Research

Foliar, Fruit, and Soil-Applied Organic Insecticides for Oriental Fruit Fly, *Bactrocera dorsalis* (Diptera: Tephritidae), in Senegalese Mangoes

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Abstract

Since its invasion in Senegal (West Africa) in 2004, *Bactrocera dorsalis* Hendel, the oriental fruit fly, has caused loss of production of mango and other commercial fruits. The use of neem products (oil, cake, and seed powder) and kaolin may be an alternative to synthetic insecticides for controlling adult flies and larval-pupal stages and may contribute to integrated pest management tactics. In the laboratory, neem oil-treated fruit had the same number of landings and time spent on treated fruit as the control but showed reduced pupal development. Females selected kaolin-treated fruits for landing less often, with reduced pupal

development. Soil treatment with neem cake and neem seed powder reduced pupal survival. In the field, neem treatment had a greater number of infested fruits and greater pupal development than the control, but fewer flies emerged. Kaolin was not different from the control in percent of infested fruits or pupal development, but fewer flies emerged. Neem cake and neem seed powder were found to lose efficacy in the field.

Keywords: integrated pest management, stone fruits, neem, kaolin, West Africa

Arrival of Oriental Fruit Fly in West Africa and Its Impact

Mango (*Mangifera indica* L., Anacardiaceae), native to the Indo-Burma region, was introduced to Senegal in 1824 (Rey et al. 2006). The Niayes zone (northwestern Senegal) and Casamance region (southern Senegal, below Gambia) are the localities in Senegal where mango is mostly grown (Mbaye 2006). The mango supply chain generates many jobs from production, to harvest, to post-harvest (Colen et al. 2012). Mango production is for both domestic consumption and for export markets. Senegal is the second largest mango exporter to European Union countries among the Economic Community of West African States (ITC 2015). However, an economic and quarantine tephritid fruit fly, the oriental fruit fly, *Bactrocera dorsalis* Hendel (formerly *Bactrocera invadens* Drew, Tsuruta, and White), entered Senegal in 2004 (Vayssières et al. 2011) and caused severe losses in fruit production (up to 80%) (Ternoy et al. 2006).

Native to Asia, *B. dorsalis* is widespread in Asia, Africa, and Hawaii. It is a generalist species, which attacks at least 34 species of fruit tree including 24 varieties of mango in Senegal (Ndiaye et al. 2012) and could infest more than 300 species of fruits and

vegetables (Plant Health Australia 2018). Tactics used to control *B. dorsalis* populations in Senegal are mass trapping, bait treatment with spinosad, and release of parasitoids (*Fopius arisanus* Sona, Hymenoptera: Braconidae) (Ndiaye et al. 2015). However, these tactics are too expensive and do not cover all mango production zones.

Neem, *Azadirachta indica* Juss. (Meliaceae), native to India, is grown in tropical and subtropical areas of Africa, America, and Australia (Schmutterer 1990). Neem has an active ingredient, azadirachtin, which has been found to have multiple modes of action affecting the behavior and physiology of insect pests. Azadirachtin is an insect growth regulator (ecdysis inhibitor), feeding deterrent, repellent, ovicide, and inhibitor of fecundity (Ascher 1993; Mordue (Luntz) et al. 2000). Neem products were found to decrease female oviposition, pupal survival, and adult longevity (Böckmann et al. 2014; Di Ilio et al. 1999; Silva et al. 2013; Singh and Singh 1998; Stark et al. 1990).

Kaolin is a natural clay mineral from weathering of aluminous minerals (ATTRA 2004). Nontoxic, kaolin is used in food processing, in cosmetics, and in health products (Rasad and Rangeshwaran 2000). In pest management, when kaolin is sprayed on crops it forms a white particle barrier that protects the crops from heat stress and pests settling, laying eggs, and feeding (Glenn and Puterka 2005). Surround WP (Tessenderlo Kerley, U.S.A.) is made of 95% kaolin clay and 5% inactive ingredients. Leskey et al. (2010) found that Surround WP was a deterrent, a repellent, and an insecticide of apple maggot, *Rhagoletis pomonella* Walsh (Diptera: Tephritidae). Surround WP prevented medfly,

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Ceratitis capitata Wiedemann, infestation on citrus and resulting decay of fruits (D'Aquino et al. 2011). Surround WP is as effective as spinosad (a spinosyn) and azinphos-methyl (an organophosphate) on apple maggot infestation (Villanueva and Walgenbach 2007).

