ECOLOGY, BEHAVIOR AND BIONOMICS

Seasonal and Vertical Distribution of *Phyllophaga cuyabana* (Moser) (Coleoptera: Melolonthidae) in the Soil Profile

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Edited by Angelo Pallini – UFLA

Neotropical Entomology 38(5):582-588 (2009)

Distribuição Estacional e Vertical de *Phyllophaga cuyabana* (Moser) (Coleoptera: Melolonthidae) no Perfil do Solo

RESUMO - O padrão de distribuição temporal e vertical de *Phyllophaga cuyabana* (Moser) foi avaliado no perfil do solo para subsidiar amostragens da população do inseto visando ao seu manejo. Em levantamentos populacionais realizados durante três anos, em de Boa Esperança, PR, *P. cuyabana* foi univoltina, com baixa sobreposição de estádios de desenvolvimento. Houve um pico populacional no verão (dezembro a fevereiro) e um declínio nos meses frios, quando as larvas estavam em diapausa. O inseto, nos distintos estágios de desenvolvimento, explorou diferentes profundidades do solo. Ovos e larvas no início do primeiro instar concentraram-se entre 5 cm e 10 cm de profundidade e, ao se desenvolverem, atingiram 30 cm de profundidade. Adultos e ovos ocorreram na primavera (outubro a dezembro), quando começaram a ser observadas as larvas ativas em amostras realizadas entre zero e 15 cm de profundidade. Larvas em diapausa e pupas foram observadas em maior concentração entre 15 e 30 cm de profundidade, do inicio do outono ao início da primavera. O número de insetos no solo (até 40 cm de profundidade) mostrou relação funcional positiva com a temperatura do ar e com a evapotranspiração. Entretanto, a relação da distribuição percentual de larvas no perfil do solo com a temperatura do solo foi positiva apenas para profundidades de zero a 10 cm. Para estimar a população de corós de novembro a abril, as amostragens podem ser feitas até 20 cm de profundidade, porém de maio a outubro a profundidade das amostragens deve atingir 30 cm.

PALAVRAS-CHAVE: Scarabaeoidea, coró, praga de solo, macrofauna de solo

ABSTRACT - *Phyllophaga cuyabana* (Moser) temporal and vertical distribution patterns were evaluated in the soil profile, in order to subsidize methodology for population sampling, aiming at its management. In insect surveys carried out during three years, in Boa Esperança County, State of Parana, Brazil, *Phyllophaga cuyabana* was univoltine, with little overlap of the larval stages. Population peaked during December-February, but declined during the colder months, when larvae were in diapause. Different developmental stages exploited distinct soil depths. Eggs and early first instars tended to concentrate between 5 cm and 10 cm deep, but they spread more uniformly through the soil profile, reaching depths up to 30 cm, as they developed. Adults and eggs occurred in the spring (October to December) when active larvae also started to be observed; feeding larvae occurred up to late-April between 0 to15 cm deep. Diapausing larvae and pupae were observed from early fall to early spring, mostly from 15 cm to 30 cm deep. Throughout the year, the number of insects in the soil (up to 40 cm deep) showed a positive functional relationship with air temperature and evapotranspiration. The relationship of percent distribution of larvae in the soil profile and soil temperature, however, was positive only above 10 cm. To estimate the insect population from November to April, samples can be collected until 20 cm deep; from May to October, however, samplings should be deeper, up to 30 cm.

KEY WORDS: Scarabaeoidea, white grub, soil pest, soil macrofauna

The American genus *Phyllophaga* Harris includes around 840 described species, and many others remain undiscovered in nature or undescribed in collections. The taxonomy of the group is poorly studied in South America, despite the importance of some species as pests of a variety of cultivated plants (Morón 1986, Aragón-García *et al* 2003). The identification of *Phyllophaga* species is not an easy task, considering that most species were described during the 19th century based on a few specimens with brief data labels ("Brazil"), and some type specimens remain unavailable (M A Morón, personal communication). A number of species are very similar and the limits of specific variation are unknown. Similarly, the geographic distribution of each species is poorly known, as well as the feeding preferences of larvae and adults. Until 2001, 31 species of *Phyllophaga* were recorded in Brazil, but the actual scenario points that 70 different species may occur in the country (Morón & Rojas 2001, Morón 2004).

