course of my ongoing research with another weevil species that attacks sweetpotato, the West Indian sweetpotato weevil, *Euscepes postfasciatus* (Fairmaire) (Coleoptera: Curculionidae), a means of significantly enhancing the attraction of the sweetpotato weevil to the male lure was discovered. It had been reported elsewhere that the West Indian sweetpotato weevil was attracted to green light[11–13]. In efforts to test the potential value of this attraction for purposes of monitoring and control of West Indian sweetpotato weevil in Hawaii, it was discovered that green light strongly synergized the attractiveness of the sweetpotato weevil male lure to the sweetpotato weevil. This paper provides documentation of this synergistic enhancement of attraction, an example of where improved control may come from incorporation of an additional attraction modality (here: olfactory and visual [that is visible at night]), rather than simply seeking an improved attractant within a specific modality.

Figure 1 | Adult sweetpotato weevil on sweetpotato root (photograph by Grant McQuate).

Results

Trial 1: Initial field trial using a low dose of male lure (12 μg) ± green light. The male lure that is typically used for sweetpotato weevil monitoring in sweetpotato fields is a rubber septum loaded with12 μg attractant. We began our testing with this dose, deployed in a funnel-type trap developed by ISCA Technologies, Inc. (Riverside, CA) that incorporated a green light (wavelength =515–520 nanometers) light-emitting diode (LED) that ran off of a battery powered by a solar cell on top of the trap. The trap had an integrated light sensor so that the light did not turn on until the ambient light dropped at dusk, then turned off at dawn (Figure 2). With traps placed in a sweetpotato field that had an established sweetpotato weevil population, we tested whether there was a difference in catch of sweetpotato weevils among traps with the sweetpotato weevil lure + green light, with only the sweetpotato weevil lure, with only green light, and with neither light nor lure. Irrespective of treatment, all weevils caught were male. Sweetpotato weevil catch differed significantly by site (F = 59.41; df = 1,119; p < 0.0001) (weevil populations varied from field to field), by treatment (F = 125.9; df = 3, 119; p < 0.0001), and in the site x treatment interaction (F = 64.60; df = 3, 119; p < 0.0001). Average weekly catch in the light + lure treatment (68.8 ± 14.0 [SEM]) was significantly greater than the average weekly catch in the lure only treatment (34.9 ± 12.2) which was significantly greater than the average weekly catch in either the light only treatment (0.50 ± 0.2) or the treatment lacking both light and lure (0.33 ± 0.066). Trap catch was higher in the light + lure treatment than in the lure only treatment in each of the ten weeks of the study. The addition of the green light to the male lure, on average, improved trap catch by 204% (range: 33.6–662% increase) (Figure 3 presents the overall treatment averages. The average catch, by treatment, for each of the 10 weeks of the trial, along with the average percentage increase in catch each week in the light + lure treatment over the lure only treatment, can be found in Supplementary Table S1 online). Weekly weather data averages over the first five weeks were 21.0 ± 0.35°C, 86.9 ± 1.4%RH, 1.46 ± 0.070 m/sec wind speed, and 63.3 ± 15.3 mm total rain.

Figure 2 | Solar powered green light funnel trap used in trials. A rubber septum, loaded with sweetpotato weevil male lure ((*Z*)-3-dodecenyl (*E*)-2-butenoate) is attached to a side column of the trap to facilitate positioning the rubber septum immediately below the green light (photograph by Grant McQuate).

Trial 2: Field trial using higher doses of male lure (12 μg, 120 μg, or 1.0 mg) ± green light. Because population control efforts based on mass trapping of male sweetpotato weevils have utilized higher doses of male lure, we also wanted to determine the extent to which the synergistic enhancement of trap catch by green light found when using a 12 μg male sweetpotato weevil lure also applied when higher lure doses (we used 120 μg and 1.0 mg doses) were used. Irrespective of treatment, all weevils caught in this trial were male, as we had found with the 12 μg dose study (Trial 1). Sweetpotato weevil catch differed significantly by site (F = 2.97; df = 3,63; p =0.0466), light (F = 212.4; df = 1,63; p < 0.0001), lure dose (F = 275.9; df = 3,63; p < 0.0001) and dose x light interaction (F = 56.7; df = 3,63; p < 0.0001). Average weekly catch in the light + 1.0 mg lure treatment (52.0 ± 6.4) was significantly greater than the average weekly catch in the 1.0 mg lure only treatment (9.4 ± 1.4). Average weekly catch in the light + 120 μg lure treatment (52.0 ± 6.4) was also significantly greater than the average weekly catch in the 120 μg lure only treatment (9.4 ± 1.4). Average weekly catch in the light + 12 μg lure treatment (3.6 ± 1.1) was greater numerically, but not statistically, than the average weekly catch in the 12 μg lure only treatment (1.1 ± 0.31). Average catch among the light plus lure treatments were significantly different in the order of 1.0 mg > 120 μg > 12 μg (Figure 4). The addition of the green light to the male lure, on average, improved trap catch by 455% (1.0 mg), 262% (120 μg), and 222% (12 μg) (Figure 4 presents the overall catch averages by treatment and the average percentage catch increase for the addition of green light for each lure dose. The average catch, by treatment, for each of the four weeks of the trial, along with the average percentage increase in catch each week for each lure dose in the light + lure treatments over the lure only treatments, can be found in Supplementary Table S2 online). These data help to explain the significant dose x light interaction by showing that the light-induced synergistic catch enhancement increases as dose increases. Weekly weather data averages over the four weeks of the trial were 21.5 ± 0.37°C, 82.6 ± 0.39%RH, 1.18 ± 0.072 m/sec wind speed, and 16.5 ± 10.1 mm total rain.

