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Comparing different methods to assess weaver ant abundance in plantation trees

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ABSTRACT. Weaver ants (*Oecophylla* spp.) are widely used as effective biological control agents. In order to optimize their use, ant abundance needs to be tracked. As several methods have been used to estimate ant abundance on plantation trees, abundances are not comparable between studies and no guideline is available on which method to apply in a particular study. This study compared four existing methods: three methods based on the number of ant trails on the main branches of a tree (called the Peng 1, Peng 2 and Offenberg index) and one method based on the number of ant nests per tree. Branch indices did not produce equal scores and cannot be compared directly. The Peng 1 index was the fastest to assess, but showed only limited seasonal fluctuations when ant abundance was high, because it approached its upper limit. The Peng 2 and Offenberg indices were lower and not close to the upper limit and therefore showed fluctuations throughout the season. The numbers of nests showed high fluctuations unlikely to reflect ant abundance, but rather reflected nest building behaviour influenced by tree phenology. In conclusion, nest counting is not recommended, whereas the Peng 1 index can track dynamics at low ant abundance and the Peng 2 and Offenberg indices can be used in most situations.

Keywords: *Oecophylla*, density index, mango, cashew, Benin

INTRODUCTION

The production of several tree crops is severely constrained by pest damage. Control methods against pests based on chemical pesticides present risks to public health and the environment (De Bon *et al*. 2014). Therefore, worldwide, research efforts have been intensified to develop alternative control strategies, including the use of biological control agents (van Lenteren 2000; Neuenschwander *et al*. 2003; Adandonon *et al*. 2009). In tropical countries, the successful application of the weaver ant, *Oecophylla* spp. (Hymenoptera: Formicidae), as an endemic natural enemy is on the rise, partly triggered by emerging markets for organic produce (reviewed by Van Mele 2008). *Oecophylla* spp. are known as the 'living pesticide' in China, and represent the earliest written record of biological control (Hölldobler & Wilson 1990). According to Dejean (1991), an ant colony with 12 nests (*Oecophylla* spp. colonies are polydomous) can capture 45,000 prey items per year. The two species of weaver ants (*Oecophylla smaragdina* and *O. longinoda*) are effective biological control agents against more than 50 different pests in many tropical crops and forest trees (Way & Khoo 1992; Peng *et al*. 1995). *Oecophylla* ants can be equally or more effective than chemical pesticides (Peng & Christian 2005a; Dwomoh *et al*. 2009; Offenberg *et al*. 2013). The effectiveness of weaver ants in controlling pests is positively correlated with ant abundance on their host trees. According to Peng *et al*. (2008), weaver ant colonies must be managed to keep abundance high and in this way obtain efficient control of pests. It is generally believed that trees are well protected against insect pests if more than 50% of tree's main branches hold weaver ant trails. Therefore, weaver ant abundance is an important factor which must be monitored frequently to gain maximum profit from the presence of ants.

Various methods to track weaver ant abundance on trees have been developed. The most common methodology is based on the "branch method" originally developed by Peng & Christian (2004), hereafter called the Peng 1 index. This index is the most simple of the branch indices and is the percentage of main branches on a tree that hold a weaver ant trail, disregarding the density of the ant trail. This method has been used in multiple studies (Peng & Christian 2005a, Peng & Christian 2005b, Peng & Christian 2006; Peng & Christian 2007, Peng & Christian 2008, Peng *et al*. 2008, Peng *et al*. 2009; Peng *et al*. 2011). Later this index was modified by dividing ant trails into two different densities thereby increasing the resolution (Peng *et al*. 2005). We refer to this as the Peng 2 index. This index was evaluated by Van Mele *et al*. (2007) and found a suitable method to assess weaver ant abundance. Apart from Peng *et al*. (2005), the Peng 2 index has been used by Van Mele *et al*. (2007), Vayssières *et al*. (2011), Olotu *et al*. (2013a) and Olotu *et al*. (2013b). Recently, Offenberg and Wiwatwitaya (2010) again modified the branch index to include a further division of ant trails in relation to the number of ants on the trails – in this case trails were divided into three different densities. This method has been used by Offenberg & Wiwatwitaya (2010), Offenberg *et al*. (2013), Christian Pinkalski *et al*. (unpublished data) and Florence Anato *et al*. (in press) and is hereafter called the Offenberg index. Another frequently used method to score ant abundance is the counting of the number of ant nests in trees. Multiple studies on weaver ants have used this method (Rapp & Salurn 1995, Peng *et al*. 1997a, Peng *et al*. 1997b, Ayenor *et al*. 2007, Dwomoh *et al*. 2009, Olotu *et al*. 2013a, Olotu *et al*. 2013b).

