Journal of Forestry Research and Management. Vol. 17(3).113-123; 2020, ISSN 0189-8418

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INSECTICIDAL POTENTIAL OF SELECTED BOTANICAL EXTRACTS AGAINST PUPARIATING LARVAE OF *BACTROCERA DORSALIS* (ORIENTAL FRUIT FLY) UNDER LABORATORY CONDITIONS

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ABSTRACT

Oriental fruit fly *Bactrocera dorsalis* Hendel is an invasive insect pest of Asian origin attacking several fruits and vegetable crops across the globe. This study evaluated the contact and residual effects of aqueous and ethanol extracts of four plant species (*Azadirachta indica, Jatropha curcas, Moringa oleifera and Affamomum melegueta)* against *B. dorsalis* pupariating larvae (third instar) under laboratory conditions of 27 ± 2°C, 70 to 80% relative humidity and L12: D12 photoperiod. The ethanol extracts were applied at 75% dilution on *B. dorsalis* pupariating larvae while aqueous extracts was applied in its crude form without dilution. Larvae mortality was recorded at 20 minutes intervals for 24hour. Data collected were subjected to Analysis of Variance (ANOVA). Results showed that all applied extracts had contact and residual effects on *B. dorsalis* pupariating larvae giving 96-100% mortality at 24hours post treatments. There were no significant differences ($p>0.05$) among ethanol and aqueous extracts of the various plants tested for both contact and residual effects on *B. dorsalis* pupariating larvae. The time of action of extracts on the larvae varied with different extracts and type of solvent used for extraction. Ethanol extract was faster in action than the aqueous extracts. Both ethanol and aqueous extracts of all the plants evaluated were potent against *B. dorsalis* pupariating larvae. Thus, adoption of these botanicals in the management of *B. dorsalis* infestations on fruit and vegetables through application on the soil targeting puparia will minimize infestations of *B. dorsalis.*

Keywords: plant extracts, mortality, pest control, oriental fruit fly

Introduction

The oriental fruit fly, *Bactrocera dorsalis* (Hendel) Diptera: Tephritidae) is a severe insect pest of many fruits and vegetables across the globe and poses a major threat to mango in particular (Ekesi *et al*., 2016; Jaleel *et al*., 2019). The production of high quality fruits globally is constrained by fruit fly infestations especially by *Bactrocera* species, with damages resulting in total losses of target crops if not controlled (Mwatawala *et al*., 2006). *B. dorsalis* has been recorded in 42 African countries including Nigeria, Benin, Cameroun, Democratic Republic of Congo, Ethiopia, Gabon, Ghana, Guinea, Kenya, Togo among others (Drew *et al.*, 2005; CABI/EPPO, 2008; Vayssières *et al.*, 2008; EPPO, 2014). In Nigeria, fruits production for exports has been hampered by fruit fly infestation (Danjuma *et al.*, 2016)

Fruit fly damage on fruits is caused by females that lay eggs on the skin of both healthy and damaged fruits, followed by subsequent hatching of the eggs and subsequent larvae feeding on the rotting fruit tissues. This causes fruit decaying leading to premature fruit droppings that leads to huge

economic losses (Ekesi, *et al.*,2003). During fruit fly development, the third instar larvae drops to the ground from the fruits and burrow into the soil to pupate (White and Elson-Harris, 1992). Soil treatment with pesticides under the host trees targeting the pupating larvae and puparia is an important aspect in fruit fly eradication and control strategies (Ekesi,*et al.*, 2003) Numerous studies have reported the management of *Bactrocera* species using synthetic pesticides (Jin *et al.*, 2011; Nadeem *et al.*, 2014). However, pesticides residues in fruits are of great concern for human health (Amaro and Godinho, 2012).The organophosphate insecticide, (diazinon), is the most widely used soil insecticide against fruit fly larvae/puparia(Ekesi *et al.* 2003). However, diazinon affects non-target organisms severely including fruit fly parasitoids due to its broad-spectrum mode of action (Croft, 1990; Purcell and Schroeder, 1996). The potential of using fungi isolate *Metarhizium anisopliae* for soil inoculation against pupariating third-instar larva of fruit flies has been reported (Ekesi, *et al*, 2003). According to Jin *et al.* (2011). Over the years *B. dorsalis* has developed high levels of resistance towards nearly all commonly used insecticide groups due to over persistence of target chemicals in the environment.