Discussion

Although discoveries and application of attraction of phytophagous insects to species specific semiochemical lures (olfactory stimuli) have been widely incorporated in integrated pest management (IPM) programs, insects are exposed to, and can respond to, multiple sensory cues[14–15]. The additional influence of trap color (visual stimulus) to insect trap response has been investigated for a wide range of insect pests (e.g., thrips [Thysanoptera: Thripidae][16]; leafhoppers [Hemiptera: Cicadellidae][17]; flower-visiting insects [Coleoptera, Diptera, Hymenoptera, Lepidoptera][18] including for sweetpotato weevil[19]. In recent years, testing of visual orientation has expanded to assessing response to light-emitting diodes (LEDs) instead of just

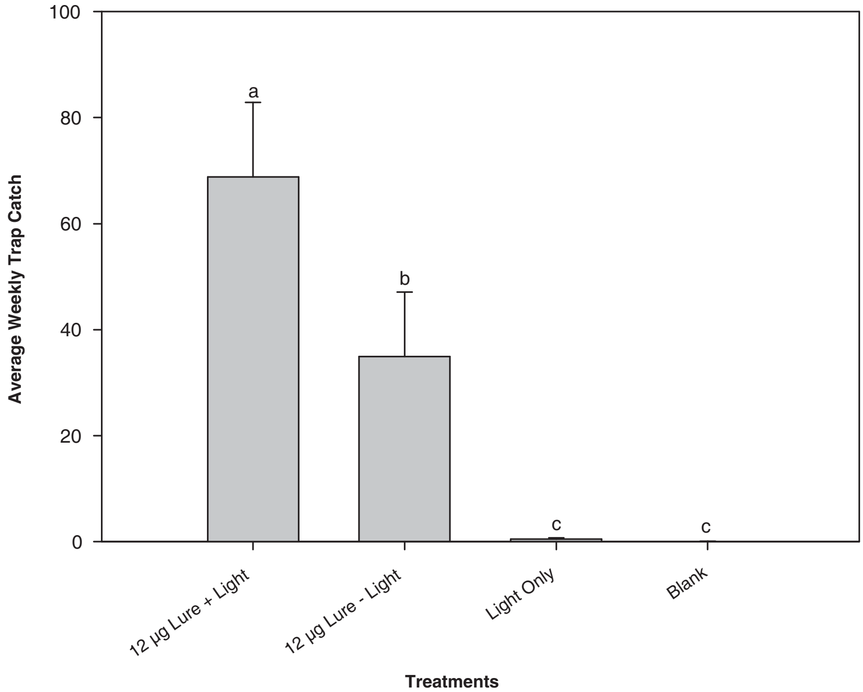

Figure 3 | Test of male sweetpotato weevil response to traps baited with male sweetpotato weevil lure (12 µg) ± green light. Average male sweetpotato weevil catch per trap per week (± standard error of the mean) in a sweetpotato field in Pepeekeo, Hawaii, in traps baited with 12 µg male sweetpotato weevil attractant and deployed with or without green light. Also shown are catch results in control traps of green light alone and traps lacking both lure and light. Trap catch of columns topped by the same letter is not significantly different at the $\alpha = 0.05$ level.

color reflected from pigmented surfaces[14] which has greater applicability to night active insects like the sweetpotato weevil. Knowledge of how arthropods respond to multiple concurrent sensory cues is limited[14]. The results presented here show that development of trapping systems for nighttime-active insects that tap both olfactory and nighttime visible visual stimuli can significantly enhance trap effectiveness. It is particularly notable that the sweetpotato weevil response to green light alone was very limited, yet the combination of the light with the strong semiochemical lure resulted in a major increase in trap response. An increase in trap catch in response to light (near UV: 390 nm) + an aggregation pheromone has also been observed with the red flour beetle, *Tribolium castaneum* (Herbst), relative to catch in traps baited with aggregation pheromone alone[20], but, in that case, there had been a good catch of beetles in traps baited with light alone and low catch in traps baited with the aggregation pheromone (a reversed relative response to light versus lure compared with what was observed in the present trial).

