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RESEARCH PAPER

SOURCES OF INFESTATION, BIOLOGY, DAMAGE BY AND CONTROL OF *MEGASELIA RUFIPES* MEIGEN (DIPTERA: PHORIDAE) ON OIL PALM SEEDS

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ABSTRACT
A study was carried out to identify sources of infestation by the Megaselia rufipes Meigen (Diptera: Phoridae) on some germinated oil palm seeds in the Seed Production facilities of the CSIR-Oil Palm Research Institute. The study also covered the identification of the pest, some aspects of the biology, cost of damage and control. It was observed that the sources of infestation was from poor sorting of seeds including broken and dead seeds mixed up with healthy seeds which were sent to the Germinator house from the Seed-store for initiation of germination process. Such unhealthy, poor quality seeds undergo fermentation during post-heating soaking process, emitting attractant(s). Adult flies which had hatched out in the Germinator room responded to the attractant(s) when such poor quality seeds were exposed in the open for air-drying. The flies' activities caused damage to seeds including rotten kennels and seeds, empty shells and dead developing embryos in transparent polyethylene storage bags. The highest infestation was on 2052 seeds out of a total production of 582,503 germinated seeds in batch number 5 and the lowest was 223 seeds out of 241,089 seeds in batch number 6. Fermenting seeds among healthy germinating seeds in improper sealed/ broken storage polyethylene bags attracted adult flies which gained access to the seeds through holes. The fly was identified as Megaselia rufipes Meigen (Diptera: Phoridae). Its pupal duration was found to be 9-10 days. Bioassay results showed that the fly could be controlled effectively by dipping the seeds in Fenitrothion insecticide.

Keywords: *fly, insect pest, Megaselia rufipes, oil palm seed, infestation*

INTRODUCTION
Oil palm, *Elaeis guineensis* Jacq., is an important commercial tree crop in Ghana. The oil palm industry provides employment to millions of Ghanaians especially in the rural communities. Palm and kernel oil are processed into various vegetable cooking oil, perfumed soaps, margarine, oleo-chemicals and bio-diesel. Sap extracted from the oil palm tree can be drank as an alcoholic beverage or processed into various types of alcohol for industrial use. The Council for Scientific and Industrial Research (CSIR)-Oil Palm Research Institute (OPRI) which is the sole producer of oil palm seeds in Ghana

has produced an estimated 40 million germinated seeds from 1967 to 2008 (CSIR-OPRI, 2008). Some of these seeds have been exported to countries such as Togo, Nigeria, Sierra Leone and Tanzania.

In 2007, some flies, suspected to be *Megaselia* sp., infested some germinated seeds in the Institute's production facility. This posed a serious challenge with regards to the quality and quantity of germinated seeds produced. Infestation by the flies on oil seeds is not very common. *Megaselia* species accounts for nearly half of the 3000 family of Phorids and are among the Phoridae which are better known insects in forensic entomology rather than in Agriculture (Smith, 1986). *M. rufipes* tends to colonize human corpses that are disposed off in exposed states (Smith, 1986). The scuttle fly, *Megaselia scalaris* Loew, is a polyphagous saprophage species that develops in an incredible diversity of host materials including meat, insects and a broad selection of decomposing plants (Disney, 1994a; Robinson, 1971), and is also associated with human remains (Leclerq and Verstraeten, 1993). The known natural history of *Megaselia* and other phorid flies was reviewed by Disney (1994a). *Megaselia* species make a major contribution to the assemblages of Phoridae, especially after disturbances or stress such as clear cuttings (Disney, 2004; Durska, 2001; 2009) and among the pioneer fauna re-colonizing such habitat.

Since there is no documentary report on fly infestation on germinated oil palm seeds, a study was initiated to identify the sources of infestation, determine the identity of the fly and investigate some aspects of the biology, damage and control with the view to managing the fly infestation. The study was also important for documentation of the fly as a major or minor pest or an insect which has some association with oil palm seeds under certain conditions.

