Leaf-Rolling: A Response Oflarval Instars of *Sylepta Derogata* [Lepidoptera: Pyralidae] to Horizontal Gas Flare at Owaza, Rivers State Nigeria

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Abstract

Six larval instars of *Sylepta derogata*, a major pest of Okra [*Abelmoschus esculentus* [L.] Moench] were sampled in Okra traditional Farms located near a gas-flared station at Owaza in March 2015. Measurements of distances from the horizontal gas flare point to the traditional okra farms were recorded as 100, 150, 200, 250, 300, 350 and 400m where the last Okra Farm was located at a temperature range of $38-48^{O}$C. Control plots were located at Oyigbo, a non-flare source. Samples were taken from okra plants in search of rolled leaves.

Introduction

Gas flaring is the burning of natural gas and petroleum hydrocarbons in flare stacks by upstream oil companies in oil fields during operations. Gas flaring is the singular and most common source of global warming and contributes to emissions of carbon monoxide, nitrogen (II) oxide and methane which have the propensity of causing environmental pollution and ecological disturbances or destruction [Ubani and Onyejekwe, 2013]. Okra is attacked by quite an array of insects prominent among them are *Sylepta derogata*, *Dysdercus superstitiosus*, *Podagrica uniforma* and *P. sjostedti*, others include *Anomis flava*, *Earias biplaga*, *Aphis gossypii*, *A. craccivora* and others [Ogbalu *et al.,* 2015]. Varieties of Okra available in the Niger Delta had been listed [Ogbalu and Ekweozor, 2004]. Attacks on okra leaves start as early as the germination stage by termites [*Macroterme* spp] and *Podagrica* species that create circular, irregular and spherical holes on okra leaves of all ages apart from punctures they make on pods. *D. superstitiosus* causes punctures on okra fruits space especially during the dry seasons in Niger Delta Area of Nigeria. Some hemipterans including *Acanthocoris* spp, *Leptoglossus australis* infest okra attacking its pods. Reports on the biology of *S. derogata* in the eastern states of Nigeria had been provided [Anioke, 1988].

Changes in temperature affect the central nervous system by differential effects on various nerves. Studies on *Periplaneta americana* group nerve chord responses are in four classes: (1) those that increase firing frequency with an increase in temperature; (2) those that show transient changes in firing pattern; (3) those that are responsive over a limited range of temperatures; and (4) those unaffected by temperature changes. The nervous system is important in the regulation of the insect's response to high temperature by: (1) involvement of the perception of environmental temperature; (2) integration of this information with other sources (i.e. motor neurons, sensilla); (3) adjusting the patterns of insect behavior which will influence activity of the endocrine glands and patterns of muscular activity of the body (Clarke, 1967). The perception of environmental temperatures by the nervous system is accomplished primarily through the sensilla, but may also occur through the antennae, arolia and pulvilli of the legs, and some chemosensors are sensitive to thermal changes (Clarke, 1967).

Another example comes from research with codling moth and the effects of pre-storage heat treatments on larval mortality (Neven and Rehfield, 1995). A ramping function heat treatment at 4°C per hour to a final temperature of 38°C and being held there for 4 days did not kill fifth instar codling moth. However, a supernumerary molt to a 6th instar was observed. With the first example, the elevated temperature could have affected the endocrine system and prevented maturation of germ cells and perhaps inhibited the deposition of vitellin in the eggs. In the case of the supernumerary molt, it is most likely that the levels of juvenile hormone were too high to permit the formation of pupae and continuation of development. There are numerous examples in the literature on abnormalities, defects, and delays in development inresponse to thermal stress (Denlinger and Yocum, 1998)**.** Many of these may be attributed to the effects on the endocrine system, whereas others may be an effect of high temperatures on DNA.

In insects, delayed mortality had been observed. The insect may seem 'alive' following a heat stress, but may fail to complete development under normal growing conditions. There has been relatively little research done on the effects of heat stress on insect cells. Inferences on the cellular effects can be drawn from other systems. As temperature increases, the pH and ion concentrations are altered, and there are also dramatic effects on macromolecules such as proteins, DNA, RNA, lipids, carbohydrates, and on cellular structures such as cell and nuclear membranes, mitochondria and ribosomes. In general, as temperature increases, pH decreases.

