Variability in seed cone production and functional response of seed predators to seed cone availability: support for the predator satiation hypothesis

Running head: Predator satiation hypothesis

Yan B. Linhart[1*], Xoaquín Moreira[2,3], Marc A. Snyder[4] and Kailen A. Mooney[2]

[1]*Department of Ecology and Evolutionary Biology, University of Colorado, Boulder, CO 80309, USA*

[2]*Department of Ecology and Evolutionary Biology, University of California, 92697 Irvine, California , USA.*

[3]*Misión Biológica de Galicia (MBG-CSIC), Apdo. 28, 36080 Pontevedra, Galicia, Spain.*

[4]*Department of Biology, Colorado College, Colorado Springs, CO 80903, USA*

[*]**Correspondence author. E-mail: yan.linhart@Colorado.edu**

Summary

1. Mast seeding is a reproductive strategy in some perennial plants defined as synchronous production of large seed crops at irregular intervals. One widely accepted theory to explain this behaviour is the predator satiation hypothesis, which states that the synchronous and variable production of seeds within a population will maximize the probability of seed survival through satiation of seed predators.

2. Although some short-term studies have documented the influence of variable and synchronized production of seeds on herbivore attack rate during one or few mast years, long-term data including multiple mast seeding years and patterns of cone production and herbivore attack on individual trees are needed to assess (i) how cone production, variability and synchrony affect individual plant fitness and (ii) the functional responses of seed predators to mast seeding events.

3. We tested these objectives, collecting long-term (29 years) data on female seed cone production and rates of seed predator attack from 217 individual contiguous trees within a *Pinus ponderosa* population.

4. Our results support the predator satiation hypothesis. Firstly, we found high interannual synchrony and variability in seed cone production and a type II functional response of seed predators to available cones. Secondly, years with high cone production (mast years) had markedly lower rates of seed predator attack than years of low production (i.e. a population-level satiation effect). Thirdly, within mast years, individuals with high cone production had markedly lower rates of attack than individuals with low cone production (i.e. an individual-level satiation effect). Finally, individual trees with greater synchrony and more variable cone production suffered lower rates of attack.

5. *Synthesis*: Our long-term data on individual trees lends strong support to the hypothesis that mast seeding in *Pinus ponderosa*has evolved in response to natural selection from insect seed predators.

Key-words: insect seed predators, mast seeding, *Pinus ponderosa*, plant–herbivore interactions, predator satiation, seed cone production, synchrony

Introduction

Mast seeding (also called 'masting') is a common reproductive strategy exhibited by some species of perennial plants, and can be defined as the synchronous production of large seed crops at irregular intervals by a population of plants (Janzen 1976; Silvertown 1980; Sork, Bramble & Sexton 1993; Herrera *et al.* 1998; Koenig & Knops 2005; Crone, McIntire & Brodie 2011). The following patterns characterize mast seeding events: high temporal variability and high synchrony in seed production among individuals of a population (Herrera *et al.* 1998; Koenig & Knops 1998; Ostfeld & Keesing 2000; Koenig *et al.* 2003; Liebhold, Koenig & Bjørnstad 2004; LaMontagne & Boutin 2007; Koenig & Knops 2013).

One of the most widely accepted explanations for the evolution of masting is the predator satiation hypothesis, which postulates that the synchronous and variable production of seeds among conspecifics within a population will increase the likelihood of seed escaping local seed predators through their overabundance (Janzen 1976; Silvertown 1980). Masting may satiate seed predators (and increase seed survival) through at least two complementary mechanisms. First, large fluctuations in seed production may prevent the buildup of seed predator populations between mast seeding events. Second, mast seeding events may increase the resource pool available to predators sufficiently to exceed their ability to consume those resources. Some short-term studies have documented the role of variable and synchronized production of seeds on herbivore attack rate during one or few mast years (e.g. Archibald *et al.* 2012). However in order to better understand how masting evolves, we need to (i) assess how total cone production, variability, and synchrony affect individual plant fitness, (ii) examine patterns of cone production over long-term spans that include several mast- and non-mast years, and then (iii) determine how those patterns influence attacks by seed predators.