The life cycle of *Phyllophaga* varies from one to four years depending on the species as well as on the geographical latitude (Morón 1986, Diagne 2004). Predominantly, *Phyllophaga* species in Latin America complete their life cycle in one year, except for some species from the *rorulenta* and the *pruinosa* groups (Aragón-Garcia *et al* 2005) and *Phyllophaga triticophaga* Morón & Salvadori in South Brazil (Salvadori & Silva 2004), which present a biannual cycle. *Phyllophaga cuyabana* (Moser) is considered a soybean and maize pest in South and Central Brazil (Oliveira *et al* 2004). Although some studies on its life cycle (Oliveira *et al* 1996) and adult behavior (Garcia *et al* 2003, Oliveira & Garcia 2003, Oliveira *et al* 2007, Zarbin *et al* 2007) were carried out in Brazil, little is known on its bionomic aspects in the field. Therefore, the objectives of this research were to study the seasonal occurrence of the *P. cuyabana* population in soybean fields and to establish the effect of climatic factors on the temporal and vertical distribution patterns of the white grub in the soil profile, to allow for adequate population sampling techniques to aid on the management of this pest on soybean crops.

Material and Methods

Study site, insects and sampling procedures. Field experiments were conducted in Boa Esperança County, State of Paraná (24° S, 53° W), South Brazil. The climate is Mesotermic humid subtropical, with rains concentrated in the summer. The clay type soil from the study area was classified as an anionic acrudox. Climatic data were recorded from a meteorological station located ca. 8 km from the study area.

Voucher specimens of immature stages and adults were deposited at the collections of Embrapa Soja, Londrina County, State of Paraná, Brazil and of the Instituto de Ecologia, A.C. Xalapa, Mexico. According to the field data, a large number of species of melolonthids were collected in Boa Esperança, but *P. cuyabana* was the only representative of the genus *Phyllophaga* (M A Morón, personal communication).

Surveys to assess *P. cuyabana* population dynamics and its distribution in the soil profile were carried out in areas selected by containing high incidence of white grubs in the previous crop season. Soybean was cultivated under conventional soil management system (one ploughing and two harrowing), followed by no-till wheat as the winter crop. Insect surveys were performed from April 1990 to May 1993, within one hectare area. Eggs, larvae, pupae and adults were recorded every 15 days on 20 soil samples randomly collected within 50 cm x 25 cm x 40 cm deep areas, at each sampling date. Surveyed insects were returned to the same soil spot, shortly after samplings were recorded.

Additional observations of the temporal population distribution were carried out from September to May (1994-2001) to record the initial flights of adults and in the diapausing larvae. Sampled larvae were classified in three active instars and in diapausing larvae (third instar with diapause characteristics, i.e., white raster, turgid body, apparent immobility, and dwelling on earthen chambers). The beginning of the adult flights were determined using light traps and by direct daily observations during twilight and early night times, during September and October of each year.

Surveys for the distribution of eggs, larvae, pupae and adults in the soil profile were conducted in two 1 ha areas. At each sampling date, the number of individuals out of four soil samples randomly collected on each block was recorded every 15 days. Soil samples were collected along the plant rows at five different depths (0-5 cm, 6-10 cm, 11-15 cm, 21-30 cm, and 31-40 cm), within the sampled area previously mentioned (50 cm in length x 25 cm wide). Soil temperature was measured with a digital thermometer, and soil moisture was measured using the thermo gravimetric method in each soil layer per sampling date, and expressed in Mega Pascal (MPa). The soil water potential was assessed by using the soil moisture characteristic curve from soil samples collected at the beginning of the study.

Statistical analyses. The number and size of samples were determined based on preliminary trials with *P. cuyabana*, considering the accuracy, objective, and effort/sample ratio (Southwood 1975).

A randomized complete block design with five replicates was used on field surveys for assessing eggs, larvae, pupae and adults on the soil profile. Regression analyses of the number of insect/m^2 and climatic data (air temperature, air relative humidity, and evapotranspiration), as well as insect distribution with soil temperature and soil water potential were performed.