Thus, different methods have been used to assess weaver ant abundance and each method may have pros and cons related to environmental conditions and the objective of a particular study. As a consequence of the multiple methods in use, it is hard to compare ant abundance between different studies and it may be difficult to decide which index is preferable for a particular study. Also, the use of different methods makes it difficult to work out a general recommendation as to when ant abundance is adequate for efficient biological control of pests. For example Peng *et al*. (2008) argue that the Peng 1 index should exceed 50% on average on plantation trees for efficient control of pests, but how does this relate to the other indices? In the present study we used the four different methods to estimate ant abundance on the same trees (mango and cashew) to compare how different estimates correlate and to assess the suitability of the different methods un-

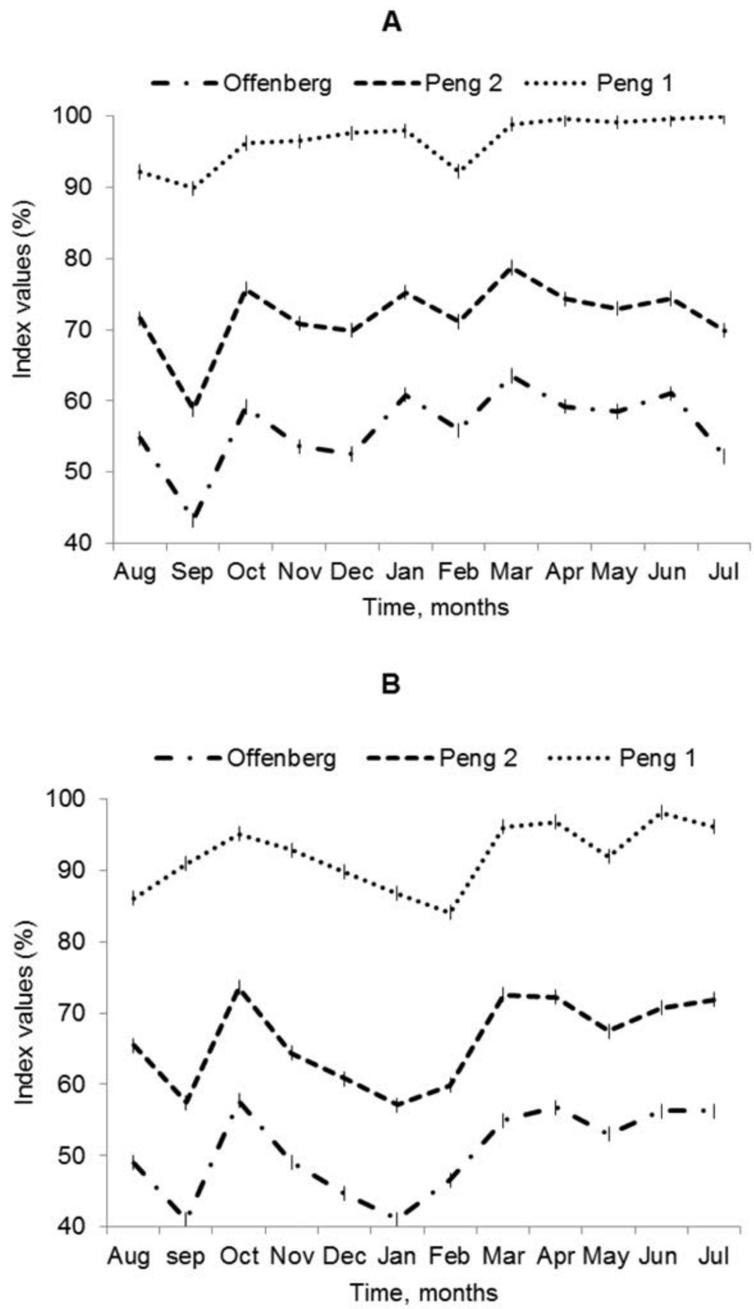


Fig. 1. The average weaver ant index scores per tree (± SE) with the Peng 1, Peng 2 and Offenberg indices by time from August 2012 to July 2013 on mango (A) and cashew (B); N = 144 trees per sampling date in each crop

der different conditions (crops and season). Specifically, we tested if the three different branch methods differed significantly in their index values and if correlations between indices can be used to convert values between the different methods. We also test if branch method estimates were sensitive to the number of main trunks on a tree, as this is a subjective measure that may vary between different observers.