Membranes of the cell, mitochondria, microsomes and nuclei are also vulnerable to thermal damage due to the effects on the phospholipid bilayer and other lipid components. Alteration of the liquid-crystaline fluidity of the membrane can alter the ionic balance of the cell, electric potential, and function of membrane-bound proteins. The cuticle is also sensitive to temperature changes. The wax of the cuticle is important in protecting the insect from its external environment and maintaining water balance. High temperatures can alter the wax complex to become more fluid and may lead to desiccation and death**.** The effects of high temperature on insect mortality in low humidity environments may be compounded with desiccation stress. However, a high temperature treatment in a highly saturated environment may lead to drowning, primarily due to the loss of cuticular protection of the spiracles leading to the tracheoles. There is no indication in the literature that can point to any one cause. However, recent research may begin to elucidate a cause.

Other work with scanning calorimetry may also help to describe energy balance of insects subjected to anoxic environments and thermal stress (Zhou *et al.,* 2000). There is an increase in the metabolic heat rate of codling moth and omnivorous leaf roller pupae as the temperature is raised from 10°C to 30°C. However, at temperatures above 40°C, there is a drop in the metabolic heat rate, indicating either a protective mechanism of energy conservation or the inability to produce an adequate supply of ATP to support elevated metabolism. It is apparent that whatever system is investigated as being affected by heat, differences will be found. The key is to determine the critical or most sensitive point in the system, which can be manipulated to make heat treatments most effective in controlling insects. The pupae and adults of *Tenebrio molitor* responded to high temperatures in laboratory by migration to the surface of maize grains [Ogbalu, 2018a]. It is also thought that coloration plays some role in the thermal economy of insects however,other opinions maintain that metallic colors in insects, like beetles, serve to reflect heat rays in sunshine.

The preferred temperature of an insect may vary during its life history. Thus in the housefly, migration from the feeding place (dung) to pupation site (ground) is due to behavioural changes in the central nervous system which modifies the temperature preferendum as the larvae grows. Other animals especially among the Invertebrates; Paramaecium, among protozoans, also exhibits selection of preferred temperature, larvae and adults of *Tenebrio molitor* react to response to higher temperature by occupying the surface levels of stored maize [Ogbalu, 2018].

This research explored and presented a method of assessing the environmental impact of gas flaring in the Niger Delta on larval forms of a phytophagous insect, *Sylepta derogata* that spent all their developmental stages on okra leaves.

MATERIALS AND METHODS

Okra plants grown at various distances from the horizontal gas flare points were sampled for leaf rolls by larvae of *Sylepta derogata* at the Owaza Gas Flare Station in March 2016. Samplers consisted of Five Final year Entomology students of 2014/2015 set who were natives. Being a traditional Okra farm, plantings were not done in a regular manner but planted in mounds with two Okra plants per mound with an irregular spacing of 15-30 cm in-between plants. Our sampling plan was the purposive type. Different samples at various proximities from the gas flare locations were taken and measurements and experimentations were meticulously carried out [Ubani and Onyejekwe, 2013]. Distances of 100m, 150m, 200m, 250m, 300m, 350m and 400metres from flared points to Okra plots were measured and recorded. Notes were made on heights of plants, number of leaves per plants, number and percentage of rolled leaves, number of eggs per leaf, number of larvae and stage of larvae per rolled leaf, number of pupae per rolled leaf, percentage defoliation [Ogbalu *et al.,* 2015].

Statistical Analysis: Statistical analyses were performed using SPSS software. A - Z-test of means was used to determine whether the mean number of larvae collected differed significantly between the nine plots of Okra. Pie charts were also used to show proportional distribution of data collected on the number of larvae that leaf-rolled from different instar stages. Correlation analysis was adopted to determine the relationship between number of larvae that leaf-rolled and distances from flared points.

The experimental Design was a Randomized Complete Block Design [RCBD]. Most of the data were analyzed using analysis of variance [ANOVA] and t-test analysis was used at 5% level of significance to establish significant difference in larval mortality in okra leaves near gas flare points and mortality in those far from gas flare points [GFP].In situations where there were significant differences between treatment combinations, Students' Newman Keule's [SNK] test, a furtherance of Duncan's multiple range test [D.M.R.T] was used to separate the means for a confirmation of significance. Pie charts were also used to show proportional distribution of data collected on the number of instars and percentage leaf rolled/mortality of larvae, in different plants.