Results

Seasonal distribution of insect phases and population fluctuation. The variation on the *P. cuyabana* population followed a similar pattern throughout the three years of experiments; there was a population peak from December to February, followed by a decrease in the population during the coldest and driest months (Fig 1). The number of insect per square meter in the soil (until 40 cm deep) showed a positive functional relationship (regression) with the air temperature

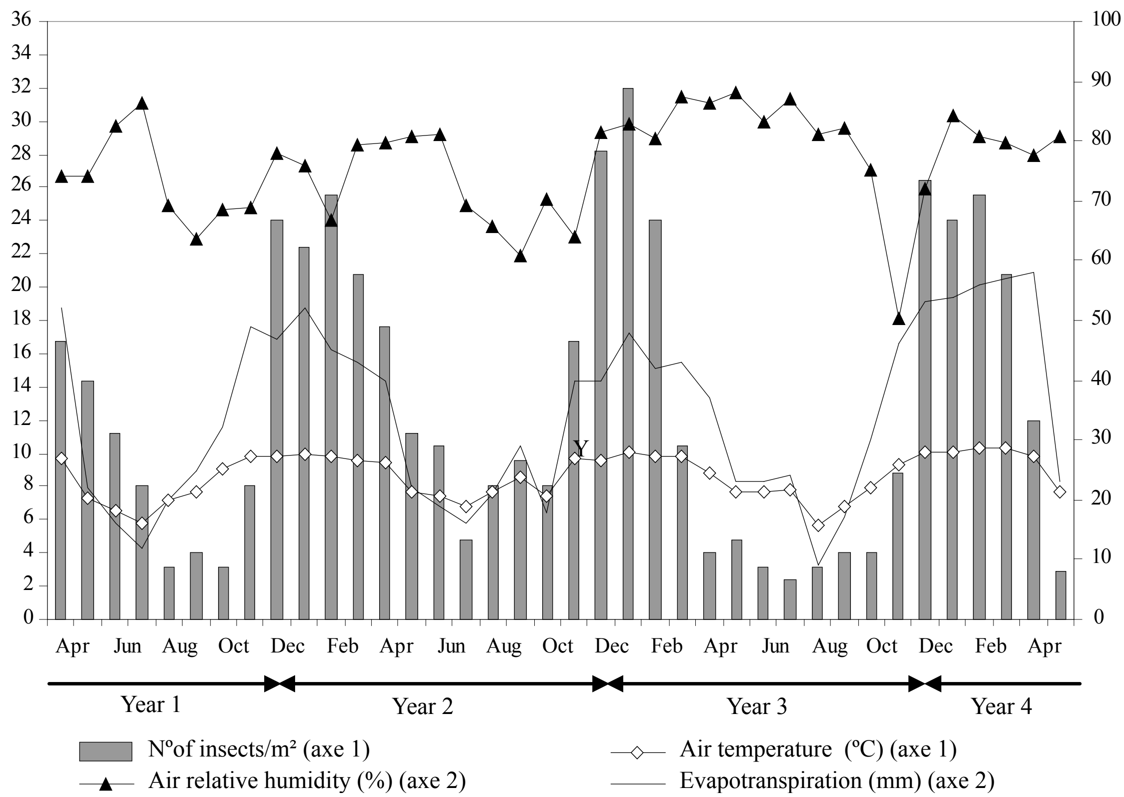

Fig 1 Climatic conditions and population dynamics of *Phyllophaga cuyabana* during 36 months in an area cultivated in a succession soybean-wheat cropping system.

($R^2 = 0.510$; $P < 0.05$) and the potential evapotranspiration ($R^2 = 0.497$; $P < 0.05$). No significant functional relationship of the insect population and the air relative humidity was observed.

The white grub fluctuation pattern within the studied area revealed that the most susceptible growth stage of the soybean plant coincides with the occurrence of the most damaging larval instars. Depending on the year, the population increase observed from mid-December to early-January coincided with the initial outbreak of the second instars, due to the eclosion of the offspring of females emerging late in the season.