MATERIALS AND METHODS

Study site and experimental design

The study was conducted in mango and cashew plantations, each of 4ha in the Parakou area in Benin (09° 22' 13"N / 02° 40'16"E). Mango trees were between 30 to 31 years old and cashew trees were between 20 to 25 years old. Each orchard had an average of 100 trees/ha at a 10 m × 10 m density and trees were homogeneous in height. In each orchard 144 trees with weaver ants (*O. longinoda*) were selected and used to monitor ant abundances. Trees were selected from a plot where ants were already present as a part of another study testing the effects of ants on crop yields.

Monitoring of weaver ant abundance

Using each of the three branch methods, weaver ant abundance was assessed fortnightly in each tree from August 2012 to July 2013 in both the mango and the cashew orchard. In total, ant densities were estimated 27 times for each tree using these methods. The counting of ant trails on the trees was conducted between 09:30 and 13:30 hours which is within the most active period of weaver ants (Vayssières *et al*. 2011). The same sampling session was used to calculate all three branch indices.

The Peng 1 index was calculated as the number of main trunks with at least one weaver ant divided by the total number of main trunks on the tree. This number was multiplied by 100 to convert it into a percentage (Peng & Christian 2004). The Peng 2 index was calculated by counting the number of main trunks with 1 – 10 ants and the number of trunks with more than 10 ants. Low density trails (1 – 10 ants) were then assigned a half trail score and the high density trails assigned a full trail score. The sum of trail scores on each tree was then divided by the total number of main trunks on the tree and multiplied by 100 (Peng *et al*. 2005). The Offenberg index was calculated by dividing ant trails into trails with 1 – 9 ants per m (low density trail), trails with 10 – 50 ants per m (medium density trail) and trails with more than 50 ants per m (high density trail). The low density trail was assigned 1/3 trail score, the medium density trail 2/3 trail score and the high density a full trail score. The sum of trail scores on a tree was then divided by the number of main trunks on the tree and multiplied by 100 to produce the index value (Offenberg & Wiwatwitaya 2010). Assessing the branch index on a tree takes approximately 40 s, 1 min and 1 min 30 s, for the Peng 1, Peng 2 and Offenberg index, respectively. Finally, weaver ant abundances were also assessed by counting the number of weaver ant nests in each tree once a month (12 times during the study period). Counting the number of weaver ant nests takes approximately 3 min 30 s per tree.

ANALYSIS

The average index value and nest numbers per tree were plotted by season for each method in each crop to assess their seasonal dynamics. Furthermore, seasonal percentage-wise fluctuations in each index were calculated as the highest (average) value during the season minus the lowest (average) value divided by the lowest and multiplied by 100 [% fluctuation = ((highest average – lowest average)/lowest average) × 100]. For each crop and each type of branch index, the average of all the 27 index values collected during the seasons was then calculated for each tree. Based on these average scores, Pearson correlation analyses between the different ant abundance indices were conducted (N=144 trees in each correlation), except in two cases where residuals were not normally distributed (Fig. 3D, 4D) and therefore Spearman correlations were used instead. Residuals were considered normally distributed if their distributions appeared symmetric by visual inspections and if their kurtosis and skewness were between −1 and 1. Scores obtained with the three different branch methods were also compared with pairwise Wilcoxon tests to test if they produced equal scores. Lastly, the number of main trunks on trees was compared with the score values obtained from the three branch methods (Peng 1 index, Peng 2 index, and Offenberg index). Values presented in the results are means (± standard errors). Statistical analyses were done with JMP 10.0.0. (SAS 1995).