High resistance in field strains of *B. dorsalis* to trichlorfon have been reported by some studies (Jin *et al.*, 2011; Khan and Akram, 2018). The use of botanicals as an alternative for the management of *Bactrocera* species has been reported to be more reliable poses less risk of developing resistance and biodegradable (Campos *et al.*, 2018). Botanicals are safe in the environment, economically cheap in production, easily applicable, have less impact to non-target organisms especially natural enemies (Potts *et al.*, 2016).

The repellency of several botanical extracts against different *Bactrocera* species such as *B. zonata* , *B. oleae* *B. cucurbitae*, and *B. dorsalis* have been reported (Ilyas *et al.*, 2017). The use of botanicals pesticides in the integrated management program against *Bactrocera species* has been reported to be a more reliable control method (Ilyas *et al.*, 2017; Hikal, *et al.*, 2017). However, there is limited information on the use of botanical extracts against the pupariating larvae of *B. dorsalis* with intention of targeting puparia on the soil under the host plant trees in the field for integrated pest management in fruit orchards. This study thus evaluated the potential of four botanical extracts (*Azadirachta indica, Jatropha curcas, Moringa oleifera and Affamomum melegueta)* against *B. dorsalis* pupariating larvae under laboratory condition

Materials and Methods

The study was conducted at the Biology laboratory of the Federal College of Forestry (FCF) Ibadan under ambient temperature of 27 ± 2°C and relative humidity of 70 to 80%. and L12: D12 photoperiod . Plant materials, that is, *A. melegueta* and *P guineense* used were purchased from Bode, market in Ibadan while *A. indica and Moringa oleifera* seeds were collected from Forestry Research Institute of Nigeria (FRIN) Ibadan. The seeds of *A. indica* and *M. oleifera* collected from FRIN were extracts from the fruits and pods, respectively and were air dried for two weeks under ambient light conditions at fluctuating temperature of 25±5^{o}C, 60-80% relative humidity and L12: D12 photoperiod. The dried seeds of *A.meleguaeta* and *P. guineense* were further air dried for two days. Each plant seeds were blended with a high-speed mill

blender. The aqueous extraction of botanicals was done by soaking 200 g each of the powdered samples of plant materials in separate conical flask containing warm water (60ºC) with a ratio of 1: 2 (w/v). The mixture was vortexed manually at intervals of 30 minutes for three hours and allowed to stay for 48 hours under laboratory conditions, the supernatant was then filtered with a Whatman filter paper(90 mm). Ethanol extraction was done by soaking 200g of each plant samples powder in a conical flask having ethanol (97%) with a ratio of 1:2 (w/v). The mixture was manually vortexed at interval of 30 minutes for three hours and allow to stay for 24 hours, the supernatant was filtered with a Whatman filter paper (90 mm) and the filtrate was left open for three hours for ethanol to evaporate before transferring to preserving bottles. All extracts were preserved in small airtight bottles in a refrigerator until used. Populations of *B. dorsalis* was reared in the laboratory at ambient temperature of 25 ± 2°C and relative humidity of 70 to 80% on *Irvingia gabonensis* fruits.

The infested fruits of *Irvingia gabonensis* were collected from the *Irvingia* plantation at Forestry Research Institute of Nigeria and were kept in a plastic cages in the laboratory and monitored for adult emergence. The emerged adults were paired in another cage for mating and oviposition. The rearing cages were supplied with fresh *Irvingia* fruits every two days after the first instar larvae emergence and the culture was maintained until the end of the experiment. Extracts were evaluated for residual action by applying 1ml of each extracts on petri dishes lined with Whatman filter paper (90 mm). Petri dishes were left for 10 minutes to drain off before five third instar larvae of *B. dorsalis* collected from the raised culture were separately introduced into each petri dish. The contact toxicity of the extracts were assessed by applying 0.1 ml of each extracts using 20µl micro pipettes on the dorsal cavity of the *B. dorsalis* pupariating larvae (third instar). Ethanol extracts was applied at 75% concentration (dilution) while aqueous extracts was applied as extracted. All assays were replicated three times in a Completely Randomized Design (CRD) at 5% level of probability. Mortality of the pupariating larvae were recorded at 20 minutes intervals until 24 hours. Data collected were subjected to Analysis of Variance (ANOVA) and significant means were separated at 5% level using Turkey's Honestly Significant Difference (HSD).