MATERIALS AND METHODS

The study was carried out from September, 2007 to November, 2008 at the CSIR-Oil Palm Research Institute (OPRI) at Kusi in the Kwaebibirim District in the Eastern Region of Ghana.

Identification of fly

Five adult insects from a collection of infested seeds were preserved in 70% ethanol. The body structure of the fly (head, antennae, thorax, abdomen, legs and wing venation) was examined under the microscope (WILD HEERBRUGG M5). The family of the pest was then determined using CABI BIOSCIENCE Identification Manual (Harris and White, 1998). Ten samples of live adult specimens were also sent to the Zoology Department of the University of Ghana for further identification.

Investigation into sources of infestation

Visits were made to the Germinator house of the Institute to trace the sources of fly infestation. Observations were carried out on the following materials and places for developmental stages of the fly:

i. A sample of 24 transparent polyethylene bags of dimension 64 X 78cm containing 1,000 oil palm seeds per bag.

ii. A sample of 24 storage bags containing 1,000 seeds per bag including some infested ones to identify possible entry points for the adult flies.

iii 10,000 soaked post-heated oil palm seeds being dried in the open at room temperature in the drying room.

iv. Storage shelves on which oil palm seeds were stored at room temperature.

v. Seeds on benches in the drying room being sorted for germinated seeds.

vi. Debris from 5,000 oil palm seeds on sorting-benches.

vii. Debris from sorted seeds in five covered petri dishes in the laboratory at room temp-

preture.

viii. Seeds in transparent polyethylene bags of dimension 64 X 78cm containing 1,000 oil palm seeds per bag undergoing heating process (mean temperature of 40^{o}C) in the heating room.

Visits were also made to the oil palm Seedstore to examine the following materials for signs of adult flies: freshly harvested fruits, fruits in storage ready for depulping, freshly depulped seeds and seeds in storage. Data on germinated seed produced and seeds discarded due to the fly damage in 2008 were obtained from the record book at the Germinator house.

Biology of the fly

Infested seeds in 10 polyethylene (1,000 seeds/ bag) bags of dimension 64 X 78cm each were examined to see where the flies lay their eggs, stages of the larvae, activities of the maggots, nature and duration of pupation, and adult emergence. For the determination of duration of pupation, hundred maggots were collected from infested seeds and placed in a 7.5cm diameter petri dish with a cover without any lining. Three samples, each of 10 maggots which pupated on the same day were collected from the petri dish and placed in 3 separate petri dishes at mean room temperature of 29^{0}C and 82% relative humidity for observation of adult emergence.

Damage assessment

One hundred seeds were sampled from 1,000 seeds in each of 19 new set of samples of polyethylene bags of infested seeds in storage. The seeds were cracked open and damage to the kernels and conditions in the cracked nuts were categorized and scored using an arbitrary scale from 0-3 (Table 1). The damage was quantified in percentage using the formula:

Percentage (%) $A = \frac{t}{T} X\ 100\%$

Where A is the percentage of the category;
t is the mean of the number of seednuts in the the category of the 19 samples from the 19 bags;
T is the mean of the total number of seednuts sampled from the 19 sampled polythene bags of seednuts.

Infested seeds in the bags were also examined to ascertain the conditions of the infested nuts and the nature of external damage on the nuts. The data on total number of seeds destroyed by the flies within the 7 week period of infestation was obtained from the record book at the Germinator house and used to calculate percentage cost of damage due to the fly.

Laboratory Bioassay

Insecticide trials comprising of two chemicals {Dursban 4E Chlorpyrifos ethyl, and Fenitrothion 50 EC Fenitrothion)} were set up in the laboratory to determine the effective chemical and dosage for control of the fly. Dursban insecticide was chosen because it was already being used for dipping of seeds before storage whilst Fenitrothion was being used at the oil palm nursery to control nursery insect pests. Ten pupae were introduced into each of the five petri dishes (7.5cm diameter each) after dipping the pupae into the following particular concentration of the two insecticides for about 30 seconds:

Distilled water: 100ml H_2O (D_0 or F_0)
1ml/ 100ml H_2O (D_1 or F_1)
2ml/ 100ml H_2O (D_2 or F_2)
3ml/ 100ml H_2O (D_3 or F_3)
4ml/ 100ml H_2O (D_4 or F_4)

D_0 and F_0 represent the distilled water control in the Dursban 4E and Fenithrothion treatments respectively whilst D_1, D_2, D_3, D_4 and F_1, F_2, F_3, F_4 represent the corresponding concentrations of treatments in the two insecticides used. The petri dishes were arranged in a completely randomized design.