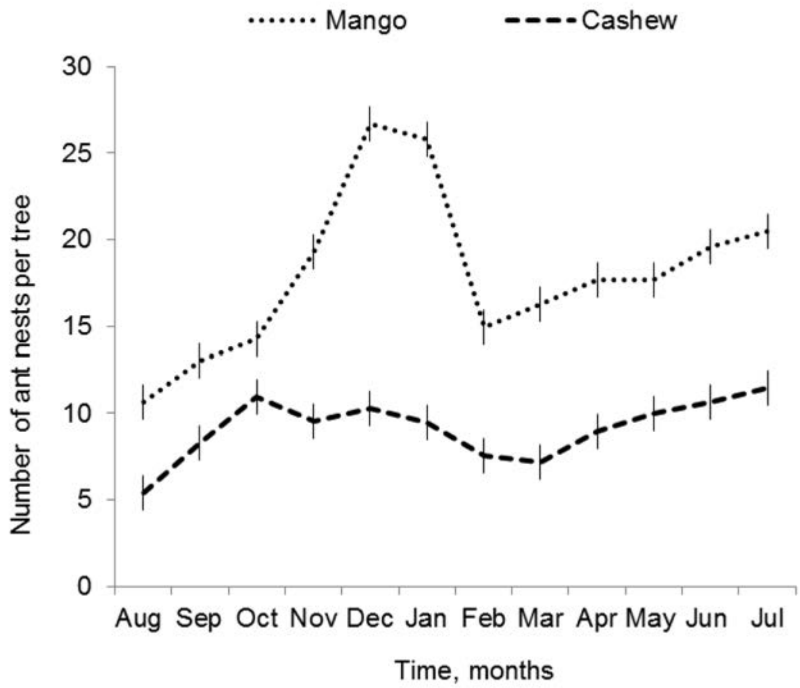


Fig. 2. Average number of weaver ants' nests per tree (± SE) on mango and cashew trees by time from August 2012 to July 2013; N = 144 trees per sampling date in each crop.

RESULTS

Mango

All average branch index values were higher than 40% throughout the season and the average number of nests per tree was not below 10 (Fig. 1 and Fig. 2). However, there were consistent differences between the different branch index values; Peng 1 (mean = 96.65 ± 0.21%) scored higher than Peng 2 (mean = 72.01 ± 0.51%), which scored higher than the Offenberg index (mean = 56.18 ± 0.57%) (Wilcoxon test; χ^2= 366.64; df = 2; P < 0.0001). In particular, the Peng 1 index showed high scores as all were above 89%. Such high scores were associated with a low seasonal fluctuation compared to the other methods. The seasonal fluctuation in the Peng 1 index was only 11%, whereas it was 40% and 49%, respectively, in the Peng 2 and Offenberg indices. Thus, the Peng 1 index showed limited fluctuations as it frequently saturated. Fluctuations in the three branch indices were limited, though, with a highest score in March which is the beginning of the wet season where mango starts to fruit in northern Benin. In contrast, the number of nests in mango trees peaked in December – January which is the dry season with flowering. Temporal variation in branch indices and nest numbers were therefore poorly aligned, and the average number of nests on trees showed high fluctuations in mango with a 2 – 3 fold increase between lowest and highest numbers (Fig. 2). This suggests that nest numbers did not necessarily reflect ant numbers.

Cashew

In most respects the dynamics in cashew were similar to those found in mango, though branch indices as well as nest numbers were generally lower in cashew (Fig. 1 and Fig. 2). Peng 1 (mean = 91.78 ± 0.59%) was higher than Peng 2 (65.97 ± 0.65%), which was higher than Offenberg (50.33 ± 0.63%) (Wilcoxon test, χ^2= 346.45; df =

2; $P < 0.0001$) and again the seasonal fluctuation was low when using the Peng 1 index. Seasonal fluctuation with Peng 1 was 17%, compared to 28% and 40%, respectively, with the Peng 2 and Offenberg indices. Nest numbers showed high seasonal fluctuations, varying more than 100% from 5.40 up to 11.50 nests per tree. The branch indices were highest from March to around June with an additional peak in October (Fig. 1b). Similarly nest numbers peaked in the same periods (Fig. 2). In October at the end of the wet season cashew trees start producing new flushing shoots, whereas from March to July cashew trees are at the end of their fruiting stage in the beginning of the wet season.