RESULTS AND DISCUSSION

Entomology data showed variations in infestation rates of the pyralid moth larvae distributions in Okra farms at Owaza.Samples were taken from nine plants out of 18-30 plants per plot. Prominent among the larvae sampled were the first instar larvae of *S. derogata*which in Fig. 1 showed their response to heat at 100 metres distance, 15% [CI, 95%; 7.5-15.5] (Fig.2). showed that at 150m distance the percentage that rolled their leaves were lower than those that responded by rolling their leaves at 100m distance from the Flare point 12%[CI, 95%; 10.5-12.5].At 200m distance, first instar larvae showed higher ability to leaf roll, 19% [CI, 4.5-19.5]. As the distances increased from Flare points larvae of different ages showed increase in their behavioural

Fig. 1. Response to Heat From Horizontal Gas Flare in 1st Instars of *S.derogata*; Distance = 100m

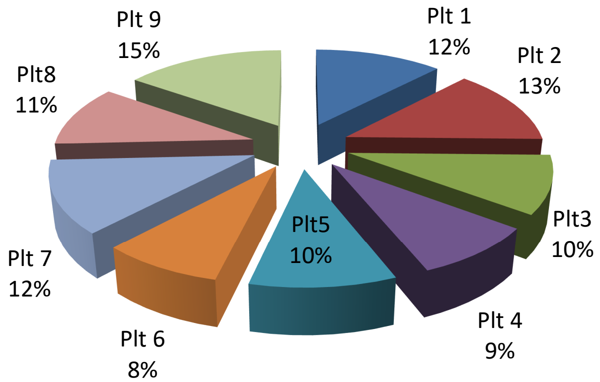


Fig. 2. Response to Heat From Horizontal Gas Flare in 1st Instars of *S.derogata*; Distance = 150m

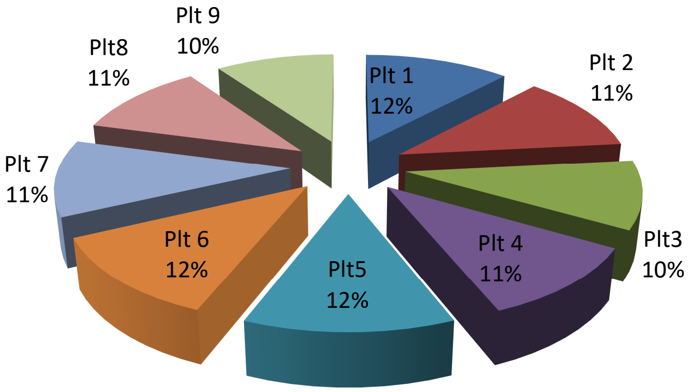


Fig. 3. Response to Heat From Horizontal Gas Flare in 1st Instars of *S.derogata*; Distance = 200m

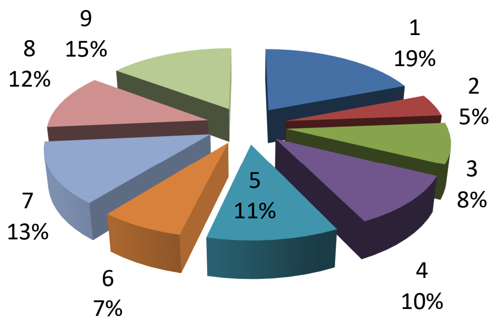
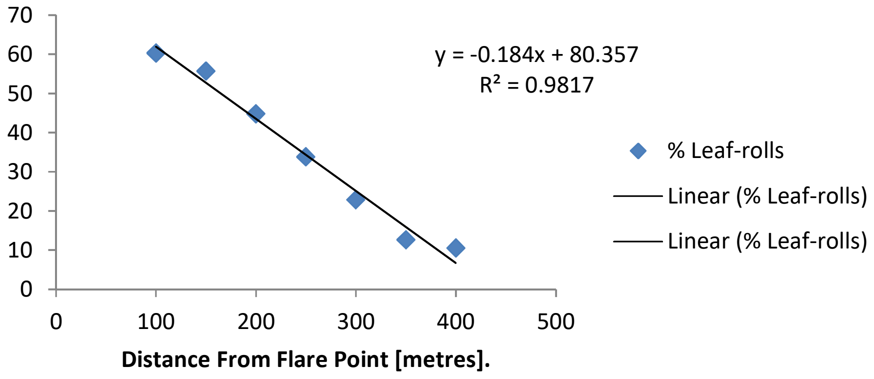