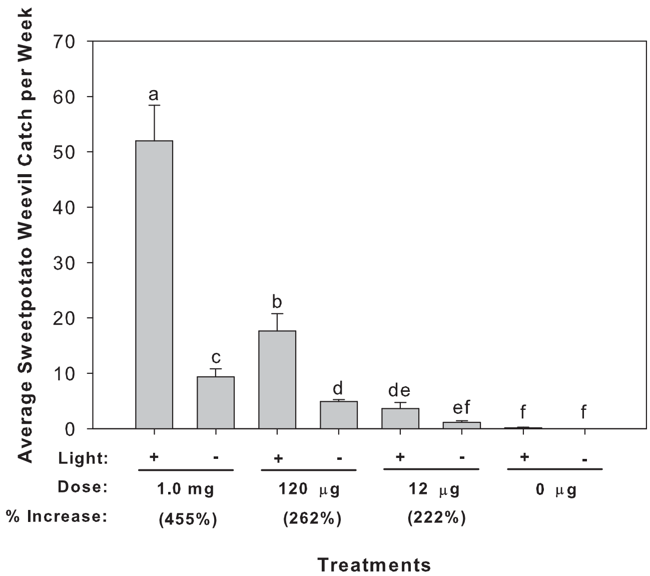

Figure 4 | Test of male sweetpotato weevil response to traps baited with different loadings of male sweetpotato weevil lure (12 µg, 120 µg or 1.0 mg) ± green light. Average catch/trap/week (± standard error of the mean) of sweetpotato weevils in traps baited with sweetpotato weevil male lure of three different loadings (12 µg, 120 µg and 1.0 mg) deployed with or without green light. Also shown are catch results in control traps of green light alone and traps lacking both lure and light. Trap catch of columns topped by the same letter are not significantly different at the $\alpha = 0.05$ level.

Testing in the present trial focused on the use of green light, the selection of which followed from prior research with the West Indian sweetpotato weevil. In that research, attractiveness of a range of LED colors (red, yellow, green and blue) was compared and it was found that light preference increased in the order of red, yellow, blue, green[13]. Although relative attractiveness of these other colors, or their relative potential for synergistic trap catch enhancement, have not been tested with the sweetpotato weevil, it can be initially hypothesized that relative response may be similar between the two weevil species, and testing of this hypothesis can be done. Another recent study reported that diffused UV light was more attractive to West Indian sweetpotato weevils than direct green light[21]. Using wind tunnel trials (GTM unpublished data), I have found no evidence for greater attraction to UV light than to green light by the sweetpotato weevil.

The mechanism accounting for synergistic sweetpotato weevil trap catch enhancement in male lure baited traps by the addition of green light has yet to be elucidated, but a hypothesis can be developed, drawing on earlier research experience with the West Indian sweetpotato weevil. In tests of attraction of West Indian sweetpotato weevil to UV light, it was observed that weevils are attracted to UV light, but are not induced to enter the trap (similarly, catch of sweetpotato weevils in traps baited only with green light tends to be quite low). An additional attractant is needed to induce the attracted weevils to enter the trap. In that study, sweetpotato bait on the inside of the trap was used to induce trap entry[21]. For sweetpotato weevil, it is hypothesized that light may be visible to the weevils at a greater distance than the male lure is readily detected. The attraction of the weevils to the green light may bring them to a closer point where they are able to detect, and respond to, the male lure, which leads to a higher catch rate.

In contrast to cases of trap catch enhancement, there have been a number of cases (with examples given below) where baiting of traps with both light and a semiochemical lure led to a loss of response to the semiochemical lure, and this phenomenon has been observed where the lure was a host odor, an aggregation pheromone or a female sex pheromone. In trials with the tropical root weevil, *Diaprepes abbreviates* (L. 1758) (Coleoptera: Curculionidae: Entiminae), weevils were attracted to green light and to the odor of its citrus host + conspecifics when the attractants were presented separately, but weevil response to the odor of its citrus host + conspecifics disappeared in the presence of a green light[22] (Otálora-Luna et al. 2013). The loss of response to an aggregation pheromone was reported for Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) when there was an addition of yellow light in a dark environment[14] (Otálora-Luna and Dickens 2011). Additionally, the loss of male response to a female sex pheromone in the presence of an incandescent light was reported for male *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae)[23]. These examples show that the effect of the addition of a lure based on a different sensory modality can vary considerably among different insect species.