The occurrence of only one generation per year was observed during the three years of study. Eggs, active larvae, pupae and adults of *P. cuyabana* in the field overlapped mostly in October-November (Fig 2), in general, with a

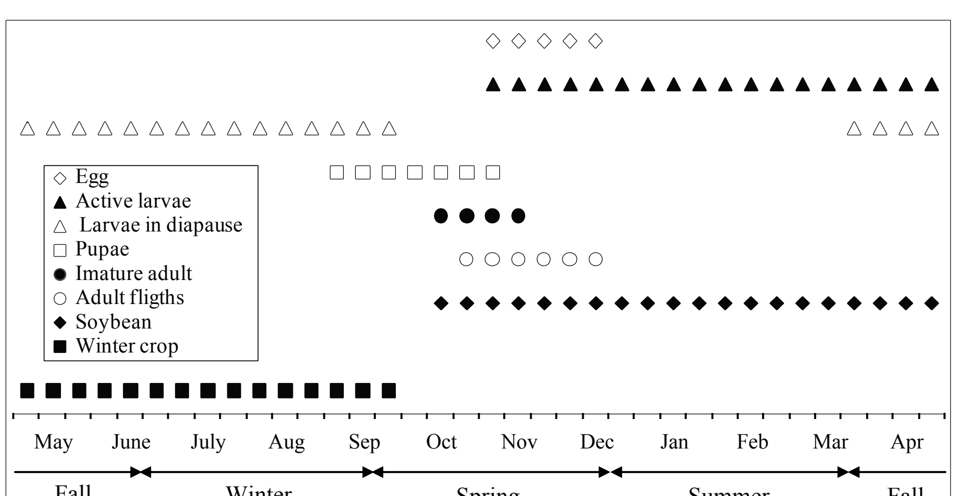

Fig 2 Annual occurrence of different developmental stages of *Phyllophaga cuyabana* in an area cultivated in a succession soybean-wheat cropping system.

predominance of one developmental stage at each month. Active *P. cuyabana* stages (adult and larvae) were observed from late-October to late-April.

Eggs were observed from November to part of December coinciding with the germination and early vegetative stages of the soybean plants (Fig 2). Active larvae occurred from November to April; first instars were common in December, and second instars in January, reaching 68.6% and 54.0% of the total recorded specimens, respectively. Third instars were active in the summer, and were firstly recorded in January, reaching 77.2% of all surveyed individuals in February, 92% in March, and 67.2% in April. No first or second instars were found from February to October, but third instars were found almost throughout the year (January to October).

During nine growing seasons (from 1990/91 to 1998/99), the first larvae in diapause occurred at early fall, i.e., between the third week of March and first week of April. In general, by the end of April, all larvae were in diapause. Pupation occurred in earthen chambers mostly (> 60%) during September and up to early-November (late winter and early spring).

Adults were observed from October to mid-December, but remained inactive inside earthen chambers from early-October to late-November (44.7% of observed specimens). Adult flights were observed from October 25^{th} to November 3^{rd}, during the spring, when the air relative humidity increased.

Population distribution in the soil profile. The percent distribution of larvae in the soil profile along the year had a positive functional relationship (regression) with the soil temperature only from 0 to 10 cm deep ($ß = 3.175$, $R^2 = 0.560$, $P < 0.01$). Significant relationship of larval proportion in the soil profile with soil temperature was also observed at the 0-5 cm ($ß = 1.441$, $R^2 = 0.727$, $P < 0.01$) and 6-10 cm layers ($ß = 1.837$, $R^2 = 0.657$, $P < 0.01$). Specimens of *P. cuyabana* were observed throughout the year at different depths in the soil profile (Fig 3). At the active stages, *P. cuyabana* was concentrated from 0 to 20 cm deep (Figs 3 a,b,c). Only from November to April, insects were recorded from 0 to 10 cm deep (Figs 3a, b).

From November to April (except in March) between 40% and 62% of the larval population was found in the 11 cm to 20 cm deep soil layer (Fig 3c). In such population, some individuals were larvae in diapause. At the 21-30 cm deep soil layer, the concentration of specimens started in March, reaching the highest proportion (more than 60% of the surveyed individuals), in August (Fig 3d), mainly as larvae in diapause. Only during the colder months of the year (May to September), larvae were observed at the 31-40 cm deep layer, but again in diapause and in a proportion lower than 15% of the total population (Fig 3e).

