Relationships between tree diversity and risk of attack by *Dendroctonus* beetles, on pines in Mexican mixed forest

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Abstract

Mexico has lost much of its natural forests during the last centuries, and the country has one of the highest deforestation rates in Latin America. Together with the forests, ecosystem services such as prevention of natural disasters and protection against soil erosion are also lost, as well as many of the organisms living in the forests. In order to mitigate these problems, extensive reforestation projects are carried out around the country. However, the biodiversity is often lower in planted forests than in natural forests. This can lead to an increased risk of large scale bark beetle outbreaks. In this study, I investigated the relationship between tree composition and the risk of attacks by *Dendroctonus spp*. The result shows that pines that grow in areas with a higher proportion of pines, the only kind of tree known to be attacked by these insects in the area investigated, are more often attacked by *Dendroctonus*. This result indicates that, in order to lower the risk of outbreaks, a mix of different tree species is preferable in future reforestation. There is, however, no relationship between the amount of pines in an area, and the risk of attacks in that same area. In addition, I found a negative relation between canopy closure and the probability of the pines to be attacked, indicating that areas with lower canopy closure are more likely to be attacked. Also, a high percentage of the canopy of the tree exposed to direct sunlight increases the probability of attack. In conclusion, to avoid large scale bark beetle outbreaks, heterogeneous landscapes are better than homogeneous, and areas with thick vegetation are better than more open areas.

Sammanfattning

täckningsgrad och sannolikheten för att tallarna var attackerade, vilket indikerar att sannolikheten för en attack är högre i områden där trädkronorna har en lägre täckningsgrad. Då en hög procent av trädkronan var exponerad för solljus ökade också sannolikheten för attack. Sammanfattningsvis är heterogena landskap bättre än homogena, och tät vegetation bättre än mer öppna områden för att undvika storskaliga barkborreangrepp.

**Introduction**

In natural ecosystems, the vegetative cover of a forest prevents soil erosion, replenishes ground water and controls flooding by enhancing infiltration and reducing water runoff (Altieri, 1999). During the last 50 years, Mexico has lost more than 500 000 hectares of forest per year, and has one of the highest rates of deforestation in all of Latin America (Gomez-Pompa & Kaus, 1999). With the loss of the forests, ecosystem services such as protection against natural disasters (Bradshaw et al, 2007) and protection against soil erosion are also lost, as well as many of the organisms living in these environments.

In order to mitigate these problems in the future, as well as to enhance biodiversity and restore natural habitats, plantations of trees are carried out around the country. However, the survival rate of the planted trees is often poor, and in many cases, exotic species are planted (Greenpeace, 2008). Moreover, plantations can sometimes lead to an increase in pest outbreaks (Raffa et al, 2008).

Reforested areas often contain little heterogeneity compared to natural forests, which may result in an increased risk of outbreaks of eruptive herbivores, such as bark beetles (*Dendroctonus spp*) (Samman & Logan, 2000). Under some circumstances, these insects can have a large impact on forests. For example, a big outbreak in British Columbia, Canada, caused the death of pines over more than 7 million ha (Aukema et al, 2006).

The beetle, *Dendroctonus spp* is present in all montane systems of Mexico (Salinas-Moreno et al, 2004), and there is a high ecological diversity in *Dendroctonus spp-Pinus spp* associations. Mexico has one of the highest pine diversities in the world, with 42 species. Most of these are attacked by at least one of the 12 species of *Dendroctonus* that are found in the country. (Salinas-Moreno et al 2010). The regions that historically have been most affected by outbreaks are the Transverse Volcanic Belt, Sierra Madre Occidental and Sierra Madre del Sur (Salinas-Moreno et al, 2010). To prevent large scale outbreaks in the future, it is important that the plantations that are carried out around the country are done in a way that does not favor these eruptive herbivores.

Below, I focus on whether the likelihood of an outbreak could be mitigated by a change in the way the plantations are carried out. I investigated what factors are affecting the risk of attacks on
pines in the forests on the slopes of Popocatetel, an area considered to have a high "Bark beetle threat index" (Salinas-Moreno et al, 2010). The results of this study could be of particular interest, since extensive reforestation is planned in this area (Mi rio Yautepec, appendix).