Determining a seed predator's functional responses, and how these are related to mast seeding, is crucial to testing the predictions of the predator satiation hypothesis, and understanding its evolution (Koenig *et al.* 2003; Fletcher *et al.* 2010). Predator satiation requires negative density dependence across some portion of the range of naturally occurring resource densities (Holling 1965). The functional response of a seed predator depends on the size, mobility, and diet breadth of the predators (Koenig *et al.* 2003; Klinger & Rejmánek 2009). For example, an individual tree would have a higher probability of escaping mobile generalist seed predators (e.g. squirrels, mice, birds) in a mast event if its cones mature synchronously with those of other trees within these predators' typically large territories. Therefore, mobile predators should select for increased among-population variation and synchrony (Koenig *et al.* 2003). In contrast, less mobile specialist seed predators (e.g. insects) may be satiated by individuals or smaller groups of trees, and might select for increased variation in individual reproduction without selecting for large scale synchrony (Koenig *et al.* 2003). Consequently, characteristics of the seed predator community are likely to play a central role in determining whether masting is advantageous, and at which spatial scales.

A central assumption of the predator satiation hypothesis is that superabundant seed production in mast years reduces proportional seed consumption by predators (satiation effect), thus increasing the proportion of seeds surviving (Koenig *et al.* 2003; Fletcher *et al.* 2010). However, the proportion of seeds consumed by predators may also be reduced at low seed densities if, for example, seed predators turn to more abundant food sources (prey-switching behaviour, e.g. Ims 1990). These opposing dynamics highlight the importance of determining the form of the functional response of predators to seed availability, so as to fully test the predictions of the predator satiation hypothesis (Ruscoe *et al.* 2005; Fletcher *et al.* 2010). Despite the potential to provide mechanistic explanations of predator satiation, the functional responses of seed predators to mast seeding events have been poorly examined (but see Ruscoe *et al.* 2005; Fletcher *et al.* 2010).

The aims of the present study were threefold. Firstly, we evaluated the variation and synchrony in seed cone production within a ponderosa pine (*Pinus ponderosa* var. *scopulorum*) population in relation to the proportion of insect-damaged cones. Secondly, we examined the functional response of specialist insect seed predators to mast and non-mast seeding events in the same pine population. Thirdly, we assessed these dynamics both at the population level, and in terms of individual trees, thus allowing inferences about possible evolutionary responses. To test these objectives we collected long-term (29 years) data on seed cone production, classified as healthy and insect-damaged cones, and calculated synchrony and variability in 217 trees. Taken together, these objectives provide a more complete understanding of masting behaviour in pines and its fitness consequences with respect to interactions with seed predators.

Materials and methods

PINE SPECIES, STUDY AREA AND DATA COLLECTION

Ponderosa pine is a native conifer in western North America where it is one of the most widely distributed pine species, especially at higher elevations (Richardson 1998). Ponderosa pine is monoecious, i.e. male and female cones occur on the same tree, which reproduces via seeds. The female cones usually require two growing seasons to mature after pollination and reach their full size by mid-summer. The cones usually open to release the seeds several months later in the fall. The seeds are small and winged, anemophilous (wind-dispersed) and rich in nutrients, which serve as food source for many animals. As in other pines, episodic mast seeding events are common in ponderosa pine populations (e.g. Linhart & Mitton 1985; Mooney, Linhart & Snyder 2011).

We carried out a long-term field study (from 1979 to 2008, except in 2004) monitoring a population of 217 ponderosa pine individuals distributed over a 2 ha area on the south-facing slope of Boulder Canyon at an elevation of 1740 m in the Front Range of the Rocky Mountains (near the town of Boulder, Colorado, 40° 00' 48"N, 105° 18' 12"W). The stand was open and park-like, typical of ponderosa pine forests in this xeric, continental setting. Ages of studied trees ranged from about 40 to over 280 years, and were estimated from cores taken at 30-50 cm above-ground. There was no evidence of logging or other disturbance at this site.

