# Ontogenetic Resistance in *Pinus ponderosa* to *Rhyacionia neomexicana* (Lepidoptera: Tortricidae): Role of Anatomical Features

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**Abstract-** The anatomical features of 10-year old saplings derived from rooted cuttings (stecklings) from sexually mature (19-33 years old) ponderosa pine (*Pinus ponderosa*) trees (ontogenetically mature), and 10-year old saplings from seedlings (ontogenetically immature) were compared to test if ontogenetic maturity was associated with resistance to southwestern pine tip moths (*Rhyacionia neomexicana* (Dyar)). Southwestern pine tip moth preferred to attack ontogenetically immature trees and differences in one-year old needle length and toughness may contribute to with this pattern

## I. Introduction

The resistance of host trees to insect herbivores is often ontogenetic or age dependent. Many insect herbivores seem to be associated with host plants of particular ages or sizes [1, 2]. Some insect guilds attack host plants only at certain ontogenetic stages or ages. For example, *Rhyacionia* (Lepidoptera: Tortricidae) tip/shoot moths are well known to attack juvenile (usually less than 20 years old) [3, 4] but not mature pines; whereas the *Dendroctonus* (Coleoptera: Scolytidae) bark beetles primarily attack mature [5, 6, 7] but not young pines. More interestingly, host trees of different ages or sizes also may create a selection pressure for those insect herbivores that have similar feeding behaviors on host trees such as pine sawflies (*Neodiprion* spp.). For example, three reported sawfly species in Arizona: *N. gillettei*, *N. autumnalis*, and *N. fulviceps* prefer needles on seedlings, sapling to mature trees, and mature trees of ponderosa pines, respectively [8].

The success of insect herbivores on host plants depends on numerous factors such as the availability of suitable nutrients, effectiveness of physical and biochemical defenses of host plants, inter- and intra-species association (e.g. competition and symbiosis), and predation pressure. Among them, the availability of suitable nutrients (e.g. nitrogen), effectiveness of physical and biochemical defenses (e.g. toughness, phenolics, fiber, and terpenes) of host plants often determine the foliage resource quality and are directly associated with plant ontogenetic stages or ages. Clancy [9] summarized 10 common mechanisms of conifer tree resistance to insect defoliators; at least three of them (e.g. toughness of needles, nutrient quality, and defensive compounds) are closely related to host ontogenetic changes or ages and also should be applicable for other insect guilds such as pine tip/shoot moths. Although there is an intrinsic relationship between tree physiology and forest insect pests [10], few studies actually demonstrated how these ontogenetic or age related changes influence the success of insect herbivores.

Southwestern pine tip moth (*Rhyacionia neomexicana* (Dyar)) is a native insect pest that occurs in ponderosa pine (*Pinus ponderosa* Dougl. ex Laws) dominated conifer forest ecosystems in the western United States. It typically attacks juvenile ponderosa pines but occasionally also other pine species including Scotch pine (*P. sylverstris* L.), jack pine (*P. banksiana* Lamb), and Austrian pine (*P. nigra* Arnold) [4,11]. The southwestern pine tip moth has one generation per year, and its larvae go through 5 instars. The early instars mine inside needles, and the later instars bore into shoots and feed on the inside tender tissues. Because pine tip moth primarily attacks the new growth of shoots and buds, it directly disrupts the normal growth pattern of pines and causes the deformation of trees including crooks, forks, multiple stems, and spike tops (dead leaders) [4].