The aims of this research were to (i) determine the effect of neem oil and kaolin on the behavior and reproduction of *B. dorsalis* and (ii) determine the effect of soil treatment with neem cake and neem seed powder on the survival of the larval and pupal stages of *B. dorsalis*. Research to address these objectives was conducted in the laboratory and in the field.

Effect of Foliar and Fruit Treatment with Neem Oil and Kaolin in Behavior and Reproduction of *B. dorsalis*

Laboratory study. Experiments were conducted in the Entomology Laboratory at the Direction de la Protection des Végétaux (DPV) in Dakar, Senegal. Two products were used: Neemland oil and Surround WP. Neemland oil (95% neem oil and 5% neem soap) was produced locally in Senegal by Neemland Ecosystem SARL. Neem oil solution was made from 2 ml of Neemland oil mixed with 200 ml of water according to the label rate (10 ml of neem oil per liter of water). Surround WP (95% kaolin and 5% other ingredients) was obtained from Tessenderlo Kerley. A kaolin suspension was made from 4.19 g of Surround WP mixed with 100 ml of water (label rate: 25 to 50 lb per 100 gal of water). An adjuvant, a wetting agent from Niayes Sarraut Dakar Company, helped kaolin to adhere to the surface of mango fruits. Mango fruits were purchased at the market, and three per treatment were treated with either neem oil or kaolin or were left untreated. Two to three applications were made to ensure that the entire surface of the mango fruit was covered with neem oil or kaolin, followed by a 1-h drying time.

Three groups of 10 gravid females were placed into separate cages (30 × 30 × 30 cm), one group per cage, the day before the trial (24 h) to acclimate. One mango from each of the three treatments (neem oil, kaolin, and control) was placed into each of the cages using a 3 × 3 Latin square design so that each mango treatment combination was located at each of three positions in the cages. The design was replicated five times, resulting in 15 mangoes tested per treatment. Immediately after placing treated mangoes in each cage, the behavior of females was video recorded with a digital camera (Panasonic, DMC-TS6) for a period of 5 min. The number of landings and time spent on each fruit were recorded. Mangoes were removed from cages after 48 h and incubated individually in containers filled with sand for pupation.

After 2 weeks, the number of pupae per mango for each treatment was recorded. The number of landings, time spent on fruit, and number of pupae were log transformed to homogenize the variance and to satisfy the assumption of normality for the analysis. An analysis of variance (ANOVA) was carried out to test for significant differences between the treatments in the mean number of landings and mean number of pupae. Data on time spent by adult *B. dorsalis* on mango fruit were analyzed using an analysis of covariance (ANCOVA) with the number of landings as a covariate. Post hoc multiple comparison of the mean responses among treatments was carried out using Tukey–Kramer HSD (Zar 2010). All analyses were carried out with JMP Pro 11 (SAS Institute, Cary, NC) at a significance level of $\alpha = 0.05$.

The result of analysis of the data from the laboratory bioassay showed that at least one of the treatments, kaolin, neem, and untreated control, had a significant effect on the mean number of female *B. dorsalis* landing on mangoes ($F = 4.4157$, $df = 2,38$, $P = 0.019$). Post hoc comparison showed that the significant treatment

effect resulted from the lower mean number of female landings on kaolin-treated mangoes compared with the control (Fig. 1). A significant difference was also observed among treatments with respect to the time spent by adult female flies on mangoes ($F = 5.5293$, $df = 2,35$, $P = 0.008$). Adult females spent significantly less time on kaolin-treated mangoes compared with neem-treated and control mangoes (Fig. 2). The mean numbers of pupating larvae were also significantly affected by treatment ($F = 26.3372$,

FIGURE 1

Mean (± SE) number of landings by *Bactrocera dorsalis* on untreated and treated mango fruit for 5 min observation in cage assays ($n = 15$, Latin square ANOVA). Bars with different letters denote significant differences at $F = 4.4157$, $df = 2,38$, $P = 0.019$.