We recorded total cone production each year (between July and October) by counting total individual seed cones throughout the crowns of all trees. Seed cones were recorded separately as cones with no signs of predator activity (healthy cones, hereafter) and cones with external evidence of insect damage (attacked or aborted cones, hereafter). In this population, the cone-feeding insects include the cone weevil *Conotrachelus neomexicanus* Fall (Coleoptera: Curculionidae), the cone moths

Dioryctria spp. (Lepidoptera: Pyralidae) and *Eucosma* spp. (Lepidoptera: Tortricidae) (Bodenham & Stevens 1981; Keefover-Ring & Linhart 2010). Adults of these species oviposit on green second-year cones in spring and early summer, and their larvae mine the interior, indiscriminately devouring scales and seeds (Furniss & Carolin 1980; Hedlin *et al*. 1981; Cibrian-Tovar *et al*. 1986). This feeding destroys all of the seeds in a cone, and the damaged cones never open (Plate 1; Linhart personal observation; Bodenham *et al*. 1976; Schmid *et al*. 1986; Pasek & Dix 1988; Blake *et al*. 1989). Such infested cones quickly die, turn reddish to dark brown, and appear stunted or deformed (Plate 1). To identify the insects responsible for this damage, we dissected cones and identified insects in situ. This was done repeatedly over the years. During one three-year sampling (1988, 1989, 1998), we dissected 715 cones at this site, and another 766 at nearby sites to familiarize ourselves with sources of cone damage (Keefover-Ring & Linhart 2010). Prior to those years and periodically afterwards, we sampled 20-30 cones haphazardly throughout the population to see if other insects might be responsible, and to verify that shrunken cones contained no viable seeds. Given that the damaged cones produced no viable seeds we also refer to these attacked cones as aborted cones. When counting cones we were able to classify them as either viable or aborted unambiguously. Early in the summer, viable cones were green, while aborted cones were brown, smaller, and often distorted and covered with resin. Later in summer and early fall, healthy cones opened to release the seeds, while aborted cones stayed closed (Plate 1). In this population, trees are short enough and the stand density is open enough that accurate counts of cones were easy to obtain. In order to ensure the continuity and consistency of data collection, one or more of the authors was present for all counts.

DATA ANALYSIS

Descriptive statistics

Mast seeding years are qualitatively defined as being those years when a heavy seed crop is produced (e.g. Silvertown 1980; Norton & Kelly 1988; Kelly 1994). As in past studies, we defined mast seeding as those years when seed production exceeds the mean by a designated amount, measured in standard deviations (LaMontagne & Boutin 2007; LaMontagne & Boutin 2009). Specifically, we calculated the population deviation from the long-term mean in standard deviations for each year as $SD_i = (X_i – X_p) / (SD_p)$, where the SD_i and X_i are the standard deviation and mean for year *i*, and X_p and SD_p are the standard deviation and mean for the population across all 29 years. 'Mast years' were then defined to be those where $SD_i \geq 0.93$, while 'non-mast years' were defined as those where $SD_i < 0.93$. This classification resulted in 6 mast years, 19 non-mast years, and 4 intermediate years (Figure S1). A threshold of 0.93 was selected in favor of 1.0 (used by LaMontagne & Boutin 2007) as this resulted in the inclusion of 2005 as a mast year, a year that was much closer in rates of cone production to the five other mast years than to the non-mast years (Figure S1). However, all of our findings are qualitatively identical if a threshold of 1.0 is used (results not shown).