Results

Residual effects of the ethanol extracts on *B. dorsalis* pupariating larvae

The mortality of the pupariating larvae commenced 40 minutes after exposure with *A. melegueta* giving the highest mortality (26.6%) among the extracts (Fig1). The mortality progressed along with increasing time, at 60 minutes post exposure the mortality rate increased with *A. melegueta* inducing the highest mortality rates(26.6%) followed by *J.curcas* (20%). The residual effects of *A. indica* and *M.oleifera* on pupariating larvae of *B . dorsalis* increased from 80 minutes post exposure, as they both recorded 20% mortality. The residual effects of *A. indica* extracts demonstrated the highest efficacy peaking at 120 minutes post exposure with 33.4% mortality. At 1440 minutes of observation, *M. oleifera* gave its highest kill of *B. dorsalis* pupariating larvae with 26.6% mortality. No mortality was observed on the control throughout the time of experiment. The residual effects of the various ethanol extracts showed significant differences ($p<0.01$) at 60 minutes of post exposure.

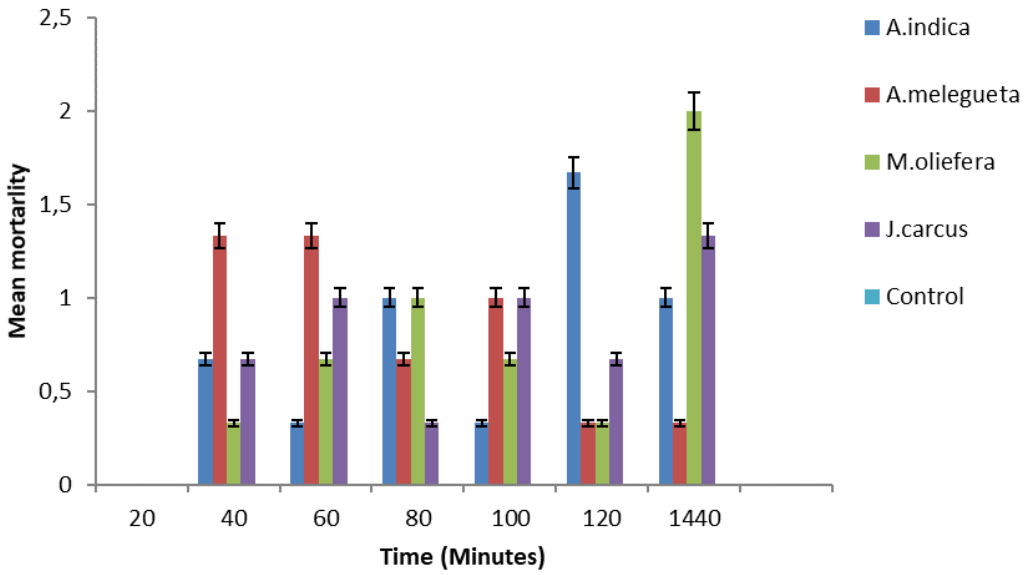

Fig. 1. The residual effects of the ethanol extracts on the *B. dorsalis* pupariating larvae mortality

Contact effects of the ethanol extracts on *B. dorsalis* pupariating larvae

The contact effects of the ethanol extracts followed similar pattern with the residual actions. The mortality commenced after 40 minutes of post exposure with *A. indica* recording the highest mortality (20%). The mortality rates of the *B. dorsalis* pupariating larvae progressed with the time of exposure with the various extracts until 1440 minutes of observations (Fig2). Mortality was recorded at diverse dilution rates by the different extracts at various time of observation. *A. melegueta* and *M. oleifera* recorded their highest mortality at 1440 minutes of exposure with 33.4% and 20% respectively. No mortality was observed in control and there were no significant differences ($p>0.05$) on the contact effects of the ethanol extracts from 40 to 120 minutes of observation. The contact effects of the extracts was significantly different ($p<0.05$) at 1440 minutes post treatments.