All the four ant abundance measures were significantly (all $P < 0.0001$) and positively correlated in both crops (Fig. 3 and Fig. 4). The strongest correlations were found between the Peng 2 and the Offenberg indices with r values of 0.98 and 0.96 in mango and cashew, respectively. In those cases the Offenberg index equaled 0.78 and 0.77 times the Peng 2 index, respectively, in mango and cashew. The correlations between Peng 1 and the two others branch indices were much weaker, with r values between 0.37 and 0.85 and therefore not allowing a reliable conversion between these estimates (Fig. 3 and 4). The correlations between the three branch indices and the number of nests per tree were even weaker with r values between 0.33 and 0.47 in mango and values of 0.32 and 0.36 in cashew. The weaker correlations found in cashew compared to mango, in these cases, probably derived from the fact that the range of the number of nests per tree in cashew were lower than in mango. The number of main trunks on the mango trees ranged from 3 to 8 with a median of 5 trunks and in cashew from 2 to 6 with the median of 4 trunks. Ant abundance index values based on the branch methods were all negatively correlated with the number of main trunks on the trees (Fig. 5). However, only correlations in mango and with the Peng 2 and Offenberg indices were significant (all $P < 0.001$), though with low r values of −0.27 and −0.28, respectively. These correlations suggest that the assessment of the number of main trunks in a tree (a subjective measure) may influence the resulting branch index values.

DISCUSSION

It is clear from this study that the values obtained from the three different branch methods cannot be compared directly. The Peng 1 index was significantly higher than the Peng 2 index which again was higher than the Offenberg index. This pattern was consistent through seasons and between crops. It is also clear that the Peng 1 index under the prevalent conditions with high ant abundance showed limited fluctuations because the index values were often saturated. Note, that its seasonal fluctuation was only 11% in mango but a little higher in cashew (17%) probably because ant abundance was lower and therefore less constrained by the upper limit of the index. Peng 1 is the fastest way to assess ant trails as only the presence of ants on each trunk needs to be determined. In terms of time investment this measure is therefore preferable. Also, this measure can be used to assess if ant abundance is high enough to attain adequate protection. If the Peng 1 index on average exceeds 50%, effective pest control may be expected (Peng *et al*. 2008). However, if population dynamics need to be tracked, Peng 2 and Offenberg indices should be preferred due to their higher sensitivity to variation. This would be at the cost of spending a few minutes more per tree during the scoring of ant trails.

The number of weaver ant nests per tree has often been used as a measure of ant abundance in plantation crops (Rapp & Salurn 1995, Peng *et al*. 1997a, Peng *et al*. 1997b, Ayenor *et al*. 2007, Dwomoh *et al*. 2009, Olotu *et al*. 2013a, Olotu *et al*. 2013b). The results from the present study, though, suggest that this may be problematic. The range of fluctuations in the number of nests was very high and unlikely to reflect actual ant abundance dynamics. The fluctuation in nest numbers was 2 – 3 fold on mango and 2 fold on cashew within a year (Fig. 2), in contrast to the branch indices that showed much less fluctuations (from 11 to 49%). Nest numbers increased from October to December on cashew and from November to January on mango (Fig. 2). These periods are the time when cashew and mango trees, respectively, produce leaf and flower flush in Benin. During this developmental stage of the host trees, ants produce numerous new small nests not necessarily because of increased ants numbers, but

