Life Cycle and Natural Enemies of *Demotispa neivai* (Coleoptera: Chrysomelidae)¹

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Abstract *Demotispa neivai* (Bondar) (Coleoptera: Chrysomelidae) causes significant palm oil loss due to the damage it causes to the epidermis when feeding on fruit of oil palm, *Elaeis guineensis* Jacq. This research was conducted to characterize the biology and natural enemies of *D. neivai*. The life cycle was studied under laboratory conditions ($28.8^{\circ}C \pm 1.2^{\circ}C$ and $76.8\% \pm 6.3\%$ relative humidity); population fluctuation was established in field conditions using a 5-yr-old oil palm plot. Natural mortality factors of *D. neivai* were recorded over a 22-week period, with sampling of bunches occurring once every 2 weeks over five phenological stages of fruit development. At each sampling, three bunches were selected per stage. The *D. neivai* life cycle was as follows: the egg stage was 7.1 ± 1.2 d, the larval stage with five instars was 21.9 ± 2.0 d, the pupal stage was 19.6 ± 3.0 d, and the adult stage lasted 268.9 ± 53.1 d. The entire life cycle was, thus, completed in 309.1 ± 54.3 d. The largest *D. neivai* populations were recorded in oil palm during the bunch-filling phenological stage. Their most important natural mortality factor was the fungus *Metarhizium anisopliae* (Sorokin) Metchnikoff, which caused, on average, 25.3% larval mortality.

Key Words oil palm, Colombia, Metarhizium anisopliae, Tetrastichus sp., bunch phenology

Demotispa neivai (Bondar) (Coleoptera: Chrysomelidae) is one of the most important pests of oil palm, *Elaeis guineensis* Jacq., in Colombia due to the damage caused by *D. neivai* when feeding on the fruit epidermis (Aldana et al. 2003, 2004; Bustillo 2014; Genty and Mariau 1973; Mariau 2001; Zenner and Posada 1992), as well as damage caused to the palm spear in nurseries and in the field (Aldana et al. 2010, Urueta 1975).

Demotispa neivai—related damage to oil palm bunches leads to losses of around 8% of the harvest due to larvae and adults feeding on the epidermis of the fruit, thereby producing damage leading to desiccation and a cork-like appearance. This, in turn, hampers ascertaining the degree of maturity of the bunch (Aldana et al. 2010). Losses of palm oil have been estimated as high as 2% (relative to extraction rate) when 80–100% of the fruit epidermis is affected (Aldana et al. 2003, 2004).

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Aldana et al. (2004) state that the distribution of damage caused by *D. neivai* on oil palm lots is not homogenous. Losses associated with harvesting and palm oil make *D. neivai* an economically important pest for oil palm growers in Colombia (Valencia et al. 2007).

For evaluating the damage caused by *D. neivai* in the field, Aldana et al. (2004) devised a qualitative evaluation scale ranging from 1% to 100% relative to bunch damage, which is as follows: Scale 1, covered bunches suffering 1–20% damage; Scale 2, 21–40% damage; Scale 3, 41–60% damage; Scale 4, 61–80% damage; and Scale 5, 81–100% damage. The damage evaluation is made at stockpiled places of bunches, taking into account the originating plot.

Demotispa neivai is a nocturnal insect. It flies to nearby palms in the late afternoon and can be seen moving over and feeding on bunches during the night. It can consume 1.5–2 cm² of the surface of one or more fruits during a single night; activity can be recorded in the copula throughout the night and even into the early hours of the morning (Aldana et al. 2004).

Its eggs are laid within the bunches on the spikes or bracts covering the fruits and can occasionally be found on the surface of the fruits, measuring 1.5 mm on average (Aldana et al. 2003). The larvae remain within bunches and feed on the spikes and the epidermis of the fruits; when completely developed, they can measure 7 mm in length and 4 mm in width (Aldana et al. 2003, Genty and Mariau 1973). The pupae can be found on all bunch structures (Aldana et al. 2010); adults are reddish, oval, and have a flattened appearance, reaching 5.5 mm in length and 2.5–3 mm in width (Aldana et al. 2003, Genty and Mariau 1973).