Spiegel and Price [12] investigated the distribution of southwestern pine tip moths among ponderosa pine trees of different age classes around Flagstaff, Arizona and found that tip moth attacks did not occur on trees that were greater than 20 years of age or taller than 4 m, which agreed with the conventional wisdom regarding tip moth attack behavior [12]. However, mechanisms of ontogenetic resistance in ponderosa pine to southwestern pine tip moth have not been tested. In this study, we will address two major research questions: 1) Is there an ontogenetic basis for tip moth preference of certain aged trees? and 2) What host plant morphological and anatomical traits (e.g. shoot and needle length, and needle toughness) may explain this pattern? We postulated that cuttings generated by stem cuttings (an asexual propagation method to produce clones) would have similar height growth as seedlings (so to remove the influence of size) but have advanced ontogenetic characteristics. We hypothesized that 1) tip moths would attack new growth of needles and shoots more on saplings from seedling origin (ontogenetically immature) than on saplings from rooted cuttings (ontogenetically mature); and 2) saplings from rooted cuttings would have shorter shoots and needles but greater toughness of needles than saplings from seedlings.

## II. Materials and Methods

*A. Tree materials*

The rooted cuttings were derived from local ponderosa pine trees and were planted in July 1992 on the Ft Valley Experimental Forest Station, which is located about 16 km north of Flagstaff, Arizona (elevation = 2,135 m), and surrounded by ponderosa pine dominated forests. The rooted cuttings were obtained through a series of asexual propagation experiments between 1986 and 1987. The 20 trees used to produce stecklings were 5.14 ± 0.32 m in height, and 25.31 ± 1.36 cm in DBH, and aged from 19 to 33 years old, with an average of 26 ± 1 years (mean ± se). No attempt to track the ortet source of the rooted cuttings was made.

Seedlings were also derived from local ponderosa pine sources and included both half and full sib progeny. The parental source for these seedlings was not tracked. Since all seeds and stem cuttings were collected from sexually matured ponderosa pine trees around Flagstaff, the possible genetic variation due to individual tree family is included as a part of tree-by-tree variation in this study. All trees were grown as 1 x 1 m spacing and have been established at the Ft Valley Experimental Forest Station for 10 years prior to data collection.

### B. Experimental design and data collection

The basic experimental design in our study was a paired design with 30 replications (n = 30 pairs or 60 trees), which allowed for selecting the best pair of saplings from seedling and steckling origin based on similar height and base diameter for removing the influence of tree size. The paired saplings of seedling and steckling origin were within 25 m of each other. Because these saplings from seedling and steckling origin were carefully paired, the possible influence on the pair of trees (i.e. experimental unit) due to tree sizes, soil fertility gradient, and microclimate was minimized.

Based on the color and position of damaged residual shoots in the whorls, we were able to distinguish the tip moth infestation from the most recent three years (e.g. 2000-2002). The percentage of infestation was determined with the number of terminal shoots attacked divided by the total number of shoots at a given age class within the top three whorls.

For each pair of saplings from steckling and seedling origin, a single one-year old shoot was sampled from the same crown position within the top two whorls in the middle of May 2003 before the occurrence of new (or current) growth. Three one-year old needles from the one-year old shoot from each sapling tree were randomly selected for length and toughness measurement. We decided to measure shoot length, needle length, and needle toughness based on their potential importance to population dynamics of tip moths. We reasoned that longer shoot represented higher growth vigor and better food sources for tip moth mining [12, 13]. Likewise, we reasoned that one-year old needle length and toughness would influence the oviposition and early instar larvae mining of tip moths. The length of needles and shoots was measured with a caliper close to 0.01 mm. The toughness of needles was determined with a high precision

penetrometer (TA-XTA Texture Analyzer, Texture Technologies Corp., Scarsdale, NY) following the procedures of Wagner and Zhang [14]. The measurement on needle toughness was performed in the laboratory within 48 hours of needle harvest.

Finally, we sampled one-year old shoots and one-year old needles from the 20 sexually mature trees from which the stecklings were derived (i.e. donor trees or ortets) to obtain shoot length, needle length, and needle toughness with the same methodology as above.