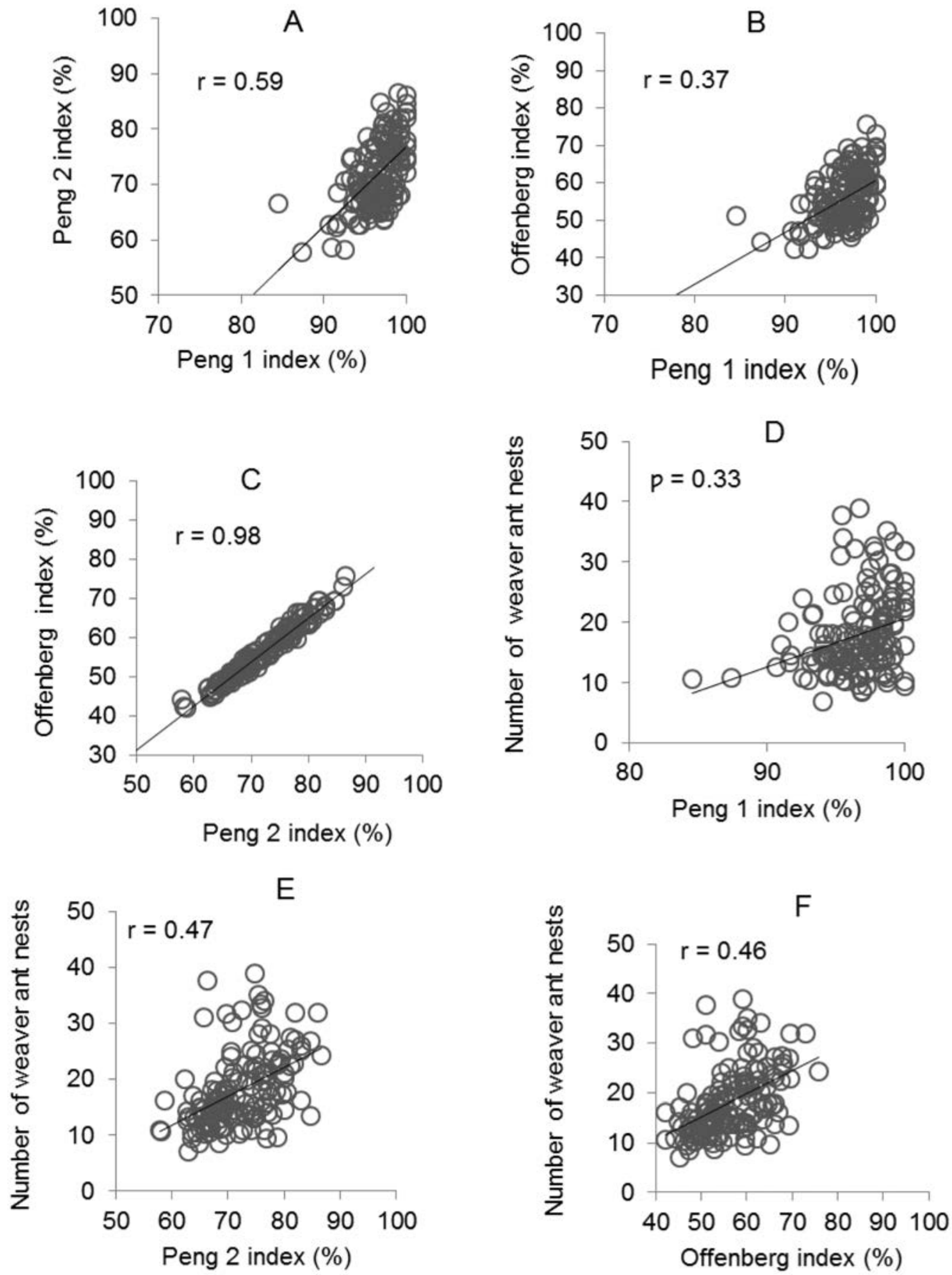


Fig. 3. Correlation analyses between branch index values and the number of ant nests on mango trees (r is the Pearson correlation coefficient and p is the Spearman rank correlation coefficient; N =144 trees; $P < 0.0001$ in all correlations).

because they prefer to build new nests on young shoots with flexible leaves (Offenberg *et al*. 2006) and since flushing shoots are often infested with honeydew producing homopterans which the ants shelter by building nests around their colonies (Lokkers 1990; Joachim Offenberg, unpublished data). In both mango and cashew, nest numbers also increased from May to July, which is the end of the fruiting season in both crops (early varities: GVN and Ifac 3). During this time fruit petioles are often infested with attended homopterans and these are also sheltered by new small ant nests. Similar observations were made by Lokkers, (1990) who showed that the number of weaver ant nests (*O. smaragdina*) peaked during seasons of maximum physiological activity of the ant´s host plants, i.e. during leaf and flower flush. The result is a high number of small nests during the flush and fruiting of host trees. Thus, an increasing number of nests do not necessarily reflect an increase in ant numbers, but more likely reflects the phenology of the host tree. Another problem with nest numbers is the fact that their size and thus the number of nests needed to sustain an ant colony depend on the leaf morphology of their host tree. E.g., *O. longinoda* build significantly smaller nests in cashew than in mango trees (Issa Ouagoussounon, unpublished data). Based on these considerations we believe that nest number is a poor predictor of ant numbers and furthermore more costly in terms of time investment compared to the branch methods as it takes considerably more time to count nests than counting ant trails. On the other hand, the volume of nests, rather than their numbers, has been shown to be a good predictor of population sizes in *O. smaragdina* (Lim 2007; Christian Pinkalski, unpublished data) and also well correlated with branch indices (Christian Pinkalski, unpublished data).