FIGURE 2

Mean (± SE) time spent by *Bactrocera dorsalis* on untreated and treated mango fruit with neem oil and kaolin per landing in cage assays ($n = 15$, Latin square ANCOVA). Bars with different letters denote significant differences at $F = 5.5293$, $df = 2,35$, $P = 0.008$.

df = 2,32, $P < 0.0001$), with a lower mean number of pupae emerging from neem- and kaolin-treated mangoes compared with the control (Fig. 3).

Field study. Field experiments were conducted in Niayes and at Djibelor Village in Casamance. Three orchards were selected at each of the two localities. In Niayes, orchards' GPS locations were 14.953N 17.032W, 14.972N 17.004W, and 14.986N 17.005W, and in Djibelor, orchards' GPS locations were 12.550N 16.321W, 12.556N 16.322W, and 12.554N 16.320W. Four trees were selected for testing within each orchard. Each of the selected trees was divided into three treatment zones around the tree canopy, with each zone separated by an untreated buffer. The treatment zones were randomly assigned to one of three treatments: neem oil, kaolin, or untreated control.

Two applications of neem oil and kaolin were applied at 2-week intervals at label rates using hand-pumped backpack sprayers. Two weeks after the second application, 10 mango fruits were randomly selected and harvested from each treatment zone of the four test trees in each orchard and placed in a tagged plastic bag. The mango fruits collected from each of the 36 treatment zones (3 zones/tree $\times$ 4 trees/orchard $\times$ 3 orchards) were incubated in 15-liter buckets quarter-filled with sand to collect pupae. Each bucket was covered with a transparent fabric to prevent escape of emerged flies. Three weeks later, the buckets were examined to determine the percent of mango fruits infested by *B. dorsalis*, the numbers of pupae, and the number of emerged adults in each of the treatment groups.

No mangoes from any of the treatment groups from the orchards at Niayes were infested by *B. dorsalis*. Therefore, only data from the mangoes collected at the three orchards at Djibelor Village, Casamance, were analyzed. Data on the percent of infested mangoes and number of pupae were analyzed using a mixed-model ANOVA with mango tree nested within orchard as a random effect. Post hoc multiple comparison of the mean responses among treatments was carried out using Tukey–Kramer HSD (Zar 2010). χ^2 analysis was used to test for significant differences in the percent of *B. dorsalis* adults emerging from pupae collected from each of

the three treatments. For this analysis, orchard was considered a blocking factor. All analyses were carried out with JMP Pro 11 (SAS Institute) at a significance level of $\alpha = 0.05$.

The percent of mangoes infested with *B. dorsalis* differed significantly among the three treatments: neem, kaolin, and untreated control ($F = 3.6014$, df = 2,22, $P = 0.044$). The mean percent of mangoes infested in the kaolin treatment did not differ significantly from either the control or neem treatments; however, the mean percent of infested mangoes in the neem treatment was significantly greater than in the control (Fig. 4). Further, the analysis showed that the mean number of pupae differed significantly among the three treatments ($F = 4.0958$, df = 2,22, $P = 0.031$), with a significantly greater number of pupae observed in the neem treatment compared with the control and kaolin treatments (Fig. 5). The analysis also determined that there was a significant difference among the three treatments in the percent of adults that emerged from pupae ($\chi^2 = 83.128$, df = 2, $P < 0.0001$). A significantly higher percentage of flies emerged from the control treatment compared with both the neem and kaolin treatments (Fig. 6).