Different developmental stages exploited different soil depths (Fig 4). Eggs (65%) and early first instars (59%) tended to concentrate from 6 cm to 10 cm deep (Fig 4a). As they develop, they spread more evenly through the soil profile, dwelling in deeper layers, with some of them reaching up to 30 cm. Most of them (77% of second instars and 82% of third instars) were observed from 2 cm to 15 cm deep (Fig 4b). *Phyllophaga cuyabana* inactive stages that occurred in the winter were mainly concentrated in depths ranging from 15 cm to 30 cm (Fig 4c). Larvae in diapause, pupae and adults (inside earthen chambers) were observed from 6 cm to 30 cm; larvae in diapause were more frequently observed (51%) from 20 cm to 30 cm deep (Fig 4c), where temperature and soil water potential were more stable (Fig 3d). Pupae were concentrated at the 16-30 cm deep soil layer, reaching 78% of the total estimated number of specimens.

In the colder months, the larvae in diapause inhabited deeper layers, most likely to survive until summer crops were established. On the other hand, when a new generation begun, the population concentrated in shallower layers (0-15 cm), where secondary roots are more abundant.

Discussion

In all the field experiments conducted with *P. cuyabana* during three years in Boa Esperança County, State of Paraná, the insect had one single generation each year. This information reinforces that in Latin America only few species of *Phyllophaga* have longer life cycle, as observed by Salvadori & Silva (2004) and Aragón-Garcia *et al* (2005).

The active part of the *P. cuyabana* life cycle is synchronized with its source of food, the soybean crop. In fact, eggs and first instars were observed right after soybean emergence until the vegetative stage. The most active and damaging individuals (second and third instars) were concentrated up to 20 cm in depth, close to plant roots, and occurred from the late vegetative stage to the harvesting of the soybean crop. Second instars were more abundant in January (late vegetative stage and flowering), while third instars prevailed in March (reproductive stage). Therefore, despite starting in the spring, the active larvae occurred mostly in the summer.

Differently, in Mexico, some specie from the genus *Phyllophaga* are active most of the year, excepting in the winter (Deloya 1993). In Boa Esperança, during part of the fall, the incidence of active larvae and third instars in diapause overlapped, indicating that *P. cuyabana* overwinters at this stage. This statement is supported by the observations that all third instars from May to August were in diapause inside earthen chambers, apparently motionless, presenting white raster and turgid body. In Louisiana, USA, according to Diagne (2004), *P. ephilida* (Say) spends the winter and part of the spring as third instars, pupating at the end of spring and emerging in the summer. Here in the studied site, the first larvae in diapause occurred between the third week of March and first week of April (late summer to early fall), and at the end of April all larvae were in diapause, during nine growing seasons (1990/91 to 1998/99).

Flights of adults were also observed for a short period, from late-October to early-November, during springtime. Deloya (1993) also observed a great abundance of emerging adults during spring in México. The relationship of climatic factors with population of *P. cuyabana* (number of insects/m^2) was quite evident, mainly with air temperature and potential evapotranspiration. Unexpectedly, no functional relationship (regression) of white grub population with air relative humidity was observed in our studies, considering that this climatic factor, in general, stimulates activity