The life cycle of bark beetles is relatively well known. The adult insects find a tree where they bore their way through the bark, to reach the phloem in the inner bark. After having copulated, they place their eggs along the tunnels they dig out in the phloem. Larvae then feed and develop inside the cortex, and finally reach the pupal stage, after which they become adults, ready to look for a new tree. During their lifecycle, the beetles introduce a number of different fungi species, that also live from the phloem (Raffa et al, 2008).

Bark beetles need recently dead tissue to reproduce successfully (Rudinsky, 1962). This can be found either by colonizing dead or wounded trees, which most species do (Rudinsky, 1962), or by killing a tree. However, this second strategy is not without risks. Living trees have defenses (Nebeker et al 1995 a,b), that the attacking beetles need to overcome. In order to exhaust the trees defensive system, beetles use pheromones to attract more beetles to the attacked tree, and thereby launching a mass attack (Wood 1982).

The interaction between the beetles and the trees is definitive, either the beetles kill the tree and can reproduce successfully, or the trees defensive system forces the beetles to abandon the tree, and almost no successful reproduction can take place (Malmström & Raffa, 2000). The outcome of the interaction is determined by the amount of beetles that are attracted to the tree, and how much defensive chemical substances the tree is able to produce (Malmström & Raffa, 2000). This means that there is a threshold that the beetles need to overcome in order to reproduce.

The trees photosynthates are allocated to different uses, and disease resistance mechanisms generally have a rather low priority in this hierarchy, since the tree first need to focus on essential functions, such as production of roots, growth and reproduction (Fettig et al, 2007). The environmental factors are thus important in determining the trees ability to create enough photosynthates to defend itself. Therefore, it is not surprising that many outbreaks are associated with droughts (Christiansen et al, 1987).

The probability that the beetles are able to overcome this threshold is determined by their ability to attract enough beetles to the attacked tree. This is largely determined by the beetle population density, which, in turn, is dependent on host abundance in the area (Raffa et al, 2008). Since bark beetles are relatively poor dispersers, good connectivity between suitable habitats is essential (Raffa et al, 2008). Forest management practices that increases the homogeneity in the landscape can therefore be aiding the dispersal of the beetles, increasing the risk of outbreaks (Raffa et al, 2008).
A high diversity in the vegetation generally lowers the risk of pest outbreaks, while a lower diversity increases the risk of such outbreaks. A few different explanations for this relationship have been put forward. In the studies of pests on agricultural plants, it has been found that the amount of pest insects is reduced considerably when the background of the crop is allowed to become weedy (Smith, 1969) or when the crop is inter cropped with another plant species (Tukahirwa & Coaker, 1982). A few explanations have been put forward, out of which three will be presented here. The first explanation is that a more complex background makes the host plants less apparent amongst the foliage of the non host plants, and thus makes the host finding process more difficult for the insects (Finch & Collier, 2000). The second explanation is that a more complex plant composition masks the odour released from the host plant (Tahvanainen & Root, 1972). A third explanation is that this might be due to enhanced biologic control, achieved since more diverse environments makes the area more suitable for a wider array of organisms, some of which might prey on the pest insect (Thies & Tscharntke, 1999; Way & Heong, 1994). There is a general belief that diverse backgrounds have more effect on specialist insects, than on generalists (Finch & Collier, 2000).

At least some of these explanations might be applicable to the relationship between *Dendroctonus spp* and *Pinus spp*. Being a host specific pest, these insects might be prone to experience hampered host search in diverse environments, as well as enhanced biological control when the complexity is increased. Also, as they are relatively poor dispersers, good connectivity between suitable host trees, offered in homogenous environments, is of big importance for *Dendroctonus spp* (Raffa et al, 2008). Previous reports have stated that efforts to prevent undesirable levels of bark beetle caused tree mortality at the landscape level must take spatial distribution of both cover types and stand ages into account (Fettig et al, 2007). Reduced habitat heterogeneity increases the risk that "thresholds", needed to be surpassed for an attack to be successful, are exceeded. Increased heterogeneity decreases this risk (Raffa et al, 2008).