The set up was replicated 5 times. Data were recorded on adult emergence under the different insecticide concentrations for the two chemicals for 20 days after which the set up

was discarded. The data were subjected to statistical analysis using SPSS version 11.5. Bar and line graphs were also used where necessary.

Table 1: Categorization of damage on oil palm seeds

Damage Categorization	Damage Score	Damage Description
Wholesome seeds	0	Kernels wholly intact and undamaged
Damage seeds	1	kernels partially eaten by larvae
Empty kernels	2	kernels wholly eaten by larvae
Rotten seeds	3	kernels rotten

Background to induction of germination
Induction of germination
Stored seeds ready for germination processing are first removed from the Seed-store and are counted in 1,000 lot /small nylon bags (50 X 50cm). The seeds are then taken to the Germinator house where they are soaked in ordinary tap water in a tank for seven days. The water in the tank is changed each day. At the end of the seven days, the seeds are treated with fungicide (Diathane M45) before air-drying at room temperature in boxes on shelves for 24 hrs. The seeds, still in 1000 lots are transferred into transparent polyethylene bags (dimension of 64 X 78cm) at 1000 seeds/bag. The open end of the bag is firmly secured by tying with cotton string.

Bags of seeds prepared thus are taken into the heating room for heat treatment at temperature between 39 and 40^{o}C for 70 days. After the heat treatment, the seeds are removed from the polyethylene bags and transferred into small nylon bags again in 1000 lots/ bag and the open end again tied with a string, and then soaked in ordinary tap water in a tank for a period of 4 days. The water in the tank is again changed each day for the duration of soaking. At the end of the 4th day, the seeds are again treated with the fungicide and air-dried at room temperature in boxes at 1,000 seeds/ box for 2 hrs. The seeds are then bagged and sealed with a cotton string again in a transparent polyethylene bag (dimension of 64 X 78cm) at 1,000 seeds/ bag and stored on shelves for 14-21 days. After the 21 days, the seeds are inspected and the germinated ones taken out. After this sorting, the process is repeated after 14 days and again 21-28 days after the second where necessary. Sorting of germinated seeds in a typical bag is done for a period of 8-10 weeks before disposal of ungerminated seeds by burning. Sorted germinated seeds receive insecticide treatment (Dursban 4E) by spraying with a hand sprayer before bagging in sealed polyethylene bags for sale or for nursery establishment.

RESULTS
Fly identification
Preliminary investigation showed the suspected insect belonged to the order Diptera and family Phoridae. Further taxonomic identification at the University of Ghana, Zoology Department, identified the fly as *Megaselia rufipes* Meigen (Diptera: Phoridae). This is the first documented report of the association of the fly with oil palm seeds in Ghana. Figs. 1 and 2 show the pictures of the head and wing venation of the fly *Megaselia rufipes.* The first record of *M. rufipes* from New Zealand was as a new species erroneously described as *M. omnivora* (Hudson, 1982). It is easily recognized by the blunt, dense spines on the tergites of the male abdomen and the presence of 5 large alular setae (Disney, 1994b).