Fig. 4. Correlation relationship between Distance [metres] from Flare Points and % Leaf-rolls in First Instar Larvae of *S. derogata*



response to radiation from the Flare point among the nine plants sampled per Okra plot. The second instar larvae of the Pyrallid followed a relatively higher trend to leaf roll 35% [CI, 15.5-35.5]; the third instar larvae maintained a higher trend of 43% [21.5 – 43.5] and later and older instars that were mature to form webs for pupation readily leaf rolled, even most of the sixth instars being the last instar stage pupated within leaf rolls with apical openings for the emerging adults. All pupae at 400m distance and in control plots pupated outside the leaf rolls on the adaxial portions of the leaves. Leaf rollings in early instars were significantly higher especially in those nearer to flare points than those at 400m distance, except in higher instars [4th-6th] that usually leaf roll in preparation for pupation. Okra plants at close proximities with flare point showed abscission of leaves, flowers and premature pod droppings.Table 1 showed that larval instars [I and II] of *S.derogata*at 100m distance had significantly higher mortality than others. Being the early instars at the closest proximity to flare source and not covered nor leaf-rolled they suffered harsh desiccation and died.The quantity of carbon emitted by the flare was about 2525000.00 tonnes of carbon per day[Ubani and Onyejekwe, 2013]. These values portray a negative result for the affected invertebrates, pollinators and other bio-agents within the environment.

Table 1. Mortality of Larval Instars of *Sylepta derogata* on Okra Plants[*Abelmoschus esclentus*] Grown at 100-400 Metres From A Horizontal Gas Flare Source.

	Plot1/ 100m	Plot 2/ 150m	Plot3/ 200m	Plot4/ 250m	Plot 5/ 300m	Plot 6/ 350m	Plot 7/ 400m	plot 8
First Instars	77b	43a	44b	23b	24b	12c	18b	12c
2nd Instars	88a	22c	30c	12d	14c	10c	13c	18b
3rd Instars	23d	44b	48a	42a	38a	56a	35a	21a
4th Instars	32c	12d	19d	16c	14c	17b	0f	0d
5th Instars	17e	7e	4e	2e	6d	4d	8d	0d
6th Instars	11f	13d	5e	0f	0e	0e	3e	0d

Figures in the same Column with different letters were significantly different from each other [D.M.R.T; SNK, $P < 0.05$].

Aging tissues (especially senescing leaves) and nodes of stems produce ethylene. The best known effect of the hormone, however, was the promotion of fruit ripening. Ethylene stimulated the conversion of starch and acids to sugars [Ogbalu, 1999]. Ethylene also triggered leaf and fruit abscission, flower fading and dropping, and promoted germination in some cereals and sprouting. Apart from the role of ethylene in the abscission of both leaves and pods, the positive role of the feeding activities of *S. derogate* larvae cannot be over emphasized. Physiologically, they fed on the basal portions of the okra pods causing dislodgements of tissues and regional porosity presenting a tendency to abscission. The feeding activities of other insects might have also accounted for defoliation and abscission [e.g. *Earias* spp, *Zonocerus variegatus*].The feeding impact of *S. derogata*larvae on Okra showed a strong correlation between number of larvae and number of leaves rolled [r = 0.94]in this study. Ogbalu *et al.,* 2015 further reported on a high correlation that existed between number of larvae and percentage of leaves rolled [r = 0.82]. Apparently, the proximity of the flare point impacted higher leaf rolling than in previous study that was conducted in a non - gas flaring environment. Although leaf-rolling has been an attribute of older instars in preparation for pupation and earlier instars leaf-rolled to protect themselves from heat of the gas flares. Fig. 4 showed a negative correlation.

Older *S. derogata* larvae exhibited higher tolerance as they had to flush out their digestive contents prior to pupation. After the period, most Lepidopterans entered into a non- feeding period with little or no activities, not increasing their energy uptake due to low or no mobility and no heat gain in this quiescent period. Moreso, their heavily sclerotized cuticle protect their internal organs from environmental heat waves. Apart from the aforementioned attributes of *S. derogata* larvae elaborated in respect to heat stress in the study, the early instars had imbibed some parental imprint behaviors that helped them to evade heat stress; also these stages [[I-VI instars] have low mobility and the first instars had just emerged from hatched eggs which were deposited on the adaxial sides of Okra leaves where the temperatures are cooler on hot days. Samplings during this study coincided with intermittent rainfalls of March in the Niger Delta which promoted survival of the larvae as environmental temperatures lowered. Other studies involving insects developmental stages showed a relatively high level of heat tolerance as in *Drosophila buzzatti* [Krebs and Loeschcke, 1995; Son and Lewis, 2005]. From our findings, first instar larvae showed the lowest leaf rolls following exposure, whereas 3rd-instar and other older instar larvae were relatively heat resistant. Apart from their ability to leaf roll, they resorted to migration to plants farther away from heat source especially at nights. Fig. 5 showed both percentage leaf rolls and abscission decreasing as distance from heat source increased except for stages that had reached pupal stage.