As branch indices produced different scores, conversion factors between them would be desirable. On average, over the entire season, the Peng 2 and Offenberg indices equaled 75 and 58%, respectively, of the Peng 1 index in mango, whereas, in cashew they equaled 72 and 55%. There was, thus, some consistency in the relationship between index scores between the two crops. On the other hand, because of the limited fluctuations in the Peng 1 index, correlations between this index and the two other branch indices were weak (Fig. 3 and Fig. 4) and therefore conversion between them should be done with caution. In contrast, strong correlations were found between the Peng 2 and Offenberg indices ($0.96 < r < 0.98$; Fig. 3 and Fig. 4), allowing a reliable conversion between the two. This conversion may be used to compare studies using the two different methods. The almost identical relationship between the two indices in the two crops (Offenberg index = 0.78 Peng 2 index and 0.77 Peng 2 index, in mango and cashew, respectively), suggests that their conversion is robust across crops. The strong correlation between the Peng 2 and Offenberg indices suggest that the extra time invested in the assessment of the Offenberg index is redundant unless ant abundance is so high that the Peng 2 index approaches its maximum and therefore loses its dynamics as seen with the Peng 1 index in the present study. In that case, the Offenberg index may still retain scores below 100% as it produced the lowest scores of the three indices.

All the three types of branch indices are based on an assessment of the number of main trunks on a tree. A main trunk, however, is not defined in any of the methods given, and is therefore a subjective measure. If ant trails converge toward the base of a tree, along with the convergence of branches, then lower level branches will be more and more likely to hold an ant trail. It may thus be expected that the inclusion of only few main branches may lead to higher branch scores compared to an inclusion of more main branches. Under these assumptions, in the extreme case, only the single base trunk is included and will then always hold an ant trail if ants are present on the tree. If the number of main trunks affect branch method scores, this needs to be taken into consideration when evaluating and comparing ant densities. As expected we found negative correlations between the tree´s number of main trunks and the branch indices. However, the effect was only significant in mango and only when using the Peng 2 and the Offenberg indices (all $P < 0.001$) and in these cases with low r values of only −0.27 and −0.28, respectively. As this effect is not strong we consider branch indexing a valid method, however, we suggest that branches are categorized in a way so that trees do not end up with more than 10 main branches per tree in total. Including more than 10 main branches may bias abundance scores.