Sources of infestation and damage
M. rufipes were found in the depulping area near the Seed-store, on harvested fruits which were yet to be depulped, freshly depulped seeds

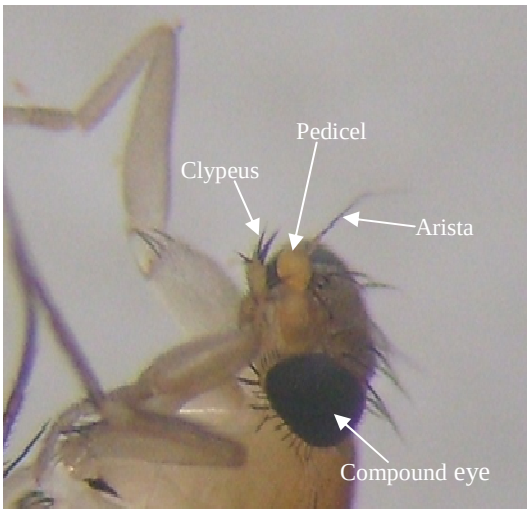

Fig. 1: Head of *M. rufipes*

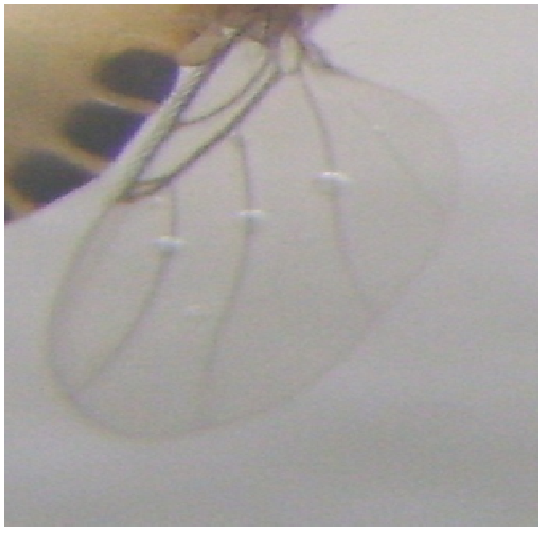

Fig. 2: Wing venation of fly

and seeds in the drying room. Inspection at the Germinator house showed that adult *M. rufipes* had not only infested germinated seeds in some bags but were also found hovering outside a mixture of newly germinated and ungerminated seeds in sealed bags awaiting sorting. They were also found on already sorted ungerminated seeds in sealed bags being stored for further sorting. Samples of 100 seeds from infested bags which were cracked-open showed many damage kernels, empty kernels, partially eaten rotten kennels and shells with their kernels completely eaten leaving only loose testa of kennels inside them. Fig. 3 below shows the damage distribution in samples of infested seeds in some sampled bags.

The percentage of undamaged seeds were above 50 and significantly different from the damaged seeds, rotten seeds and empty shells. Total seeds damaged by the fly within the initial 7 weeks from the record book at the Germinator house was 10,500 (percentage cost of damage was 1.29% amounting to GH¢2,730) out of a total production of 811,715 from September to December, 2007.

Fig. 4 shows the distribution of production of germinated oil palm seeds in 2008 and infested/ damaged seeds due to the pest. The highest infested seeds occurred on batch 5 in October and lowest on batch 6 in November.

Inspection of seeds stored in sealed bags in the drying room did not reveal any *M. rufipes* (maggots, pupae and adults). Those seeds had passed through induction process of heating and water treatments for germination process to commence in the bags. However, infestation of seeds in some bags was noticed in the drying room at the Germinator house a few days after sorting of germinated seeds. Infestation and damage on seeds in such contaminated bags increased with subsequent sorting.

Close observation showed that adult flies hovering in the Germinator room readily gained access to the seeds during sorting on benches in the drying room. The flies lay many eggs on such nuts especially decaying ones. The Seed-store was found to play a major role in the pest infestation in terms of quality of seeds sent to the Germinator house. Inspection of seeds in storage boxes at the Seed-store showed that such seeds included cracked seeds due to poor

sorting. Such cracked seeds go through fermentation and decaying during the processing of germination initiation and provided ideal condition for adult flies to lay eggs on them during drying period and sorting of geminated seeds. Such fermented and decayed seeds also provided ideal condition for fungal growth which spread disease among the germinated seeds.