Neem oil did not repel or deter oviposition by *B. dorsalis*. Females of *B. dorsalis* landed and laid eggs on neem-treated mango fruits. The repellent and deterrent effect of azadirachtin (Ascher 1993; Mordue (Luntz) et al. 2000) was not evident in this study. Neem toxicity may vary among species even within the same family. Stark et al. (1990) found *B. dorsalis* was less sensitive than the melon fly, *Bactrocera cucurbitae* Coquillett (Diptera: Tephritidae), to neem oil. The repellency of neem is related to the concentration of neem in the formulation (González-Gómez et al. 2012). Low concentrations of neem were shown to not prevent bee pupae from becoming infested by *Varroa destructor* Anderson and Trueman (Acari: Varroidae). Vanranden and Roitberg (1998) reported that neem insecticide did not act as an ovipositional deterrent for the western cherry fruit fly, *Rhagoletis indifferens* Curran (Diptera: Tephritidae), under laboratory conditions. However, the mean numbers of pupating larvae were significantly lower on neem-treated mangoes compared with the control in our study. The number of eggs that hatched decreased when females of the peach

FIGURE 3

Mean ($\pm$ SE) number of *Bactrocera dorsalis* pupae per untreated and treated mango fruit in cage assays ($n = 15$, Latin square ANOVA). Bars with different letters denote significant differences at $F = 26.3372$, df = 2,32, $P < 0.0001$.

FIGURE 4

Percent of infested mango fruits treated with neem and kaolin, compared with untreated control (Djibelor field, $n = 120$, mixed model ANOVA). Bars with different letters denote significant differences at $F = 3.6014$, df = 2,22, $P = 0.044$.

fruit fly, *Bactrocera zonata* Saunders (Diptera: Tephritidae), laid eggs on neem-treated fruits (Mahmoud and Shoeib 2008). Their laboratory results support our laboratory and field results, in which the number of pupae and the number of emerged flies, respectively, were significantly lower on neem-treated fruit compared with the control (Figs. 3 and 6). Vijayalakshmi et al. (2014) reported that efficacy of neem oil depends on the source of manufacture and doses. In addition, they found that neem oil was less efficient in the field than in the laboratory.

Surround WP discouraged female *B. dorsalis* from landing and ovipositing on treated fruits in our laboratory conditions. When the

Surround WP was sprayed on fruit, the white coating from kaolin diminished visual attractiveness and served as a tactile deterrent. However, kaolin has shown variable efficacy among other tephritids. Leskey et al. (2010) observed that apple maggot, *Rhagoletis pomonella* Walsh (Diptera: Tephritidae), spent less time on kaolin-treated fruit relative to untreated fruit. However, the efficacy of Surround WP (kaolin) was found to decrease in high *R. pomonella* populations. Likewise, in this current field study, efficacy of Surround WP decreased. Lo Verde et al. (2011) found that infestation by the Mediterranean fruit fly, *Ceratitis capitata* Wiedemann (Diptera: Tephritidae), was not significantly different in kaolin-treated versus untreated clementines at high pest densities and when there were no host plants besides clementine.

Effect of Soil Treatment with Neem Cake and Neem Seed Powder on Survival and Development of the Larval and Pupal Stages of *B. dorsalis*

Neem products used in this study were neem seed cake (Neemland Rakkal) and neem seed powder (Neemland Sol). Both products were locally manufactured by Neemland Ecosystem Company in Senegal. Neem cake is the solid waste of pressed seed after oil extraction, it differs from neem seed powder by the absence of oil. Label rates were 30 to 50 g/plant or 50 to 100 g/m² (field) for neem seed cake and 10 g/plant or 100 g/m² (field) for neem seed powder.

Laboratory study. This study was conducted at the Entomology Laboratory of DPV, Dakar. Three treatments (neem seed cake, neem seed powder, and an untreated control) were used to assess the effect of neem products on the survival of larvae and pupae of *B. dorsalis*. Soil samples treated with either neem seed cake or neem seed powder were compared with untreated soil. Soil (30 g) was mixed with either 30 g of neem seed cake or 10 g of neem seed powder. Each replicate consisted of one treatment container (either neem seed cake or neem seed powder) and an untreated container (control). A group of 17 randomly selected third-instar larvae was placed in each container, and treatments were replicated six times. Containers were covered with a transparent fabric to prevent escape of larvae or emerged flies. Two weeks later, the percent of emerged flies was recorded.