To assess synchrony and variability in reproduction and the proportion of attacked cones, we calculated the following population-level metrics: (i) annual variation in total seed cone production by the population (CV_p), calculated as the coefficient of variation for total population cone production over 29 years (LaMontagne & Boutin 2007; Mooney, Linhart & Snyder 2011); (ii) total cone production for each year T_p, calculated as the total number of cones produced each year across all trees (n = 29); (iii) the proportion of seed cones attacked in the population each year (P_p), calculated as the mean proportion of attacked cones across all trees for each year (n = 29). We in turn calculated a set of parallel statistics for each individual tree (n = 217): (i) total cone production for each tree T_i, calculated as the total number of cones produced across all years; (ii) individual variability CV_i, calculated as the coefficient of variation across 29 years for each individual tree (Herrera 1998); (iii) individual synchrony r_i, calculated as the Pearson's correlation coefficients for seed cone production between each tree and total cone production for all other trees across years (Crone, McIntire & Brodie 2011; Mooney, Linhart & Snyder 2011); and finally (iv) individual proportion of seed cones attacked over 29 years P_i, calculated as total attacked cones divided by total cone production over 29 years.

Functional response: Population- and individual-level tests

Holling (1959) reported three general forms of predator functional responses to prey density which we can translate to seed consumption by predators (Figure 1a): (i) Type I responses, where the proportion of consumed seeds remains constant, independently of the level of seed availability; this does not support the predator satiation hypothesis; (ii) Type II responses, where the proportion of seed consumed by predators is highest at low levels of seed availability; and (iii) Type III responses, where the proportion of seed consumed by predators is highest at some intermediate level of seed availability and then declines towards zero. Type II and III functional responses both support the predator satiation hypothesis because the proportion of seed consumed by predators decreases with increasing seed availability across at least some range of seed density.

Distinctions among type I, II and III functional responses are best tested with a statistical model in which the proportion of resource consumed is modeled as dependent upon the linear and quadratic effects of resource availability using the logistic regression (Trexler, McCulloch & Travis 1988; Juliano 2001; Fletcher *et al.* 2010). Because the distribution of seed cone production among trees was extremely skewed, we used the $\log_{10}$ (x+1)-transformed number of seed cones as the independent variable. Type I functional responses were identified based on non-significant linear and quadratic coefficients of available cones; type II responses were identified by a negative linear coefficient of available cones, and type III responses were identified by positive linear and negative quadratic coefficients of available cones (Trexler, McCulloch & Travis 1988; Juliano 2001; Fletcher *et al.* 2010).

First, we calculated the functional response at the population-level, regressing the proportion of attacked seed cones for the population each year (P_p, dependent variable) onto total cone production for each year (T_p, independent variable). Second, we determined the form of the functional response at the individual level across both mast- and non-mast years. Here we regressed the proportion of attacked seed cones for each tree (P_i, dependent variable) onto total cone production for that tree (T_i, independent variable), where each tree was represented twice with values of P_i and T_i taken separately from mast ($P_{i\text{-mast}}$, $T_{i\text{-mast}}$) and non-mast years ($P_{i\text{-non-mast}}$, $T_{i\text{-non-mast}}$), and including individual tree as a fixed effect. This approach thus tests for the individual level benefit of masting across the full range of cone production (Fletcher et al. 2010). Finally, we tested whether variation in cone production among trees influences cone attack within mast years ($n = 6$), regressing individual proportion of seed cones attacked over all mast years ($P_{i\text{-mast}}$, dependent variable) onto total cone production for each tree over all mast years ($T_{i\text{-mast}}$, independent variable). A parallel analysis within non-mast years (based upon $P_{i\text{-non-mast}}$ and $T_{i\text{-non-mast}}$) was not possible because the range of cone production among trees was too low to accurately assess seed predator functional response.

Associations among individual-level descriptors

To test the predictions of the predator satiation hypothesis, i.e. that high variability and synchrony maximize the probability of seed escape, we used multiple logistic regressions to test the effects of (a) individual synchrony in seed production (r_i), (b) the variation at the individual-level in seed cone production (CV_i), and (c) the total cone production for each tree (T_i) upon the individual proportion of attacked seed cones (P_i).