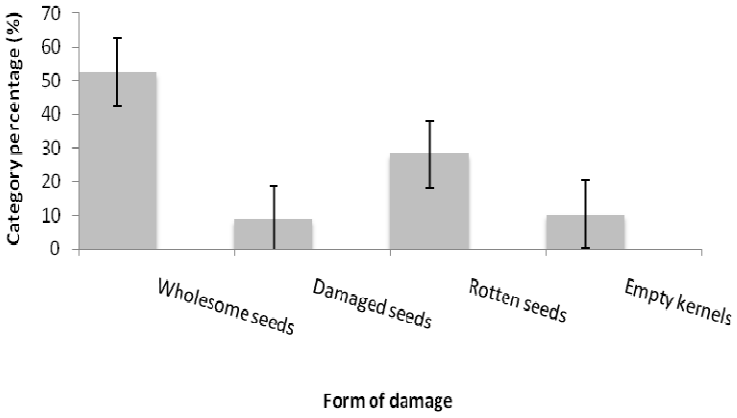

Fig. 3: Mean damage of oil palm seednuts by *Megaselia rufipes*

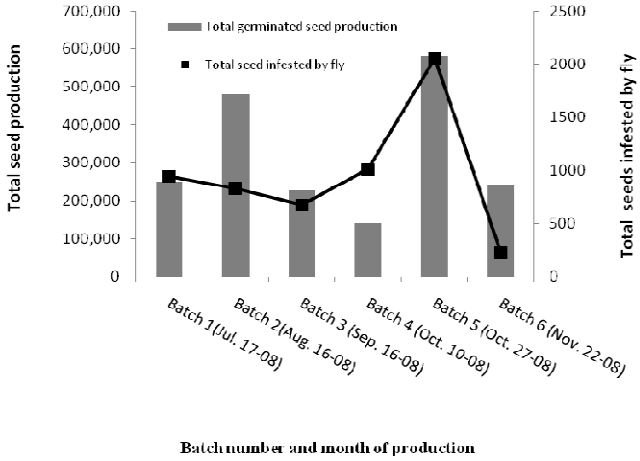

Fig. 4: Distribution of germinated seed production and fly infestation in 2008

Eggs were also laid on kernels through cracked seeds. Eggs thus hatched out in the unsorted left-over seeds in bags and possibly some of the nuts bagged for sale.

Depending on the duration of storage, the flies multiply in the warm moist condition in the storage bags and attack healthy seeds causing damage or destruction to the kennels and embryos in the bags. Adult insects also gained access to sealed bags through holes sustained through poor handling or poor sealing of bags. In one particular instance, adult flies were found in freshly unopened polyethylene bags of seeds and close examination of the polyethylene bags revealed holes which served as entrance for the flies. Maggots, pupae and adult insects were noticed in cracked seeds kept in a covered petri dish in isolated places in the laboratory for 3 days. The hatched larvae developed in the seeds by feeding on the kernels. They later emerged through the germpoles of the seeds and then pupated on the seeds and inner surface of the polyethylene bags. It was also noticed that the surface of the bench on which sorting of germinated seeds was carried out was disinfested only once a day and just before sorting. Both infested seeds and seeds from uninfested polyethylene bags were thus sorted out on the same bench one after the other without disinfesting the bench between them. This could have resulted in contamination of fresh seednuts.