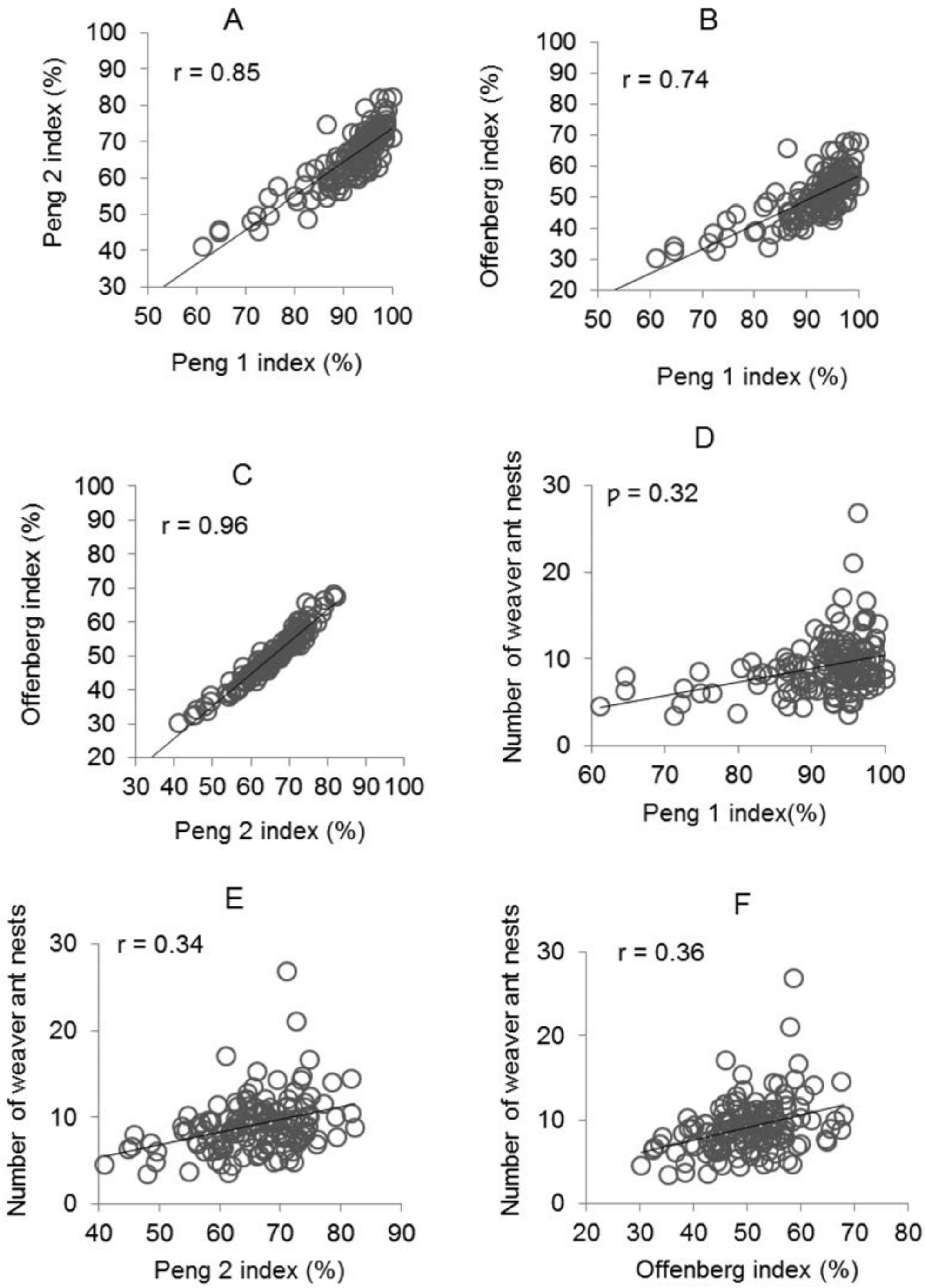
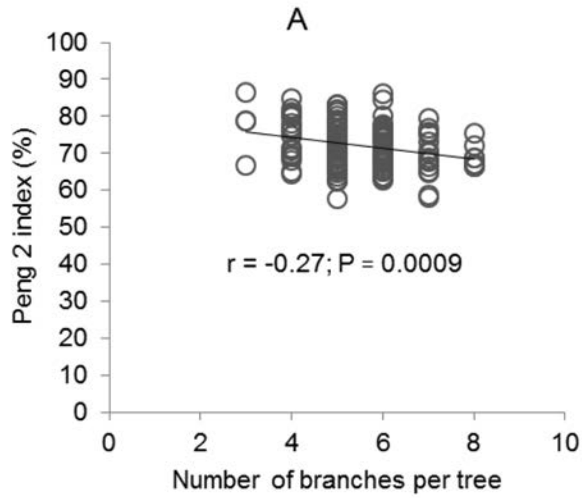


Fig. 4. Correlation analyses between branch index values and the number of ant nests on cashew trees (r is the Pearson correlation coefficient and p is the Spearman rank correlation coefficient; N = 144 trees, $P < 0.0001$ in all the cases)



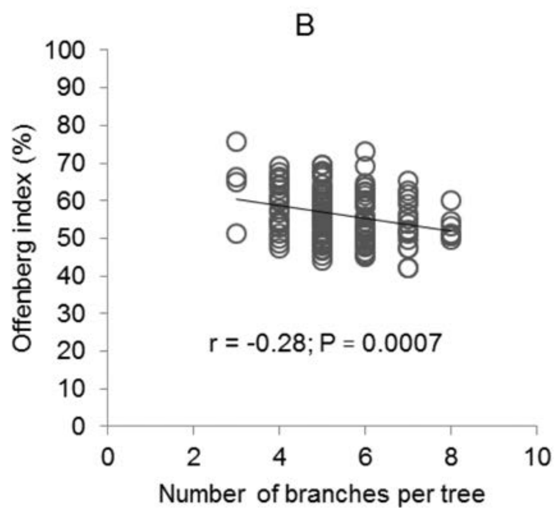


Fig. 5. Correlations between the Peng 2 and the Offenberg indices values and the number of main branches on mango; N = 144 trees in both cases (r is the Pearson correlation coefficient).

In conclusion, the use of the Peng 1 index is recommended in cases where the objective is to assess if ant abundance is adequate to achieve effective biological control or in cases where weaver ant abundances are low. In contrast, the Peng 2 and the Offenberg indices are to be preferred over the Peng 1 index in cases where it is of importance to track seasonal variations in population numbers. We further recommend not using nest numbers as a measure of weaver ant populations unless nest volume is also taken into account.

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