Developmental stages of *D. neivai* are affected by different natural enemies, such as two pupal parasitoids, *Tetrastichus* sp. (Hymenoptera: Eulophidae) and *Psychidosmiera* sp. (Hymenoptera: Chalcididae) (Aldana et al. 2003, Corley and Tinker 2003, Genty and Mariau 1973); the fungus *Beauveria* sp. (Aldana et al. 2004, Valencia et al. 2007); and the predators *Hololepta* sp. (Coleoptera: Histeridae), various species of *Chrysoperla* spp. (Neuroptera: Chrysopidae), *Alcaeorrhynchus grandis* (Dallas) (Hemiptera: Pentatomidae) nymphs, and jumping spiders from the family Salticidae (Aldana et al. 2004). Likewise, ants from the genera *Crematogaster* and *Odontomachus* are considered very important for regulating *D. neivai* populations (Aldana et al. 2004, Corley and Tinker 2003).

The life cycle occurs on oil palm bunches during their development, filling, and maturation phases. The fruit development and filling phase is covered by phenological growth Stages 700, 701, 703, 705, and 709 on the Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie (BBCH) phenological scale developed for *E. guineensis*. Stage 700 is characterized by pollinated flowers and is the initial stage of the development and filling phase. Stage 701 begins a week after anthesis, fruit reaching 30% of its final size. Fruits have green mesocarp, and neither the endocarp nor the endosperm can be seen. Stage 703 covers the 28- to 31-d period after anthesis, fruit reaching 50% of its final size. Fruits have green mesocarp; the endocarp can be differentiated and the endosperm is in the aqueous stage. Stage 705 occurs 59–61 d after anthesis, fruit having reaching 80% of its final size and having green mesocarp; well-differentiated endocarp and the endosperm is in the colloidal stage. Stage 709 (the last development and filling phase) begins 79 d after anthesis, fruit reaching 90% of its final size (Forero et al. 2012, Forero and Romero 2012, Hormaza et al. 2010).

The bunch maturation phase covers Stages 800, 805, and 807. Stage 800 covers the initial stage of the fruit and bunch maturation phase and occurs 84 d after anthesis. The fruit reaches 100% of its final size during this stage and has light green mesocarp; the endocarp is coffee-colored and well differentiated, and the endosperm is in the solid stage. Stage 805 is the stage prior to the best time for harvesting (i.e., 112 d after anthesis). Fruits have orange-colored mesocarp; the endocarp is dark coffee-colored and well differentiated, and the endosperm is in the solid stage. Stage 807 (the best time for harvesting) is when fruit begins to become absorbed from the apical part of the bunch (i.e., 126 and 140 d after anthesis) (Forero et al. 2012, Forero and Romero 2012, Hormaza et al. 2010).

Demotispa neivai infestations and the relationship of its natural enemies with palm fruit development phenology must be fully understood. Such knowledge is fundamental for developing effective and environmentally sustainable management plans regarding this pest affecting Colombian oil palm plantations.

Materials and Methods

Study area. This study was conducted at Palmeras Yarima Plantation, using the facilities of the entomology laboratory ($28.8^{\circ}C \pm 1.2^{\circ}C$ and $76.8\% \pm 6.3\%$ relative humidity [RH]) and on Peroles Farm of Palmeras Yarima Plantation in San Vicente del Chucurí, Santander, Colombia (N 6°59′23.6″, W 73°39′18.0″), on an oil palm plot with ASD Costa Rica. Palms were 5 yr old, and growing conditions were 27.2°C $\pm 4.3^{\circ}C$ and 79.2% $\pm 18.8\%$ RH, with 533 mm of rainfall during the duration of the study.

Life cycle. The life cycle was determined in laboratory conditions. The cohorts were obtained by placing *D. neivai* adults in 314 polyvinyl chloride (PVC) tubes (8 cm diameter \times 5 cm high) with ends covered with muslin. Two oil palm fruits were placed in each tube as a food source and oviposition substrate. Fruits were monitored daily. Eggs were collected, placed individually in PVC tubes, and monitored daily to record survival, development, and time elapsed. Descriptive statistics were used for analyzing the life-cycle information.