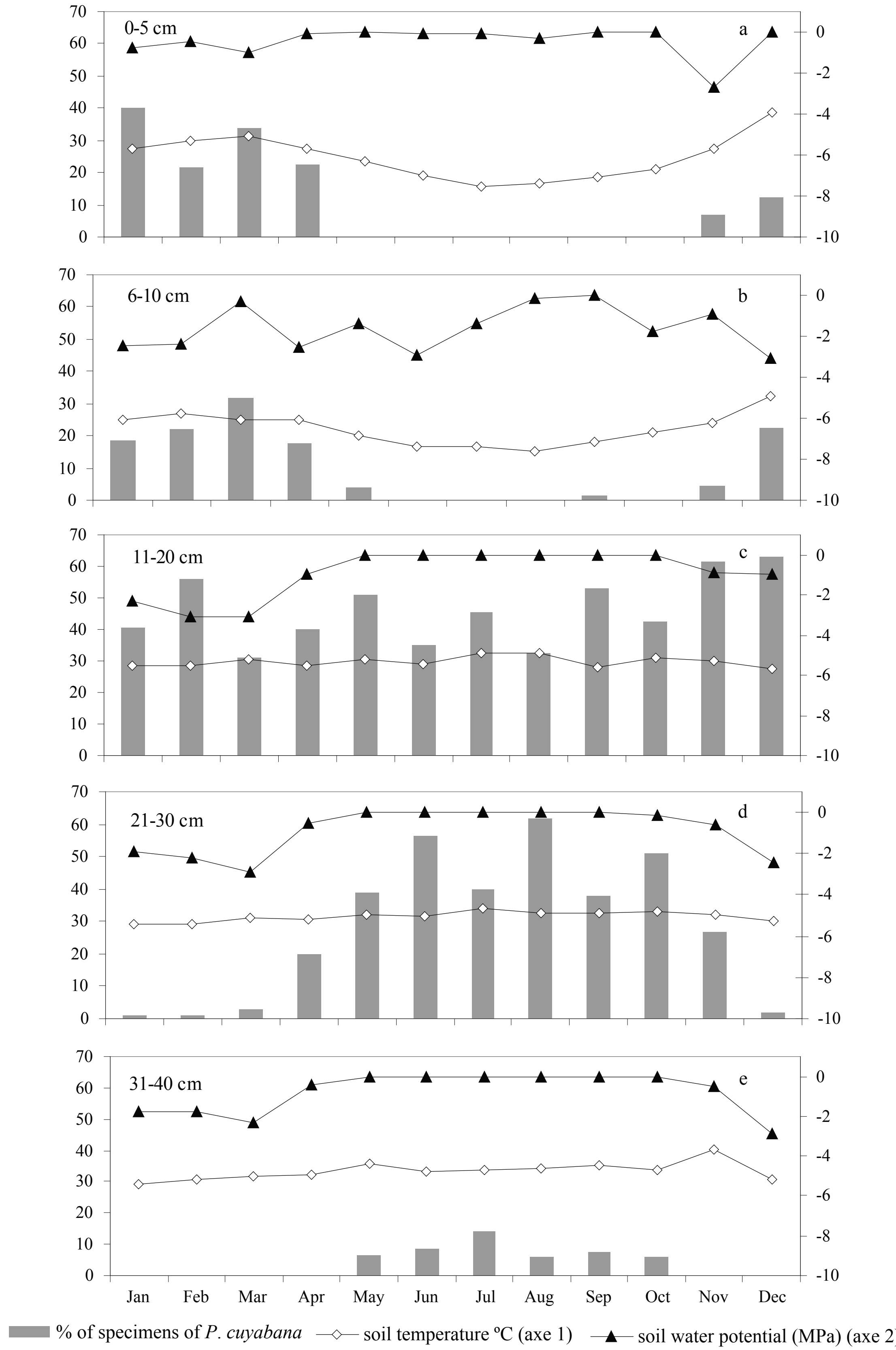

Fig 3 Frequency of *Phyllophaga cuyabana* specimens (%) on the soil profile, soil temperature and soil water potential.