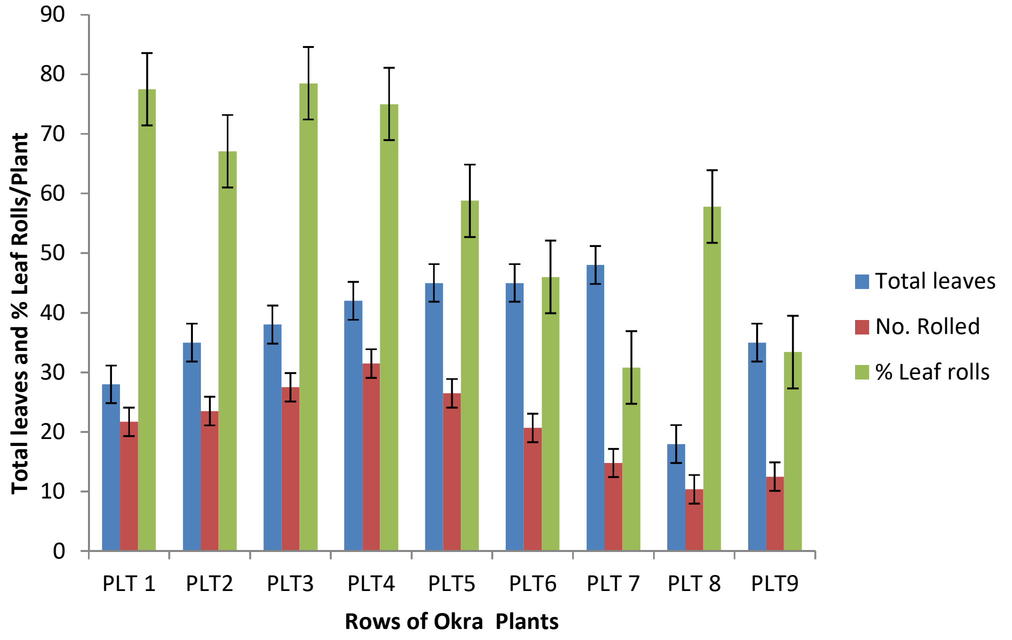


Fig. 5. Assessment of Leaf Rolls by *Sylepta derogata* in Okra Plants Exposed to Gas Flare Heat, Owaza.

All the aforementioned parameters of abscission of leaves and pods, defoliation of leaves, leaf rolling and skeletonization of leaf areas were presented as some of the factors that causing reduction in photosynthetic activities in Okra plants [Ogbalu *et al.,* 2015]. Also the oviposition patterns of females *S. derogata* on okra leaves reduced photosynthetic activities as the deposited eggs cover a reasonable area of the leaf [Ogbalu *et al.,* 2015]. However the number of eggs observed in the present study was very insignificant as it appeared the eggs might have hatched prior to samplings, predated on by ants [*Oecophylla longinoda*] or that the gravid females resorted to deposition of eggs within the leaves or in Okra flowers. Definitely, higher infestations affected the physiological functions of plants' tissues impacted by the gas flare. During evolution, insects evolved physiological and behavioural responses by elevating antioxidant defenses and synthesizing heat shock proteins. Also, physiological responses to temperature change in insects during development, however some studies have assessed the sensitivity of different developmental stages to heat stress through the measurement of survival in insects. In this study, we measured leaf rolling as a response to heat stress in larval instars of *S. derogata*; heat exposure to larvae in the field was resisted by both early and later instars depending on their proximity to the flare source. *S. derogata* are known to cope with heat stress as during the dry seasons, they are engaged in early leaf rolls to escape from excessive heat. Other factors such as oxygen depletion, excessive increase in environmental temperature might have contributed different responses from insects' larvae [White, 1981; Whitting et al. 1991].In the Pyralids, *S. derogata* larvae resorted to leaf rolling of okra leaves which were about 35 times their body size.

Conclusion: Our study was able to establish that Gas Flaring in the Niger Delta affected crops of economic importance resulting in premature abscissions of both leaves and pods causing the mortality of insects that aided pollination. Also both early and older instars of *Sylepta derogata* exhibited heat resistance in the field through leaf- rolling and other behavioural procedures.

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