Population dynamics with plant phenology. *Demotispa neivai* dynamics and those of its natural enemies corresponding to bunch phenology were determined in field conditions. This portion of the study was conducted on a commercial oil palm plot (1 ha with 143 palms) infested with *D. neivai*. Every 2 weeks over a 22-week period, three were randomly selected and examined. The phenological stages studied were 701, 703, and 705 from the bunch development and filling phase and 800 and 805 from the bunch maturation phase, according to BBCH scale for *E. guineensis* (Forero et al. 2012, Forero and Romero 2012, Hormaza et al. 2010).

Phenological stages were determined in the field by cutting one fruit from each bunch and verifying endosperm and mesocarp characteristics, according to Forero and Romero's scale (Forero and Romero 2012). Bunches selected from each stage were cut and the damage produced by *D. neivai* was qualitatively rated using Cenipalma's damage evaluation scale (Aldana et al. 2004). Each bunch was then packed in a sack or canvas labeled with the bunch's phenological stage and code number and taken to the laboratory where they were emptied before recording live

and dead *D. neivai* individuals in the sample, separating by developmental stage (e.g., eggs, larvae, pupae, adults).

Live individuals were placed in $25 \times 15 \times 7$ -cm plastic containers with palm fruit as a food source after which they were monitored daily for 15 d to determine mortality factors. Dead individuals were dipped in 1% sodium hypochlorite for 1 min and then placed in sterile distilled water before placing them in containers to determine entomopathogen-related infections.

The microorganisms found were sent to Cenipalma's Entomopathogenic Microorganism Laboratory in Bogotá to be identified and added to the collection. The parasitoids found were identified and preserved in 70% ethanol for identification and incorporation into Central Region Collection of Insects.

Results and Discussion

Life cycle and description of developmental stages. The mean $(\pm SD)$ duration of the entire life cycle under laboratory conditions was 309.1 \pm 54.3 d. The duration of the eqg stage was 7.1 \pm 1.2 d (n = 314). Eqgs are oval and creamcolored, having a smooth surface and are placed by adults on bracts or epidermis of the fruits (Fig. 1A). The larval stage lasted 21.9 \pm 2.0 d with five instars (n = 143). Larvae are oval, striated, having legs attached ventrally and completely concealed from the dorsal view. Larvae were initially cream-colored or yellow, becoming reddish or purple during the last instars (Fig. 1B–F). The mean duration of the pupal stage was 19.6 \pm 3.0 d (n=77). Pupae were oval, similar to last-instar larvae. They were initially cream-colored or yellow, the center of the pupa becoming dark-colored towards the end of the stage when the adult is ready to emerge (Fig. 1G). Mean adult longevity was 268.9 \pm 53.1 d (n=19). Adults were flattened, oval-shaped, and reddish or brownish when newly emerged, becoming brown to dark red with age. They had filiform antennae with 11 antennomeres. The first seven segments are red and the last four are darker-colored. The scutellum was small, pentagon-shaped, the pronotum was wider than it was long, and the elytra were oval-shaped with rows of parallel points from the pronotum to the apical end (Fig. 1H; Table 1). Egg- and pupal-stage duration recorded during this study was similar to that reported by Aldana et al. (2003).

We found that adult *D. neivai* can live 9 months, on average, in laboratory conditions. However, individuals sometimes lived longer than 1 yr, which agrees with Aldana et al. (2004). Adult morphological characteristics agreed with Staines (2009).

Population dynamics. Altogether, 165 bunches of the five phenological stages were studied, with 83.6% damaged by *D. neivai*. During Stage 701 (initial bunch development and filling phase), 51.5% of the bunches had no damage caused by *D. neivai*, and 42.4% had Scale 1 damage caused by *D. neivai* (1–20% of bunch surface affected). *Demotispa neivai* adults begin to infest oil palm bunches at the beginning of fruit development and filling phase (Stage 701) when the bracts covering the fruit open (Aldana et al. 2004), accounting for the low (0–20% of bunches) damage on the bunch surface during Stage 701 (Table 2).

In Stage 703, 33.3% of bunches were at Scale 1, followed by bunches at Scale 5 (81–100% bunch surface damaged). Stage 705 was equal to that for both bunch



Fig. 1. Developmental stages of *Demotispa neivai*: egg (A); first instar (B); second instar (C); third instar (D); fourth instar (E); fifth instar (F); pupa (G); adult (H).