### C. Statistical analysis

The paired t-test was performed with PROC TTEST in SAS [15] to compare the means of two independent groups (saplings from seedling versus steckling origin) for each of the following variables: 1) tip moth infestation percentage in 2000, 2001, and 2002; 2) one-year old shoot length; 3) one-year old needle length; and 4) one-year old needle toughness. If the t statistical value is greater than the two-tail critical t value at the significant level $\alpha = 0.05$, the null hypothesis that the mean difference between saplings from steckling and seedling origin was zero would be rejected. Also, the measures on shoot length, needle length and needle toughness were subject to the one-way analysis of variance (ANOVA) to compare the differences among three groups of trees (ortets, saplings from steckling origin, and saplings from seedling origin). The means of main effect (tree group) was separated with the least significant difference (LSD) at the level of $\alpha = 0.05$.

### III. Results

#### A. Tip moth damage on saplings of seedling and steckling origin

The tip moth damaged approximately 40, 35, and 11% of shoots on saplings from seedling origin in 2000, 2001, and 2002, respectively but only roughly 10, 17, and 2.5% of shoots on saplings from steckling origin in 2000, 2001, and 2002, respectively. Paired t test showed that saplings from seedling origin had significantly greater percentage of tip moth infestation than the saplings from steckling origin over consecutive years from 2000 to 2002 (t-value ≥ 2.401, p ≤ 0.012, d. f. = 29) (Fig. 1).

Moreover, there existed significant year-to-year variation in the tip moth infestation on saplings from both seedling and steckling origin. The tip moth infestation of 2000 and 2001 did not differ but both were significantly greater than that of 2002 pooled over all saplings (p < 0.001).

#### B. Shoot and needle length

There was no significant difference in one-year old shoot length (in 2002) between saplings from steckling and seedling origin (t-value = 0.91, p = 0.370). One-way ANOVA showed that there was no significant difference in

the shoot length among ortet, saplings from steckling origin, and saplings from seedling origin (F = 1.08, p = 0.344). The average shoot length was 7.74 ± 0.46 cm (mean ± se) for ortets, 6.72 ± 0.43 cm for saplings from steckling origin, and 7.35 ± 0.54 cm for saplings from seedlings origin. It indicated that shoot length was similar among three groups of ponderosa pine trees, and shoot length alone was not likely responsible for the difference in tip moth infestation between saplings from seedlings and steckling origin. The one-year old needle length was greater in saplings from steckling origin (8.13 ± 0.29 cm) than in saplings from seedling origin (6.62 ± 0.41 cm) (t-value = 2.46, p = 0.021). However, both were significantly shorter than the one-year old needle length in ortets (12.37 ± 0.37 cm) with the ANOVA (F = 60.75, p < 0.001) (Fig. 2).
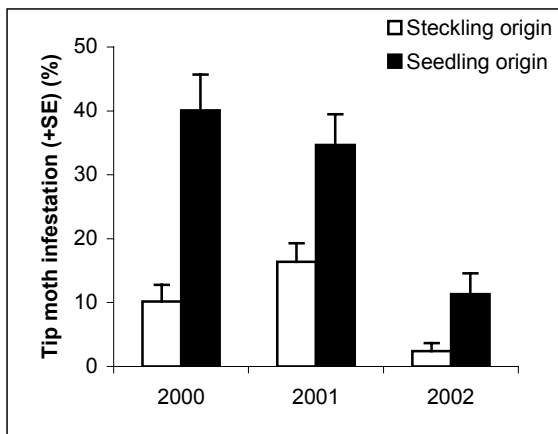


Fig. 1. The average southwestern pine tip moth infestation percentage on saplings from steckling origin (open bars) and from seedling origin (solid bars) at Ft Valley Experimental Forest Station, Flagstaff, AZ. Saplings from seedling origin showed greater tip moth infestation than saplings from steckling origin over three consecutive years from 2000 to 2002 (*p* ≤ 0.012).
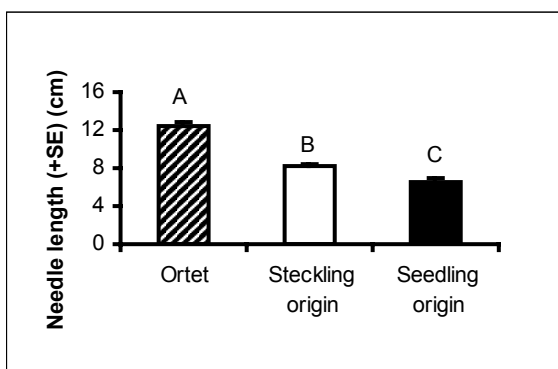


Fig. 2. Comparison of the average one-year old needle length among ortets, saplings from steckling and seedling origin; the ortets had greatest needle length, followed by saplings from steckling origin, and saplings from seedling origin; bars with different letters showed significant difference at α = 0.05.