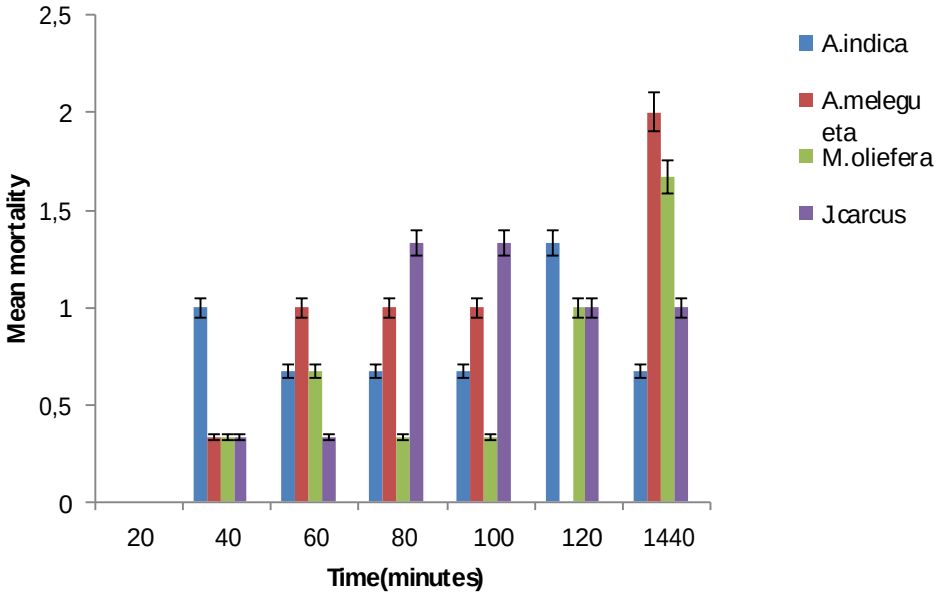

Fig. 2. The contact effects of the ethanol extracts on the *B. dorsalis* pupariating larvae mortality.

Residual effects of the aqueous extracts on the mortality of *B. dorsalis* pupariating larvae

The mortality of commenced from 60 minutes of post exposure with only *M. oleifera* recording 6.6% mortality. The mortality with the various extracts progressed with time but with slow rates compared with their ethanol extracts (Fig.3). The aqueous extract of *M. oleifera* commenced insecticidal activity from 80 minutes post exposure with 26.6% mortality while *J. curcas* commenced at 100 minutes of post exposure with 33.4% mortality. The residual effects of *A. indica* aqueous extract was observed at 1440 minutes post exposure with 100% mortality. Control recorded no mortality at the end of the assay. There were significant differences ($p<0.05$) on the residual effects of the various extracts on pupariating larvae from 100 to 1440 minutes post exposure.

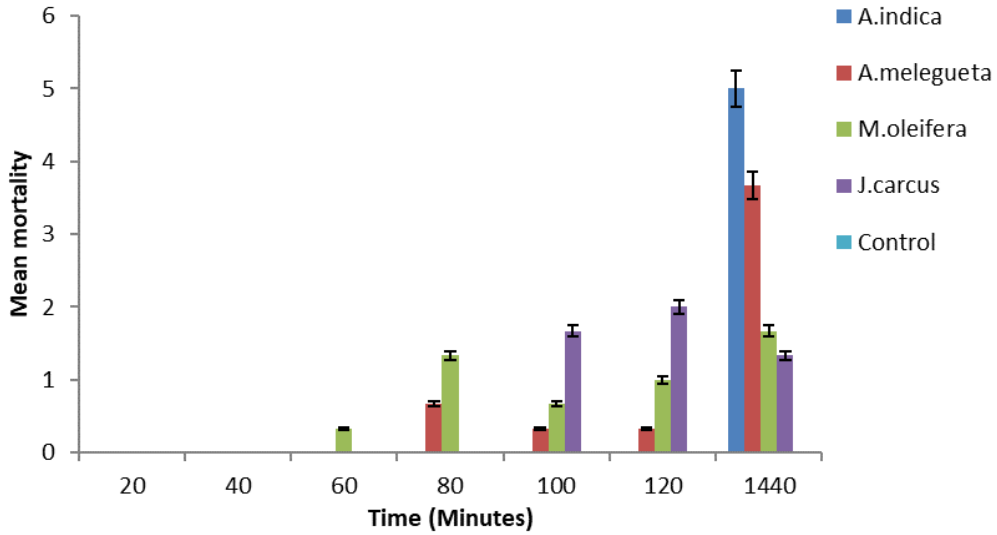

Fig. 3. The residual effects of the aqueous extracts on the mortality of *B. dorsalis* pupariating larvae .