Developmental Stage	Number of Individuals	Average (d)	Cl* (1 - α = 95)	Mode (d)	Interval (Minimum-Maximum) (d)
Egg	314	7.1	0.1	7	4–12
Larva	143	21.9	0.3	23	15–27
1st instar	248	3.4	0.1	4	2–7
2nd instar	223	4.3	0.1	4	3–6
3rd instar	196	4.2	0.1	4	3–5
4th instar	172	4.7	0.1	4	3–7
5th instar	143	5.2	0.1	5	3–9
Pupa	77	19.6	0.7	17	16–28
Total to adult	77	48.4	1.1	54	38–58
Adult	19	268.9	23.9	236	229–454
Total cycle	19	309.1	24.4	287	257–492

Table 1. Duration of developmental stages of fruits scraper *Demotispa neivai* under laboratory conditions ($28.8^{\circ}C \pm 1.2^{\circ}C$ and $76.8\% \pm 6.3\%$ relative humidity).

* CI = confidence interval.

maturation phase Stages 800 and 805, and most bunches were rated as being Scale 5 (Table 2).

The four *D. neivai* developmental stages were found in all sampling due to overlapping pest generations (Fig. 2). Eggs were not affected by any organism during any sampling. Mortality of larvae and adults occurred in all samplings. The mean natural mortality was 33.6% for larvae, 20.1% for pupae, and 10.3% for adults (Fig. 3).

The highest larval mortality recorded was 61.1% \pm 23.5% (confidence interval [CI]: 1 – α = 95). The fungus *Metarhizium anisopliae* (Metchnikoff) Sorokin (Ascomycota: Hypocreales) was the most important mortality factor impacting larvae (maximum mortality recorded = 58.3%/sampling). This isolate was coded strain CPMa1502 and incorporated into Cenipalma's entomopathogenic collection at Cenipalma's Entomopathogenic Microorganism Laboratory in Bogota.

The highest *D. neivai* pupal mortality was $40.0\% \pm 27.7\%$ (CI), associated with predation by ants and *Hololepta* sp., thereby agreeing with reports by Aldana et al. (2004). Pupae were parasitized by *Tetrastichus* sp. (Hymenoptera: Eulophidae) in two of the samples (4.8%), which was also reported by Aldana et al. (2004), who postulated that, since most *D. neivai* pupae are located inside the bunches, the probability for parasitism is low and only occurs when pupae are close to the surface of a bunch. Genty and Mariau (1973) and Genty (1978) recorded *D. neivai*

		Percentage of Bunches (%)					
Phenological Stage	п	No Damage	Scale 1 (1–20% of the Surface Affected)	Scale 2 (21–40% of the Surface Affected)	Scale 3 (41–60% of the Surface Affected)	Scale 4 (61–80% of the Surface Affected)	Scale 5 (81–100% of the Surface Affected)
701	33	51.5	42.4	0.0	3.0	3.0	0.0
703	33	6.1	33.3	12.1	12.1	15.2	21.2
705	33	18.2	30.3	0.0	6.1	12.1	33.3
800	33	6.1	3.0	15.2	9.1	18.2	48.5
805	33	0.0	12.1	12.1	12.1	15.2	48.5
Total	165	16.4	24.2	7.9	8.5	12.7	30.3

 Table 2. Percentage of oil palm bunches having different levels of damage caused by *Demotispa neivai*, rating based on Cenipalma's damage scale (Aldana et al. 2004).

as having two parasitoids from the order Hymenoptera: one each from Eulophidae and Chalcididae; however, only Eulophidae was recorded in our study.

The highest adult mortality was $29.1\% \pm 22.1\%$ (CI) associated with predation by spiders or death due to longevity. Aldana et al. (2004) indicated that spiders are one of the most frequently occurring natural enemies controlling *D. neivai* populations.

The fungus *M. anisopliae* infected both *D. neivai* larvae and adults. This is the first record of this in Colombia. Other researchers have found *Beauveria* sp. infecting *D. neivai*, however, studies aimed at demonstrating its potential efficacy have not been successful (Aldana et al. 2004, 2010; Valencia et al. 2007).

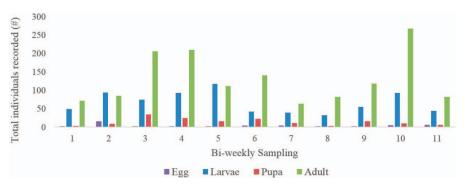


Fig. 2. *Demotispa neivai* developmental stages recorded by biweekly sampling over a 22-week period on an oil palm plot.