Here I investigate whether there is a relationship between tree species diversity in an area in which pines grow, and the risk of attack on these pines. I consider the proportion of pines in an area as well as the total amount of pines in the investigated areas. Other factors, such as ground vegetation, canopy closure and heterogeneity in diameter, will also be taken into account. If a relationship between any of these factors and the probability of attacks can be found, this can hopefully be used to plan future plantations, in order to decrease the risk of pest outbreaks, particularly in areas with a high "Bark beetle threat index", such as the one investigated. If the projections of increased frequency and severity of drought in Mexico in the future (Wehner et al, 2011) are correct, it might be particularly important to take attack risk into account when planting trees, since outbreaks are often associated with droughts (Christiansen et al, 1987).
I tested the hypothesis that a higher diversity of species, i.e. a lower proportion of pines, is linked to a lower risk of attack by beetles, by comparing attacks in areas with low (0-25%), medium low (26-50%), medium high (51-75%) and high (76-100%) proportions of pines. Since areas with a high proportion of pines should make it easier for *Dendroctonus spp* to find the trees, the frequency of attacks is expected to go up when there is a higher proportion of pines. I also analyzed whether the total amount of pines in an area is related to the attack frequency, regardless of what other trees are present. This might be the case, because a high amount of pines in an area means a lot of suitable habitats for these insects.

I measured the diameter of the pines, and then calculated the standard deviation for each area. A higher homogeneity, indicated by a lower standard deviation, in thickness could be related to a bigger probability of attacks, since a more homogenous landscape is preferable for the *Dendroctonus spp*. However, the thickness of the trees is also important. Thicker trees are expected to be attacked more frequently than thinner. This is expected because thicker trees are probably older, and so their defense might be weaker. Another factor leading to this hypothesis is that thicker trees probably holds more sap, which would make them more desirable for the insects. Therefore, areas where the pines are generally thicker might be prone to more attacks.

For each tree, I measured the proportion of the canopy that is not shaded by other trees, and so gets direct sunlight. A higher proportion of the canopy reached directly by sunlight is expected to lower the attack frequency, since trees that are able to photosynthesize more probably have better defenses, and therefore might be avoided by beetles. On the other hand, the trunks on trees in more open areas are probably exposed to more sunlight, which would mean better conditions for insect activity.

I measured the coverage of ground vegetation in two categories, low and high, in percent. It is expected that a high percent of ground cover will be related to a higher attack frequency. That is expected because the ground vegetation competes with the pines about resources, and therefore a higher amount of ground vegetation can lead to more stress for the pines in the area.

A final factor I measured was the canopy closure, also measured in percent. A higher degree of canopy closure is expected to be related to a lower attack frequency, since the shade keeps the beetles environment cooler, and thereby slower their metabolism.
Material and methodology

In the field

My investigation was carried out on the slopes of the volcano Popocatepetl, near the village Atlautla in the State of Mexico. In these forests, 60 sample areas were investigated. These areas were divided equally between 4 categories, 15 samples in each category. The categories are low (0-25 %), medium low (26-50 %), medium high (51-75 %) and high (76-100 %) proportion of pines.

According to local people working with tree plantation in this area, *Pinus ayacahuite* is not known to be attacked by *Dendroctonus spp* in this area, and therefore this species is not included when calculating proportion of pines.

The location of the samples was chosen by following a road or path for 5 minutes, and then going 100 steps away from the road in a random direction. The following criteria had to be fulfilled for an area to classify as a sample area. First of all, pines needed to be present, because these are the targets of this study. Since this investigation is aiming to aid future reforestation, the areas investigated also needed to be forested. For this reason, the canopy closure needed to be at least 30 % for an area to classify as a sample area. Lastly, the area also needed to be practically possible to investigate, excluding very slopy areas, as well as some private properties. Also, the sample areas needed to be separated from the other sample areas by at least 100 meters.

Each sample area was a square, 30*30 meter. Within this area, all the trees were recorded, in order to tell the tree composition in the area. However, only the pines within the central 15*15 meters of the sample area were investigated to measure the attacks in the area. The reason why only pines in the core of the sample area were investigated for attacks is that the attack frequency of pines in the outer parts of the sample area might be affected by the tree composition in the surroundings.

15 meter long ropes were used to mark out the core area, within which the pines were investigated for attacks by *Dendroctonus spp*. All trees within 7,5 meters from these ropes were counted, as well as all the trees within this core area, in order to calculate the tree composition. In each sample area, canopy closure, vegetation < 0,5 meter high and vegetation 0,5 – 2 meter high was estimated in percent. A GPS was used to determine the coordinates, as well as the altitude. All the samples were situated in the range between 2319 and 2630 meters above sea level.

The diameter of all the pines (except *P. Ayacahuite*) within the core area was measured 1,3 meters above ground, using a measuring tape. For each of these pines, the proportion of the canopy being directly exposed to sunlight was estimated in percent. Finally, each of these pines within the core area was investigated, in order to see if they are, or have been, attacked by *Dendroctonus spp*. An
Attack is indicated by the presence of round holes in the bark, with a diameter of a few millimeter, as well as the tunnels they dig within the cortex of the tree.