The results reported here provide promise for improved management of sweetpotato weevil, both through improved detection and improved suppression in mass trapping programs. Further research, though, is needed to more completely document the weevil response relative to variations in the magnitudes of the two sensory modalities, such as changes in light intensity and lure dose. Further research is additionally needed to ascertain the degree of improvement that can be achieved in sweetpotato weevil population monitoring and reduction of weevil damage to sweetpotato roots using this new trapping system.

Methods

Attractant. For Trial 1 (see below), rubber septa, each loaded with 12 micrograms of (*Z*)-3-dodecenyl (*E*)-2-butenoate (Suterra, LLC [lot no. 129008190]), were obtained from Great Lakes IPM, Inc. (Vestaburg, MI). For Trial 2, rubber septa holding 12 μg, 120 μg or 1.0 mg of sweetpotato weevil (SPW) male lure, (*Z*)-3-dodecenyl (*E*)-2-butenoate, were obtained from Scentry Biologicals, Inc., Billings, MT. Septa were deployed in solar-powered green light (515–520 nm) LED traps developed by ISCA Technologies, Inc., Riverside, CA, USA (Figure 2). The green light bulb had a luminous intensity of 16,000–22,000 millicandelas (mcd), which provided an average illuminance of 17.7 (± 2.9 SEM) lux at 10 cm from the trap and 0.39 (± 0.07) lux at 50 cm from the trap.

Trial 1 – Initial field trial using a low dose of male lure (12 μg) ± green light. Beginning 24 Oct., 2012, three completely randomized blocks, each with 4 treatments (green light only, green light + male SPW lure, male SPW lure only, and trap without light or lure) were set out in a sweetpotato field in the vicinity of Pepeekeo on the Hamakua Coast of the Big Island of Hawaii (Universal Transverse Mercator [UTM] grid[24]: Easting 0276964, Northing 2194451, Zone 05 Q). Distance between blocks was at least 20 m, with a spacing of 5.0 m between traps within each block. Each trap held 0.5 ml Dawn Ultra dishwashing liquid (Procter & Gamble, Cincinnati, OH) in 300 ml water to drown attracted insects. Traps were serviced after one week, and all recovered weevils were sexed and counted. Following servicing, used rubber septa were replaced with fresh 12 μg loaded rubber septa and traps were re-deployed with a newly randomized complete block (RCB) design in another field. Traps were serviced and redeployed in this manner for 5 consecutive weeks, with the last service on 28 November, 2012. A second set of 5 weekly trap catches was begun on 15 January, with the last service on 19 February, 2013, giving a total of ten replicates, overall. A weather station was set up in the vicinity of the fields where traps were deployed to collect data on rainfall, temperature, relative humidity, and wind speed over the course of the first five week trapping period.

Trial 2 – Field trial using higher doses of male lure (12 μg, 120 μg, or 1.0 mg) ± green light. Beginning 19 March, 2013, two traps of each of 8 treatments (12 μg SPW rubber septum, 12 μg SPW rubber septum + green light, 120 μg SPW rubber septum, 120 μg SPW rubber septum + green light, 1.0 mg SPW rubber septum, 1.0 mg SPW rubber septum + green light, green light only, and no light and no lure) were set out in an RCB design in a sweetpotato field in the vicinity of Pepeekeo on the Hamakua Coast of the Big Island of Hawaii (UTM grid[24]: Easting 0279869, Northing 2191349, Zone 05 Q), with blocks spaced at least 5.0 m apart and traps spaced 2.0 m apart within each block. Each trap held 0.5 ml Dawn Ultra dishwashing liquid (Procter & Gamble, Cincinnati, OH) in 300 ml water. Traps were serviced after one week, and all recovered weevils were sexed and counted. Following servicing, used rubber septa were replaced with fresh rubber septa of the same lure loading and traps were re-deployed with a new RCB at least 80 m distant in another section of the same field, where trapping had not previously been done with the sweetpotato crop that was present. Traps were serviced for 4 consecutive weeks, with the last service on 16 April, 2013. A weather station was set up in one corner of the field where traps were deployed to collect data on rainfall, temperature, relative humidity, and wind speed over the course of the four week trapping period.

Statistical Analysis. Significance of differences of average sweetpotato weevil trap catch among the four treatments (Bioassay 1) or eight treatments (Bioassay 2) was tested using analysis of variance (ANOVA), following square root transformation (sqrt[catch +0.5]). For Bioassay 1, a two way ANOVA was used which permitted testing for significance of site and as well as differences in trap catches among treatments. For Bioassay 2, a three way ANOVA was used which permitted testing for significance of site and presence/absence of light as well as differences in trap catches among treatments. For both bioassays, Tukey HSD was used to test for mean separation. Statistical analyses were performed with JMP 10.0.0 (SAS Institute Inc.). Untransformed data are presented in the summary charts.

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Additional information

Supplementary information accompanies this paper at http://www.nature.com/scientificreports

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