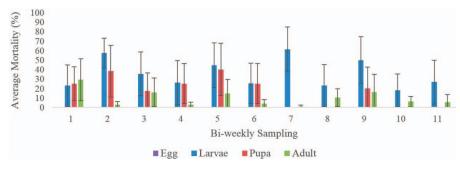


Fig. 3. Total natural mortality of *Demotispa neivai* by developmental stage over a 22-week period on an oil palm plot.

Analyzing *D. neivai* stages regarding bunch phenological stages revealed higher *D. neivai* population in Stage 703, followed by 705 (bunch development and filling phase). *Demotispa neivai* eggs were only found in Stages 701, 703, and 705. *Demotispa neivai* larvae were recorded in all bunch phenological stages (Table 3), pupae were found in all but Stage 805 (bunch maturation phase) (Table 4), and adults were present in all the phenological stages studied (Table 5).

Fruit had reached 50% of final size during Stage 703 and 80% in Stage 705 (Forero and Romero 2012, Hormaza et al. 2010), thereby providing *D. neivai* larvae and adults with a greater food source when feeding on the epidermis of fruit as this becomes exposed as the fruit grows and is no longer protected by the spathes (Aldana et al. 2004, 2010).

		Average Larval Mortality					
Phenological Stage	<i>n</i> *	Number of Total Larvae	Infected by <i>M. anisopliae</i> (%)	Cl** (1 – α = 95)	Other Causes (%)	Cl (1 – α = 95)	
701	33	30	11.1	11.4	0.0	0.0	
703	33	419	11.7	6.6	1.1	1.0	
705	33	238	17.7	10.7	5.7	3.9	
800	33	24	62.5	16.5	29.2	15.4	
805	33	17	60.0	18.7	20.0	15.3	
Total	165	728	25.3	5.9	8.3	3.7	

 Table 3. Total Demotispa neivai larvae and average mortality caused by Metarhizium anisopliae and other undetermined causes during each phenological stage regarding bunches in an oil palm plantation.

* Amount of bunches evaluated.

** CI = confidence interval.

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		Average Pupal Mortality				
Phenological Stage	<i>n</i> *	Number of Total Pupae	<i>Tetrastichus</i> sp. Parasitoids (%)	Cl** (1 – α = 95)	Other Causes (%)	Cl (1 – α = 95)
701	33	5	0.0	0.0	0.0	0.0
703	33	72	0.8	1.1	0.8	1.1
705	33	69	0.0	0.0	24.8	12.5
800	33	9	0.0	0.0	71.4	16.6
805	33	0	0.0	0.0	0.0	0.0
Total	165	155	0.3	0.3	19.8	5.7

Table 4. Total Demotispa neivai pupae and mortality caused by Tetrastichussp. and other causes, discriminated by a bunch's phenologicalstages in an oil palm plantation.

* Amount of bunches evaluated.

** CI = confidence interval.

In conclusion, *D. neivai* was found in all bunch phenological stages; however, the stages during which the highest populations were recorded were in the fruit development and filling phase. Such knowledge will enable development of a more effective and sustainable *D. neivai* management program for oil palm grown in Colombia. The fungus *M. anisopliae* CPMa1502 strain, isolated from *D. neivai*

		Average Adult Mortality				
Phenological Stage	n*	Number of Total Adults	Infected by <i>M. anisopliae</i> (%)	Cl** (1 - α = 95)	Other Causes (%)	Cl (1 - α = 95)
701	33	307	0.7	0.0	4.1	6.6
703	33	664	0.0	0.0	2.6	1.6
705	33	326	0.3	0.5	12.2	9.3
800	33	73	0.0	0.0	22.8	12.1
805	33	64	0.0	0.0	12.9	8.5
Total	165	1,434	0.2	0.3	10.1	3.7

Table 5. Total Demotispa neivai adults and mortality caused by Metarhizium
anisopliae and other causes, discriminated by a bunch's phenolog-
ical stages in an oil palm plantation.

* Amount of bunches evaluated.

** CI = confidence interval.

larvae, is a promising microorganism for the biological control of *D. neivai* and should be considered for future research concerning this pest.

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