![Diagram of sample areas](image)

Figure 1 is a map over a sample. In the sample area (the big square), the tree composition was investigated. In the core area (the small square), the pines were investigated for attacks, diameter and exposition for direct sunlight.

**Analysis**

Open office.org Calc was used to sort the data, and SPSS was used to analyze them.

The response variable I analyzed was attacked and non-attacked sample area. I did this because the number of pines in each sample area was too low (2-16 pines per sample area) to analyze the degree of attack in each sample. I therefore decided to put each sample area in one of the two categories attacked or non-attacked.

A binary logistic regression was used to analyze the relationship between pine proportion and risk of attack, as well as the relationship between the total amount of pines and the risk of attack (Table 1). This same test was also used to analyze the relationship between diameter of the individual pines within the core area, and risk of attack, as well as the relationship between the proportion of canopy direct exposed to sunlight for the individual pines within the core area and the risk of attack (Table 1). Figures were created in SPSS, to illustrate these analyzes.
Table 1. Variables that I investigated with binary logistic regression.

<table>
<thead>
<tr>
<th>Primary variables</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Pine proportion</td>
<td>The proportion of all the trees in the sample that are pines. Measured in percent.</td>
</tr>
<tr>
<td>Total amount of pines</td>
<td>The amount of pines in the sample, regardless of what other trees are found in the sample.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Secondary variables</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Diameter of the individual pines</td>
<td>The diameter of the individual pine 1,3 meter above ground. Measured in cm.</td>
</tr>
<tr>
<td>Proportion of the canopy direct exposed to sunlight</td>
<td>The proportion of the individual pines canopy direct exposed to sunlight. Estimated in percentage.</td>
</tr>
</tbody>
</table>

SPSS was also used to carry out a binary logistic regression test with multiple variables, in order to investigate whether any of the secondary variables, canopy closure, vegetation < 0,5 meter, vegetation 0,5 – 2 meter and the homogeneity (standard division) of diameter amongst the pines within the core area were related to the attack frequency (Table 2).

Table 2. Variables that I investigated with binary logistic regression with multiple variable.

<table>
<thead>
<tr>
<th>Secondary variables</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Canopy closure</td>
<td>The proportion of the sample area covered by canopy. Estimated in percent.</td>
</tr>
<tr>
<td>Vegetation &lt; 0,5 meter</td>
<td>The proportion of the forest floor in the sample covered by vegetation &lt; 0,5 meter high. Estimated in percent.</td>
</tr>
<tr>
<td>Vegetation 0,5 – 2 meter</td>
<td>The proportion of the forest floor in the sample covered by vegetation 0,5 - 2 meter high. Estimated in percent.</td>
</tr>
<tr>
<td>Homogeneity of diameter amongst the pines within the core area</td>
<td>The standard deviation of the diameter of all the pines within the core area.</td>
</tr>
</tbody>
</table>

Results

A Binary logistic regression (Figure 2) showed a relationship between pine proportion and attack frequency (p=0,016, n=60). Attacked pines were found in 9 of the 60 sample areas. All of the samples where attacked pines were found had a pine proportion of 50 % or more (Figure 3).
No statistically significant relationship between the total amount of pines and attack frequency was found.

A binary logistic regression with multivariables showed that two of the tested variables can be used to predict probability for an area to be attacked. Again, pine proportion had a relationship with risk of attack ($p=0.022$, $n=60$). The other variable that can be used to predict probability to find attacked trees in an area was canopy closure ($p=0.021$, $n=60$). There was no significant correlation between pine proportion and canopy closure. None of the other variables tested, vegetation $< 0.5$ meter, vegetation $0.5 – 2$ meter and homogeneity in diameter in the pines, could be used to predict the probability for an area to be attacked.

A significant correlation was found between the amount of an individual trees canopy exposed to direct sunlight and the probability for that tree to be attacked (Figure 4). Trees with a higher percentage of the canopy direct exposed to sunlight are more likely to be attacked ($p=0.002$, $n=399$).

A binary logistic regression showed no correlation between the diameter of the individual trees, and the probability for them to be attacked.