## C. Needle toughness

The pattern of needle toughness was similar to the pattern of needle length for the three groups of trees. That is, ortets had the greatest one-year old needle toughness (104.9 ± 2.09 g), followed by saplings from steckling origin (75.7 ± 2.25 g), and saplings from seedling origin (67.2 ± 2.09 g) (F=74.6, p < 0.0001) (Fig. 3). This once again indicated that the needle toughness of saplings from steckling origin was more similar to that of ortets. The needle toughness may account for the difference in tip moth infestation between the saplings from steckling origin and saplings from seedling origin.
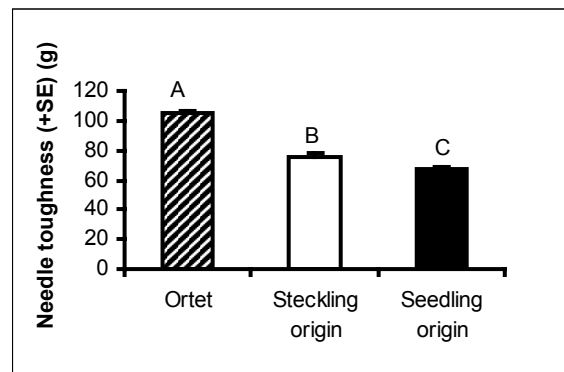


Fig.3. Comparison of the average one-year old needle toughness among ortets, saplings from steckling and seedling origin; the ortets had greatest needle toughness, followed by saplings from steckling origin, and saplings from seedling origin; bars with different letters showed significant difference at α = 0.05.

## IV. Discussion and Conclusion

Our study showed that the saplings from rooted cuttings (stecklings) were still not free from the attacks by southwestern pine tip moths regardless of their advanced ontogenetic stages (> 20 years). This is because the height of saplings from steckling origin falls in the range of 1.8-2.4 m tall, which is most susceptible to southwestern pine tip moth [4]. Why tip moths favor younger or shorter trees is perhaps due to the flying habit of tip moths [16]. The female adults fly near the ground and oviposit on upper crown terminal and lateral shoots, so the shorter trees become primary targets [16]. An alternative explanation for tip moth attacking host trees of a certain height could be due to the competition for an ecological niche. In addition, the tip moths need to descend along the tree bole to the soil for overwintering during the pupation; the longer distance of this descent (on taller trees) will likely increase the risk of predation by natural enemies.

Significant year-to-year variation (from 2000 to 2002) in the average tip moth infestation for saplings from both types could be due to the fluctuation of tip moth population that may be associated with the severe drought in 2002, which

was the most severe drought as defined by the Plamer Drought Severity Index (PDSI) in recorded history. The shoot length was similar for ortets, and saplings from steckling and seedling origin and thus seemed not responsible for the difference of tip moth infestation between saplings of stecking and seedling origin. The southwestern pine tip moth lays eggs on one-year old needles and the first two instars of larvae primarily mine the one-year old needles [4]. Therefore, both the one-year old needle length and toughness very likely influences the initial success of early instar larvae on host trees.

Conclusively, the southwestern pine tip moths showed a preference to attack saplings from seedling origin over saplings from steckling origin in spite of their similar size; and this pattern was consistent over three consecutive years. Our field experimental results further verified the conventional wisdom that the tip moths primarily attack juvenile pines. The one-year needle length and toughness better explain this pattern than one-year shoot length.

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