Relative effects of individual vs. site-wide cone production

If large seed crops reduce rates of seed predator attack on individual trees, such effects are likely driven by both seed cone abundance within the individual's canopy, as well as seed cone abundance within the canopies of all other trees. To assess the relative contributions of these two factors, we used multiple logistic regression. In this analysis, the proportion of cones attacked on a single tree within a single year was regressed onto both the total cone production for that tree within that year, as well as the mean number of cones produced by all other trees within the population for that year, with individual tree included as a fixed effect. With this approach, the partial regression coefficients quantify the relative influences of variation in individual vs. population-wide cone production for the rate of seed predator attack.

Results

Contributions to total seed cone production

A total of 183,015 cones were produced at the site over the 29 years of study for a site-wide average of 6,100 ± 1,464 cones per year (mean ± SE). Cone production varied extensively among the 217 trees, ranging from 29 year totals of 0 to 25,842 total cones produced. Across all 29 years, the 27 most productive trees (12% of trees) produced 50% of all cones, while the 29 least productive trees (13% of trees) only contributed

0.16% of all cones. Site-wide cone production varied extensively among the 29 years, ranging from 0 in non-mast years to 26,040 seed cones in one mast year (Figure 2). The contribution of individual years to this 29-year site-wide total was highly skewed because 76% of cones were produced during six mast years (Figure 2) while the 10 least productive years accounted for less than 5% of total cone production. A total of 39,339 cones were aborted and produced no viable seeds because of attacks by weevils and moths over 29 years, with the site-wide proportion of aborted cones per year ranging from 0.038 to 0.953 (Figure 2). In accordance with the predator satiation hypothesis, the proportion of attacked cones at the site was significantly lower in mast years (mean = 0.123 ± 0.116) than in non-mast years (mean = 0.444 ± 0.063) ($F_{1,24} = 5.90$; $P = 0.023$).

Variability and synchrony in seed cone production

Seed cone production was variable among years at both the population and individual level, and reproduction among trees was highly correlated. The coefficient of variation at the individual level (CV_i) ranged from 1.13 to 5.48 (mean = 2.01 ± 0.05) while the coefficient of variation at the population level (CV_p) was 1.26. The synchrony in seed cone production at the individual level, (i.e., r_i) involved all pairwise Pearson's correlation coefficients for seed cone production between each tree and total cone production for all other trees across years, and ranged from -0.09 to 0.96 (mean = 0.73 ± 0.01).

Determining the form of functional response of seed predators

The functional response of seed predators at the population level (n = 29 years) was a type II response, as demonstrated by the negative and significant linear coefficient of untransformed and $\log_{10}$-transformed available cones (Table 1a). The proportion of attacked seed cones strongly decreased as available seed cones increased, that is, the maximum proportions of aborted cones occurred at low levels of available cones (Fig. 1b). The functional response of seed predators at the individual level (n = 217 individual trees) across both mast- and non-mast years was also a type II response, as demonstrated by the negative and significant linear coefficient of untransformed and $\log_{10}$-transformed available cones (Table 1b). The functional response of seed predators at the individual level (n = 217 individual trees) in mast years was also a type II, based on the negative and significant linear coefficient of untransformed and $\log_{10}$-transformed available cones (Table 1c). Again, the proportion of attacked seed cones strongly decreased as available seed cones increased (solid dots in Fig. 1c). These results indicate that individual trees benefit from masting across the full range of cone production (Fletcher *et al.* 2010).

Associations among individual-level descriptors

Based upon the type II functional response of seed predators, we used a logistic multiple regression to assess how the proportion of attacked seed cones for individual trees (P_i) was influenced by individual variation in total cone production (T_i), interannual variability in seed cone production (CV_i) and synchrony with other trees in the population (r_i) (Table 2). P_i was negatively associated with CV_i, r_i, and T_i (Table 2). So, in accordance with the predictions of the predator satiation hypothesis, trees that produced more cones (T_i), were more synchronized with neighbors (r_i), were more variable (CV_i), and suffered lower levels cone attack.