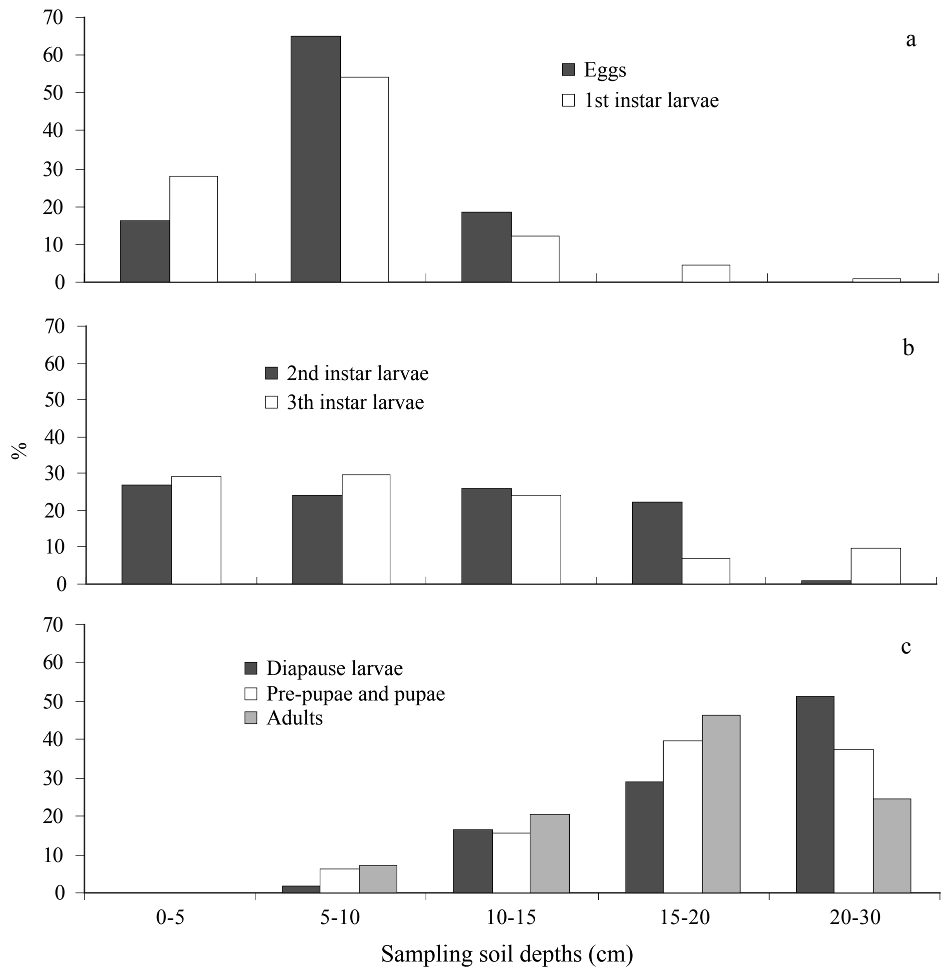

Fig 4 Distribution of the developmental stages of *Phyllophaga cuyabana* in the soil profile.

of dwelling insects above the ground. For example, Riis & Esbjerg (1998) observed that the *Cyrtonemus bergi* Froeschner (Hemiptera: Cydnidae) flight was induced by high air relative humidity, regardless the level of the soil moisture. However, in the observations reported herein, the highest air relative humidity was observed during July and August (winter), and adult flights were observed from October to December (spring).

Other climatic factors as soil moisture levels on the egg laying sites are essential for *Phyllophaga* larval development (Watschke *et al* 1995). Likewise, besides egg development, *Phyllophaga crinita* (Burmeister) first instars survival also requires adequate levels of soil moisture (Gaylor & Frankie 1979). Under conventional soil management system, some *P. cuyabana* larvae at active stage were able to exploit the soil profile up to 30 cm deep, most likely searching for adequate climatic conditions. However, Santos (1992) observed that in a no-tillage area the active larvae were more frequent no deeper than 10 cm. This author also reported that the soil compaction at the 10 cm to 20 cm deep soil layer impaired the larva movement in the soil profile.

White grubs were able to exploit different soil profiles at different stage of development and climatic conditions. In general, with high air temperature in spring and summer, adults, eggs and active larvae were observed concentrated more superficially than the diapausing larvae and pupae. At these stages, that occurred in the winter, when the air temperature was low, the insects preferred deeper soil layers where the soil temperature was more stable and higher.

The ability of *P. cuyabana* larvae to move downward in the soil profile probably makes them less susceptible to environmental stress and, possibly, in deeper soil layers they face less competition for physical space and food. This behavior was also observed by Villani & Wright (1988) in other Scarabaeidae, as *Popillia japonica* Newman and *Rhizotrophus majalis* (Razoumowsky). The soil moisture is also likely to influence insect movement, and insects are attracted to moistened conditions (Riis & Esbjerg 1998).

In our observations, the soil water potential near zero was possibly one of the determining factors for the vertical movement of *P. cuyabana* larvae, mainly in winter, when the soil temperature in deeper strata was higher and more stable than on the superficial ones. As this type of movement is very important for *P. cuyabana* survivorship and it is related to climatic factors, this trait must be considered when choosing sampling dates (Globoza *et al* 1998); on hot days and in dry periods, the population may be underestimated if only the superficial layers of the soil are sampled.

In conclusion, our data corroborate that *P. cuyabana* is univoltine, with little overlap among developmental stages in spring (October-November), mainly as eggs, active larvae, pupae and adults. Data obtained in this study suggest that samples to estimate at least 70% of *P. cuyabana* population should be collected up to 20 cm deep, from November to April. From May to October, however, deeper sampling should be taken, i.e., up to 30 cm deep.

Acknowledgments

To M A Morón (Instituto de Ecología, A.C., Mexico) for the identification of voucher specimens of *P. cuyabana* and for the kind help with the details of this insect's systematics. To CAPES for the doctoral fellowship granted to LJO.

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Received 22/I/08. Accepted 17/VII/09.