Fig. 5: Cracked seeds infested with maggots

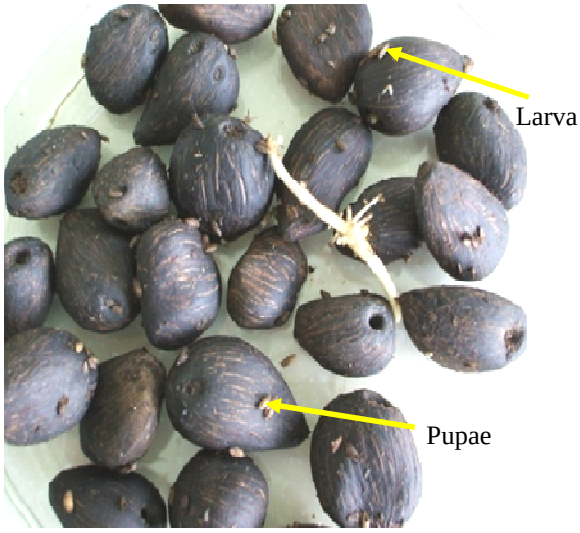

Fig 6: Seeds infested with fly stages

Some description and biology of *Megaselia rufipes*

Fig. 7A-D shows pictures of eggs, larvae, pupae and adult *M. rufipes*. The whitish spindle-shaped eggs (Fig. 7A) were laid indiscriminately on any surface. They were found on shells of seeds, kernels through cracks, broken seeds and on the inner surface of polyethylene storage bags. The larva (Fig. 7B) is creamy-white and about 3mm long. The body is broad at the posterior end which tapers towards the anterior end. The pupa (Fig. 7C) is light-brown, boat-shaped and about 2mm in length. The adult (Fig. 7D) is greater than 2mm in length. It has brown thorax with black hair, black and light-brown striped abdominal tergite. The abdominal sternite is cream-white. The femur of the hind leg is thick. The compound eyes are black.

This study showed that the larvae developed within the shells, on surface of the seeds, rotten

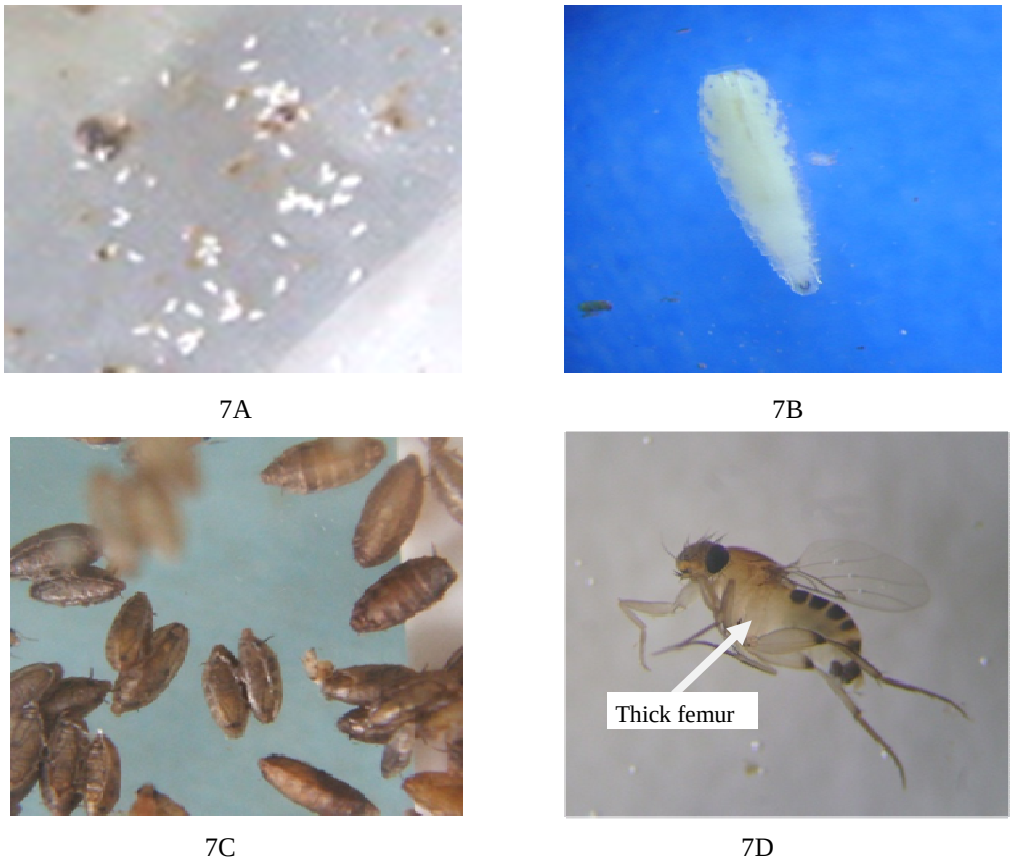

Fig 7: Pictures of eggs (A), larva (B), pupae(C) and an adult *Megaselia rufipes* (D)

roots and debris in storage bags. Majority of the larvae were found to pupate on the inner surface of polyethylene bags containing the seeds. The pupation period of the fly ranged from 9-10 days. Few pupae were found inside the shells of the cracked seeds (Fig. 8). The adults are highly mobile.