Relative effects of individual vs. site-wide cone production

Both seed cone abundance within an individual canopy and seed cone abundance within the canopies of neighboring trees significantly reduced seed predator attack to individual trees (Table 3). Based on a comparison of these parameter estimates, a tree increasing cone production by one cone reduced its own seed predator attack by an order of magnitude more than the reduction in attack that occurred when all other trees in the population each increased cone production by one cone.

Discussion

Three noteworthy results from our field 29-year-study provide strong support for the hypothesis that predator satiation can influence the evolution of masting. Firstly, we found high interannual variability and synchrony in seed cone production at individual and population levels, and a type II functional response of seed predators to available cones; these patterns are all predicted by the postulates of masting behaviour and predator satiation. Secondly, we found that years with high cone production (defined as mast years) have markedly lower proportions of seed cones aborted due to insect predators than years of low production (satiation effect). Thirdly, we found that the proportion of attacked seed cones decreased as total cone production, variability, and synchrony in seed cone production at the individual level increased, thus providing the opportunity for natural selection to shape patterns of reproduction.

The defining feature of masting patterns is the intermittent and synchronous production of large seed crops (Silvertown 1980; Koenig & Knops 2000; Kelly & Sork 2002; Koenig *et al.* 2003). We found just such a pattern, which includes high interannual variability in seed cone production at both the population ($CV_p = 1.26$) and individual level (mean $CV_i = 2.01$). Plant species exhibiting mast seeding behaviour have been commonly identified as those in which the coefficient of variation of year-to-year seed cone production is greater than 1.0 (Silvertown 1980; LaMontagne & Boutin 2009), but for plant species that exhibit "true" masting could even exceed 2.0 (sensu Kelly 1994). We also found high interannual synchrony in seed cone production within our ponderosa pine population, with a mean correlation of 0.73. These levels of within-population synchrony are even higher than values observed in previous studies with ponderosa pine and other tree species, which typically range from 0.35 (Mooney, Linhart & Snyder 2011) to 0.5, (Liebhold *et al.* 2004) and 0.6 (LaMontagne & Boutin 2007). Both results clearly indicate that masting and synchrony were at work in this population.

Plant species exhibiting masting behaviour have several fitness advantages. Those for which there is strongest evidence include increased pollination success (Smith, Hamrick & Kramer 1990), increased probability of seed dispersal (Norton & Kelly 1988) and reduced seed predation (Kelly & Sullivan 1997; Kon *et al.* 2005; Fletcher *et al.* 2010). However, disadvantages have also been associated with masting, including depletion of stored nutrients (Sala *et al.* 2012). Here we provide two strong forms of evidence that, in this ponderosa pine population, predator escape is an important factor favoring the evolution of masting behaviour. Firstly, we found that predator satiation is at work: seed predation was dramatically reduced in the most productive years (25,842 produced cones and 12% attacked in 1984; 24,936 produced cones and 7% attacked in 2001; 24,291 produced cones and 6% attacked in 2006) as compared to non-masting years, when seed predation ranged from 50 to 90 % in 9 of the years recorded (Fig. 2). Secondly, these mast-seeding years usually followed several inter-mast years of very low or no seed cone production (up to 12 years between 1984-1997; Fig. 2), with the exception of two consecutive mast years in 2005 and 2006 (Fig. 2).