A χ^2 analysis was used to determine whether there were significant differences in the percent of emerged *B. dorsalis* adults among the three treatments. All analyses were carried out with JMP Pro 11 (SAS Institute) at a significance level of $\alpha = 0.05$.

Neither neem seed cake nor neem seed powder significantly decreased *B. dorsalis* larval development into pupae. All of the larvae in the three treatments developed to the pupal stage. Neem seed cake and neem seed powder, however, significantly decreased the percent of pupal survival, and few adult flies emerged compared with the control ($\chi^2 = 262.5$, $df = 2$, $P < 0.0001$; Fig. 7). Pupae that did not result in adults when expected were kept under observation for late-emerging adults, which did not appear.

Field study. This study was conducted in a mango orchard at Djibelor (Casamance) and at DPV (Dakar) after mango fruit season to ensure that there were no pupae in the soil. At each of the two localities, emergence cages (50 × 50 × 50 cm) were installed under three mango trees to test the effect of neem seed cake and neem seed powder on the survival of the oriental fruit fly larvae and pupae. Pupal survival was measured by adult emergence. Cages were covered with a black fabric, and a transparent bottle was inserted at the top of the cage so that, at emergence, flies followed the light and were captured in the bottle. Three cages, one for each treatment (neem seed cake, neem seed powder, or untreated control) were placed randomly in the shade under a randomly selected mango

FIGURE 5

Mean (± SE) number of *Bactrocera dorsalis* pupae in 10 mango fruits per treatment (Djibelor field, $n = 120$, mixed model ANOVA). Bars with different letters denote significant differences at $F = 4.0958$, $df = 2,22$, $P = 0.031$.

FIGURE 6

Percent of adults emerging in treated and untreated mango fruit (Djibelor field, $n = 120$, χ^2 analysis). Bars with different letters denote significant differences at $P < 0.001$.

tree. Each cage was placed into a hole dug under the tree, and the bottom 10 cm of the cage was filled with the soil that had been removed from the hole. At the top of the soil within one of the cages, 100 g of neem seed cake (4× field label rate) was poured and mixed gently. This rate was used to be conservative, because this was a new method of application, in an area of high oriental fruit fly pressure. If successful, we could try different rates in a subsequent study. Likewise, 100 g of neem seed powder (4× field label rate) was poured onto the top of the soil inside another cage and mixed gently. The soil inside the third cage was stirred and left untreated to serve as a control. At the laboratory at DPV, uninfested mangoes were placed into cages with gravid *B. dorsalis* females for 48 h. The following day, three mango fruits were randomly selected and placed in each of the three emergence cages located under each of the mango trees. After 10 days, emerged fruit flies were collected daily over a 2-week period.

A nonparametric Wilcoxon rank sums test (Zar 2010) was used to test for significant differences in the number of emerged *B. dorsalis* adults among the three treatments at the two localities. For the initial analysis, locality was considered a blocking factor. However, because the analysis showed that locality was not significant, subsequent analyses were conducted separately for each locality used in the experiment. All analyses were carried out with JMP Pro 11 (SAS Institute) at a significance level of $\alpha = 0.05$.

Results showed that there were no significant differences in emergence of *B. dorsalis* adults between untreated soil and soil treated with neem seed cake or neem seed powder ($\chi^2 = 1.065$, $df = 2$, $P > 0.05$). There were also no significant differences among the treatments in the emergence of adult flies at either of the two study locations (Fig. 8).