Bioassay

Fig. 8 below shows the results of the insecticidal bioassay using the insecticides Dursban 4E and Fenitrothion on the pupae of the fly. It shows a decline in adult emergence as the chemical dosage increased from D_0 to D_4 or F_0 to F_3 exception being F_4 which showed slight increase over F_2 and F_3. With respect to Fenitrothion, the dosage F_2 and F_3 had the same adult emergence. There were no significant differences ($p>0.05$) between D_0 and D_1 and between D_1 and D_2. The emergence due to these doses however differed significantly ($p<0.05$) from the emergence due to D_3 and D_4. There were no significant differences ($p>0.05$) between the emergence due to D_3 and D_4. There were significant differences ($p<0.05$) between the adult emergence of the control F_0 and F_1 to F_4 dosages. Significant differences ($p<0.05$) also existed between adult emergence of F_1 and F_2 to F_4 dosages. F_2, F_3 and F_4 were not significantly different ($p> 0.05$) among themselves.

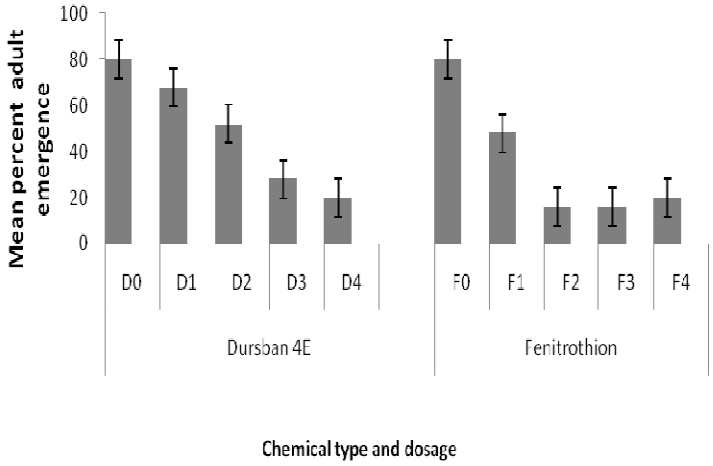

Fig. 8: Mean percent adult *Megaselia refipes* emergence from pupae after chemical treaments

DISCUSSION

M. rufipes is opportunistic because certain conditions such as decaying seeds make the seeds in storage vulnerable to attack if the storage bags have access holes on them. The polyphagous nature of *Megaselia* species might explain the reason why *M. rufipes* infested the oil palm seeds. The life history of *Megaselia* included scavenging, herbivory, predation and parasitism. *M. aurea* Aldrich is a widespread scavenger easily attracted to and reared from dead insects. The female has an unusual behaviour of forming mating swarms (Sivinski, 1988). The adult fly was attracted to odour emanating from damaged oil palm seeds undergoing fermentation and decay. The exposed nature of the seeds during drying after post-heating soaking process provided ready access to dead or damaged decaying seeds on which gravid female adult flies laid their eggs. The warm humid moist condition in the storage polyethylene bags during storage of the seeds provided suitable micro-climate for hatching of the eggs and the development of the larvae, pupae and emerged adult flies using the kernels as food source. The flies thus multiply rapidly and attack and cause damage to other healthy seeds and germinating seeds in the storage bags. Larvae of *M. scalaris* have been reported to be developing on a wide variety of host materials including a broad selection of decomposing plants (Robinson, 1971). Fig. 3 showed that damage on seeds including rotten and empty seeds caused by stages of the fly exceeded 45%. Although the percentage undamaged seeds were significantly different ($p< 0.05$) from the damaged, rotten seeds and empty kernels, the percentage difference was very small indicating the serious losses the pest could cause to seed production.