Predictions of the predator satiation hypothesis assume that synchronous and variable seed production will satiate seed predators and therefore increase the probability of seed escape, but the size, mobility, and degree of specialization of predators may influence these predictions to a great extent (Koenig *et al.* 2003). In this study, we tested the predictions of the predator satiation hypothesis by relating the variability and synchrony in seed cone production at the individual level against the proportion of seed cones attacked by insect predators. Our results showed that high variability and synchrony at the individual level drastically reduced the probability of predation, as would be expected given the relatively localized movement/foraging of the insect seed predators in this population (Bodenham & Stevens 1981), and for satiation within individual trees. If seed predators were not movement-limited, variation in cones among individuals within mast years would not affect rates of attack (i.e. a cone would be a cone, whether it was on one tree or another). The idea of satiation at small scale (e.g. within individual trees) provides further support for the relatively localized scale of synchrony among populations observed in our previous study of seven ponderosa pine populations in Boulder County (Colorado), including the one studied here (Mooney, Linhart & Snyder 2011). All these populations are attacked by the same suite of insects (*Conotrachelus neomexicanus*, *Conophthorus ponderosae*, *Dioryctria* spp. and *Eucosma* spp.) which are known to restrict their movements to one or a few adjacent trees (Bodenham & Stevens 1981; Keefover-Ring & Linhart 2010). If the spatial scale of seed predator movement was larger than the spatial scale of reproductive synchrony, then seed predators might recruit to locally abundant seeds, reducing or eliminating the benefit of masting behaviour as a reproductive strategy for predator satiation (Curran & Leighton 2000; Ostfeld & Keesing 2000). Supporting our results, some previous authors also found that individual trees are able to satiate relatively immobile specialist predators (i.e. weevils and small moths), and in these cases selection acts to increase individual variability in seed production. For example, Janzen (1978) observed this for *Cassia grandis* and its insect seed predators and Nilsson & Wästljung (1987) for *Fagus sylvatica* and the beech moth (*Cydia fagiglandana*, Tortricidae). Despite the possible benefits of satiation within individual trees, the benefits of synchrony and satiation at larger spatial scale can be seen in the fact that predator attack during non-mast years was higher even when controlling for total cones produced by the individual (Fig. 1c).

The proportion of seed cones aborted because of insect predators decreased strongly with increasing seed cone availability in our population (Fig. 1). The functional response of seed predators to different densities of seeds (consumed vs. available seeds) has been widely studied during the last 60 years (Solomon 1949; Holling 1959, 1965; Fletcher *et al.* 2010), but empirical studies determining the form of the functional responses of seed predators across low and high levels of seed cone production in natural plant populations are still rare (but see Ruscoe *et al.* 2005; Fletcher *et al.* 2010). Evaluating the form of the functional response is crucial to examine the predictions of the predator satiation hypothesis. Here we found a type II functional response of insect seed predators to available seed cones. Type II responses are satiating functional responses because the proportion of predated seeds decreases at high levels of available seeds and this behavioural response is typical of relatively immobile specialist predators (e.g. insects) that specialize on one or a few resources (Holling 1959, 1965). In a previous study with a natural population of white spruce, Fletcher *et al.* (2010) also detected a satiating type II functional response of red squirrels to available seed cones across the entire study years (one mast and three non-mast seeding years). However, these authors detected a satiating type III functional response when analyses were restricted to the three non-mast seeding years (Fletcher *et al.* 2010). Type III functional responses are commonly typical of mobile generalist predators (e.g. squirrels, mice, birds) which readily switch from one resource to another (Holling 1959, 1965).

The evolutionary dynamics of mast seeding in trees are difficult to detect, given the long life spans of the plants involved. However, we provide evidence that the type II functional response of predators we observe might select for masting behaviour in our pine population. Specifically, we found high individual variation in synchrony and variability, and this variation was in turn associated with the proportion of cones damaged, and therefore individual fitness. In addition, we showed in a previous study of this population that the 50 most fertile trees (highest total cone production) are significantly different genetically at 3 electrophoretic loci from the 50 least fertile trees (Linhart & Mitton 1985). Fertility is in turn positively correlated with both variability and synchrony in cone production (Table 2), suggesting a genetic basis to these traits and thus the possibility for selection. These differences are between members of two groups that grow intermixed on a site of uniform slope, exposure, and soils within a small area (2 ha), so the genetic differences detected among those trees are not due to differences in physical conditions of their habitat such as soils, exposure or competition. Other studies also provide convincing evidence that individual trees benefit in various ways from being highly synchronous with their neighbors, and in these studies, it is either known or inferred that trees which are the most fertile also differ genetically from other members of the population (e.g. Wolgast 1978; Koenig *et al.* 1994; Visser *et al.* 2011; Archibald *et al.* 2012). This combination of results indicates that differential reproduction has a significant heritable basis and is therefore amenable to natural selection in forest trees.