Contact effects of aqueous extracts on the mortality of *B. dorsalis* pupariating larvae

The contact effects of the aqueous extracts on the pupating larvae of *B. dorsalis* followed similar pattern with the residual effects (Fig. 4) . Mortality commenced from 60 minutes post treatment and continues in a slow rate until 1440 minutes. The contact effects commenced with *A.melegueta* and *J.carcus* with both recording 6.6% mortality and at 80 minutes with 20% and 26.6% mortality respectively. The contact effects of *A. indica* was the same as in residual effects, it commenced at 1440 minutes with 100% mortality. The contact effects of the various extracts on the pupariating larvae were significantly different ($p<0.05$) at 80, 120 and 1440 minutes of post treatments. No mortality was observed in control experiments.

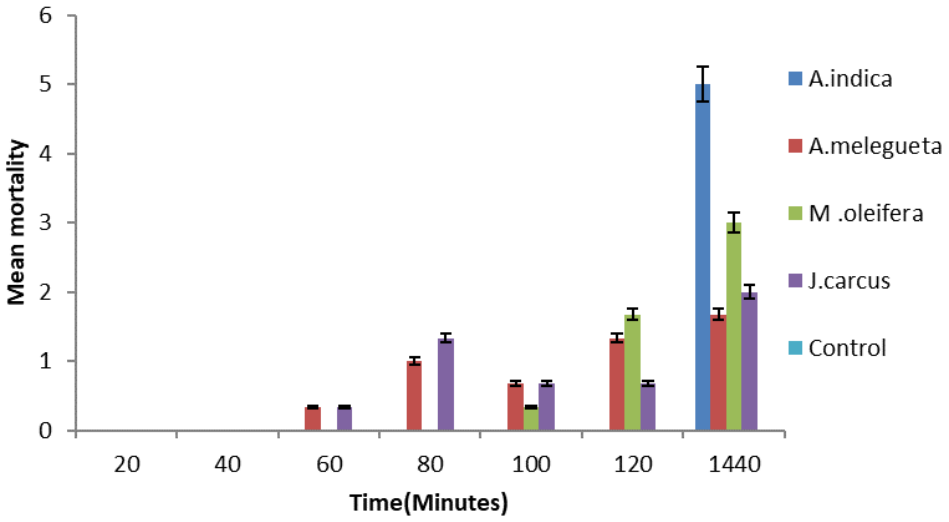

Fig. 4. The contact effects of aqueous extracts on the mortality of *B. dorsalis* pupariating larvae

Percentage mortality of *B. dorsalis* 24 hours post application of ethanol and aqueous extracts

The percentage mortality of the ethanol and aqueous extracts 24 hours post application is shown in Fig. 5. The ethanol and aqueous extracts of the screened plants proved effective against pupating larvae of *B. dorsalis* under laboratory condition. All the extracts for both contact and residual assay gave 100% mortality of *B.dorsalis* pupariating larvae in the laboratory except for contact ethanol extracts where *A. melegueta* recorded 96% mortality. Control experiment had no mortality at 24 hours post observation.