Figure 2 is illustrating the relationship between pine proportion and attack frequency. On the x-axis is the proportion in percent. On the y-axis is the categories YES (attacked pines were found in the sample) and NO (no attacked pines were found in the sample).
Figure 3 is illustrating the pine proportion for the samples where attacked pines were found. On the x-axis is the proportion of pines for each of the attacked area. On the y-axis is the amount of sample areas were attacked pines were found.

Figure 4 illustrates the relationship between the proportion of the trees canopy exposed to direct sunlight and the risk of attack. On the x-axis is the proportion of the canopy exposed to direct sunlight. On the y-axis are the categories YES (the tree is attacked) and NO (the tree is not attacked). N=399.
Discussion

The risk of attack by *Dendroctonus spp* increased with increasing pine proportion in the sample areas. The result indicates that in order to prevent outbreaks in the future, a mix of different tree species is preferable when reforesting this area. Below, I will present three possible explanations for this relation.

One reason for this relationship could be the fact that more complex environments usually make it harder for the insects to encounter the trees (Finch & Collier, 2000). A more complex environment not only hides the trees visibly, but the odours from other trees can also make the odour of the pines harder to detect (Tahvanainen & Root, 1972). Since these insects are not very good dispersers (Raffa et al, 2008), this might be an important factor.

Another possible explanation could be the enhanced biological control that is usually found in more complex environments (Thies & Tscharntke, 1999; Way & Heong, 1994), because such environments allows more possible predators to thrive. Species predating on *Dendroctonus spp* include woodpeckers (*Picidae spp*), ant beetles (*Thanasimus spp*) and flower bugs (*Anthocoridae spp*) (Moore 1972).

A third possible explanation could be the higher total amount of pines in areas with a high pine proportion. More pines also means more suitable habitats for the bark beetles. There was, however, no statistically significant relationship between the amount of pines and the risk of attack.

Another variable that proved to be a significant indicator of attack frequency was the canopy closure. Areas that have a higher percentage of canopy closure are less likely to be attacked. In other words, *Dendroctonus spp* prefer more open areas. Individual trees with a higher percentage of the canopy directly exposed to sunlight are more likely to be attacked, which further stresses this point. Below, I propose three alternative, not mutually exclusive explanations. Note that both the canopy closure and the proportion of the canopy of the individual trees directly exposed to sunlight are estimates, and these results should therefore be used as such, rather than exact results.

Firstly, the beetles may prefer more open areas because a higher amount of sunlight penetrating the canopy means more direct sunlight reaching the trunks, which in turn leads to a warmer environment for the beetles. A warmer environment increases their metabolism, and makes larvae growth faster. It should be noted here that there was no measurement of the proportion of the trunk exposed to direct sunlight in this study, and that future investigations could take this into account, in order to further investigate this factor.

Secondly it might be easier for the beetles to find their hosts in more open areas. Other studies
have shown that more complex environments make it harder to find their hosts (Finch & Collier, 2000), and this conclusion could be used to explain this result as well.

Thirdly, it could be due to the increased sugar production in these trees. When a bigger part of the canopy is exposed to direct sunlight, the trees possibility to photosynthesize increases, and this means that there is more sugars available for the beetles. This could also mean that the trees are more difficult to conquer, because trees that are able to photosynthesize more would be expected to be able to allocate more energy to defense. However, they can still be more likely to attract beetles, because the reward for a successful conquest is bigger.

I did not distinguish between successful and unsuccessful attacks, but only considered whether a tree is attacked or not. A study on the relationship between the proportion of the canopy exposed to direct sunlight and the proportion of attacks that are successful would therefore be of interest.

I did not find any significant relationship between the homogeneity of diameter of the pines in an area and the risk of attack. Nor was there any significance in the relationship between diameter of the individual pines and attack frequency. However, no attacked tree had a diameter less than 21 centimeters, which possibly can indicate that only pines above a certain thickness are attacked. There is also the possibility that the relatively low number (16 of 399) of attacked trees found is too small to find such a relationship.

The ground vegetation was another factor not significantly related to the attack frequency. Competition with other plants is probably an important factor, but other factors, such as soil quality, might be even more important. Areas with good soil could be favourable for the pines even if such areas have a thicker ground vegetation.

My study indicates that the risk of attack by *Dendroctonus spp* increases with increasing pine proportion. It also indicates that open areas are more likely to be attacked. According to these results, a mix of different tree species is preferable when reforesting this area, in order to prevent outbreaks in the future. It is also desirable to create forests with dense vegetation.

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