In our laboratory study, neem seed cake and neem seed powder did not prevent larvae from developing into pupae. However, they significantly decreased the percent of pupal survival, and few adult flies emerged compared with the control. Neem cake has a larvicidal

FIGURE 7

Effect in the laboratory of soil treated with neem seed cake (NSC) or neem seed powder (NSP) and untreated soil (control) on number of emerged *Bactrocera dorsalis* ($n = 102$ larvae, χ^2 analysis). Bars with different letters denote significant differences with $\chi^2 = 262.5$, $df = 2$, $P < 0.0001$. Lines denote standard error.

effect on some Diptera such as *Aedes albopictus* (*Stegomyia albopicta*) (Diptera: Culicidae) because of salannin, an active ingredient different from azadirachtin (Nicoletti et al. 2012). Regardless of toxicant, in our study, neem cake did not have a larvicidal effect on *B. dorsalis*. Silva et al. (2011) found that neem cake did not affect the development of Mediterranean fruit fly larvae to pupae but caused pupal mortality. Pupal mortality was 100% for mixtures higher or equal to 75% in the neem cake. Singh (2003) found that neem seed kernel extract significantly decreased the percent of emerged oriental fruit flies, and Stark et al. (1990) showed that emergence of oriental fruit fly, melon fly, and Mediterranean fruit fly adults was decreased in sand treated with neem seed extracts. These results support our laboratory results, in which all of the larvae in the three treatments developed to the pupal stage and percent of *B. dorsalis* adults was significantly decreased in neem seed cake and neem seed powder treatments compared with the control (Fig. 7).

In the field, neem seed cake and neem seed powder did not affect survival of emerged flies. There were no significant differences among the treatments in the emergence of adult flies. Weaver ants, *Oecophylla longinoda* Latreille (Hymenoptera: Formicidae), were observed in control cages but not in the treated cages at Djibelor field. Weaver ants were not observed in any cages at Dakar. Weaver ants are predators of larvae and adults of *B. dorsalis* and other tephritid fruit flies on mango (Vayssières et al. 2016). Diamé et al. (2015), for example, found low populations of *B. dorsalis* in mango orchards in the presence of weaver ant colonies. Neem was found to be a repellent to red ants, *Oecophylla smaragdina* Fabr. (Formicidae: Formicinae), by Vishwakarma et al. (2012). Perhaps, in the current

FIGURE 8

Effect in the field (Dakar and Djibelor) of soil treated with neem seed cake (NSC) or neem seed powder (NSP) and untreated soil (control) on number of emerged *Bactrocera dorsalis*. Data were analyzed using a nonparametric Wilcoxon rank sums test. Means with different letters denote significant differences at $\alpha = 0.05$. Lines denote standard error.

study, cages treated with neem seed cake and neem seed powder discouraged predation of emerged flies by weaver ants. Although we did not observe ants in the Dakar trial, it is still possible that there was predation on larvae and emerged flies by ants, especially because the laboratory colony of fruit fly at DPV was attacked by weaver ants in 2014 and 2015. The effect of sunlight on the neem product also needs to be considered. Vijayalakshmi et al. (2014), for example, reported a rapid decrease of neem efficacy in the field owing to sunlight. Therefore, it is possible that despite the placement of cages under the shade of mango trees, the efficacy of the neem products used in this study was affected by sunlight.

Conclusion

Laboratory and field experiments on foliage and fruit showed that neem oil and kaolin could decrease infestation and populations of the oriental fruit fly. However, work remains to be done to make field applications effective. In the future, neem oil and kaolin field trials should be repeated with different test conditions to determine the best treatments and treatment frequency that will negatively affect oriental fruit fly behavior and development. Research may focus on the optimal intervals between applications in the field. A benefit of using these products is that applications can be made until shortly before harvest if needed.

The laboratory soil treatment showed that neem seed cake and neem seed powder could be promising organic insecticides to manage oriental fruit fly populations while in the soil. This would aid in control independent of tree size, unlike foliar treatments. Future research may focus on the efficacy of neem seed cake and neem seed powder in the field by excluding ants, the effect of neem seed cake and neem seed powder on weaver ants, the effect of temperature and sunlight on efficacy of neem seed cake and neem seed powder, and the number of applications of neem seed cake and neem seed powder that would cause a decrease of emerged flies in the field.

Surround WP, neem oil, neem seed cake, and neem seed powder are harmless organic insecticides that should be added to the integrated pest management tools for *B. dorsalis* in order to avoid pest resistance and chemical residue in soil, to protect natural enemies, and to manage immature and adult stages of *B. dorsalis*.

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