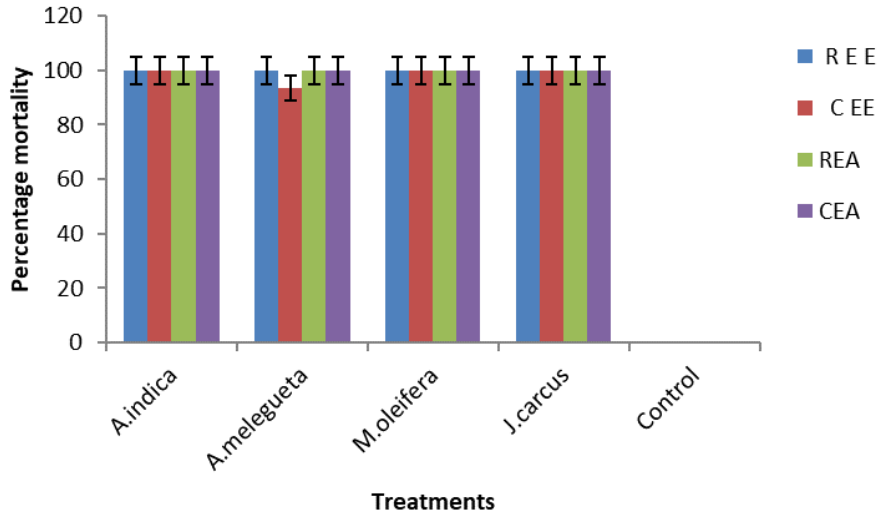

Fig.5. The percentage mortality of ethanol and aqueous extracts on *B. dorsalis* 24 hours post treatments (REE= Residual effects of ethanol extracts, CEE= Contact effects of ethanol extracts, REA= Residual effects of aqueous extracts, CEA= Contact effects of aqueous extracts).

Discussion

The study showed that crude extracts of *A indica, J curcas, M. oleifera and A. melegueta* demonstrate insecticidal properties with potential abilities of inducing mortalities in *B. dorsalis* pupariating larvae**.** The bioassays of the extracts evaluated has established that the mode of action of ethanol and aqueous extracts of the selected plants on *B. dorsalis* pupariating larvae could be contact or through residual actions. All the plant evaluated showed 96- 100% mortality of pupating larvae of *B. dorsalis* for both contact and residual effects assays at 24 hours post exposure. This has confirmed the potential of the plant extracts tested for the control of *B. dorsalis.* Several plant extracts have been used effectively against a wide range of insect pests (Isman, 2006). Some *Bactrocera* species like *B. zonata* have been reported to show growth inhibition by extracts from some plants such as *Acorus calamus Azadirachta indica*, *Curcuma longa Peganum harmala*, *Saussurea lappa* and *Valeriana jatamansi* in Parkistan (Akhtar *et al.*, 2004). Similarly, Siddiqi *et al.* (2006) reported that petroleum ether, acetone and ethanol extracts of turmeric plant were effective in repelling and inhibiting growth of *B. zonata*. *A. indica* was reported to be very effective in reducing the oviposition rate of *B. zonata*, *B. dorsalis* and *B. olae* (Rehman *et al.*, 2009a). High doses of *Azadirachtin* an active ingredient of *A. indica* was found very effective in reducing the oviposition rates of oriental fruit flies on the melon plants (Khan *et al.*, 2007). *Piper nigrum* was found very effective in repelling *B. correcta* and *B. dorsalis* (Jaleel *et al.*, 2020). The botanicals from neem oil, neem seed powder solution, tobacco leaf and Eucalyptus leaves were reported to show repellent effects on *B. zonata* (Solangi *et al.* 2011). Aqueous extracts of *A. indica, P. guineese* , *M. oleifera* and *A. melegueta* were found effective against third instar larvae of *B.dorsalis* larvae under laboratory condition

(Ugwu and Nwaokolo, 2020). According to Salimond and Abdullah (2008) *Jatropha curcas* possess insecticide or antifeedant properties that affect various insects families. Sabbour,and Abd-El –Raheem (2013) reported that *Jatropha curcas* oil deterred oviposition and influenced fecundity of *Callosobruchus maculatus* (F.) and *Callosobruchus chinensis* (L.) adversely. Similarly Abdoul habou (2014) reported that *J. curcas* seeds' oil was toxic on the adults of *C. maculatus* and *Bruchidius atrolineatus* reduced oviposition both species by 85 to 90%. *Jatropha curcas* was also found effective in reducing the infestation of legume pod borer and flower thrips population (Ugwu, 2020).

Conclusion

This study elucidated the residual and contact effects of four botanicals after 24 hours in the laboratory, although the residual effects of botanicals pesticides endured for a longer period. All the extracts proved effective against *B. dorsalis* pupating larvae under laboratory condition and both aqueous and ethanol extracts were suitable. The study provides information to support future works on the contact and residual effects of *A. indica*, *A. melegueta* *M. oleifera* and *J. curcas* against *B. dorsalis* in the field. More work is needed to ascertain other modes of actions of the various botanicals tested as well as their efficacy at farm and orchard especially as soil treatment targeting *B. dorsalis* puparia in the field.

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