Though studies on other biological aspects of the fly were still on-going, the pupation period was determined to be 9-10 days. The studies showed that the larvae developed within the shells using the kernels as food source thus causing damage and rottening of the kernels. Most of the larvae emerged through the germpoles of the seeds when about to pupate. A few pupate on the surface of the seeds. Majority of the larvae were found to pupate on the inner surface of the storage polyethylene bags con-

containing the seeds. Few pupae were found inside the shells of the cracked seeds. The behavior of the larvae in pupating outside the shell and the few pupae found within the shells may suggest restricted space within the shell for the many larvae ready to pupate.

The bioassay results using the insecticide Dursban 4E and Fenitrothion 50EC on the pupal stage of the fly showed a decline in adult emergence as the chemical dosage increased from D_0 to D_4 or F_0 to F_3 exception being F_4 which showed slight increase over F_2 and F_3 although there were no significant differences ($p>0.05$) among them (Fig. 8). The application of Dursban 4E on the pupae showed a steady decline in adult emergence as chemical dosage increased (Fig. 8). However, with the application of Fenitrothion, there was a cut-off after F_1 whereby any increase in dosage did not bring any decline or significant change in adult emergence. It must be noted that almost all adult flies which emerged after Dursban 4E application lived for at least a day before dying whilst adult flies which emerged after Fenitrothion application especially F_2-F_4 appeared weak and died shortly or a few hours after emergence. Fenitrothion thus has more adverse effect on the flies than Dursban 4E. Indeed, earlier control measure using Dursban 4E at dose rate of 4 ml in 100 ml water against the flies on seeds in storage bags could not destroy the flies when infestation was first detected. Between the two insecticides therefore, Fenitrothion is the preferred option and the recommended dose rate should be the lowest after the cut-off dosage i.e. F_2 or 2 ml in 100 ml water.

The large number of seeds (10,500) destroyed within 7 weeks showed how negatively the flies can potentially impact on germinated oil palm seed production over a longer period of time at the CSIR-OPRI, Kusi. This could lead to grievous consequences for the Institute to meeting its supply commitment both locally and internationally. This highlights the urgent need to prevent the flies from establishing at the Seed Production Unit.

CONCLUSION

Information on studies of fly infestation on oil seeds especially oil palm is very scanty but vital. The studies on this opportunistic fly infestation on oil palm seeds thus succeeded in the identification of the fly. Some aspects of the biology, sources of infestation and opportune conditions that allow the fly to infest the oil palm seeds are now known. Cost of damage to oil palm seed production can be very high if control measures are not taken. The fly can be controlled by insecticides locally available.

RECOMMENDATION

As stated above, substantial quantities of seeds have been lost through the action of these flies and urgent action is needed to manage the flies in the Germinator room. While investigations on other biological aspects are still on-going, the following measures are recommended to bring the situation under control:

a. The surface of the sorting bench should be disinfected with 70% ethanol after every sorting of seeds from each polyethylene bag.

b. The Germinator House should be fumigated with Fenithrothion 50 EC using Swingfog machine to control the adult flies hovering in the room on Friday between 3 - 4 pm when workers have closed from work. The weekend holiday will allow the chemical to disperse before the workers resume on Monday, thus avoiding contamination of the workers.

c. The windows in the drying room should be covered with fine nets to avoid re-invasion after fumigation of the room. The doors should also have net screens to prevent adult flies from entering the room. The doors can also be rotating/ trap doors to reduce entry of flies.

d. All poor quality seeds including cracked and damaged seeds should be removed and destroyed during sorting in the Seed-store before they are sent to the germinator

house to induce germination.

e. All infested seeds should be burnt.

f. Yellow sticker tape traps should be hanged in the sorting room to trap insects hovering around and also for monitoring insects in the room for quick identification of insect pests.

g. Fenitrothion insecticide should be used to treat seeds by dipping before open-air drying after post-heated soaking.

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