In summary, we provide strong support for the predictions of the predator satiation hypothesis in a long-term field study. In keeping with past studies, we show how the proportion of seed cones that escaped insect seed predators was much greater in mast years. We in turn provide mechanistic detail to the dynamics underlying this pattern; we show how insect seed predator attack follows a type II functional response, and that variation among individual trees in year-to-year fertility, variability and synchrony of seed cone production is associated with increased fitness. These results provide strong evidence that the evolution of mast seeding and predator satiation strategies of ponderosa pine have been influenced, at least in part, by insect predator activity.

.

Acknowledgements

We gratefully acknowledge the more than 60 individuals who have participated in collecting data in the field. This research was supported by National Foundation Science grants BMS 75-14050, DEB 78-16798, BSR 8918478, and BSR 912065. XM received financial support from Postdoctoral Fulbright/Ministry of Education grant program.

References

Archibald, D.W., McAdam, A.G., Boutin, S., Fletcher, Q.E. & Humphries, M.M. (2012) Within-season synchrony of a masting conifer enhances seed escape. *The American Naturalist,* **179,** 536-544.

Blake, E.A., Wagner, M.R. & Koerber, T.W. (1989) Relative effect of seed and cone insects on ponderosa pine in northern Arizona. *Journal of Economic Entomology*, **82**, 1691–1694.

Bodenham, J., Stevens, R.E. & Tatcher, T.O. (1976) A cone weevil, *Conotrachelus neomexicanus*, on ponderosa pine in Colorado: life history, habitats, and ecological relationships (Coleoptera: Curculionidade). *Canadian Entomologist,* **108,** 693-699.

Bodenham, J. & Stevens, R.E. (1981) Insects associated with second-year ponderosa pine cones, Larimer and Boulder Counties, Colorado. *Southwestern Naturalist,* **26,** 375-378.

Cibrian-Tovar, D.B., Hebel, B.H. Yates, H.O. & Mendez-Montiel (1986) *Leptoglossus occidentalis* Heidemann. *Cone and seed insects of the Mexican conifers* (eds D.B. Cibrian-Tovar, B.H. Hebel, H.O. Yates & J.T. Mendez-Montiel), pp. 55–58. USDA Forest Science, Southeastern Forest Experiment Station, Ashville.

Crone, E.E., McIntire, E.J.B. & Brodie, J. (2011) What defines mast seeding? Spatio-temporal patterns of cone production by whitebark pine. *Journal of Ecology,* **99,** 438-444.

Curran, L.M. & Leighton, M. (2000) Vertebrate responses to spatiotemporal variation in seed production of mast-fruiting Dipterocarpaceae. *Ecological Monographs,* **70,** 101-128.

Fletcher, Q.E., Boutin, S., Lane, J.E., LaMontagne, J.M., McAdam, A.G., Krebs, C.J. & Humphries, M.M. (2010) The functional response of a hoarding seed predator to mast seeding. *Ecology,* **91,** 2673-2683.

Furniss, R.L. & Carolin, V.M. (1980) Western fore st insects. *U.S. Department of Agriculture Forest Service Miscellaneous Publication* **1339**, 654 pp.

Hedlin, A.F., Yates III, H.O., Cibrián-Tovar, D., Ebel, B.H., Koerber, T.W. & Merkel, E.P. (1981) Cone and seed insects of North American conifers. Canadian Forestry Service, U.S. Forest Service, Secretaria de Agricultura y Recursos Hidraulicos, Mexico. 122 p.

Herrera, C.M. (1998) Population -level estimates of interannual variability in seed production: what do they actually tell us? *Oikos,* **82,** 612-616.

Herrera, C.M., Jordano, P., Guitián, J. & Traveset, A. (1998) Annual variability in seed production by woody plants and the masting concept: reassessment of principles and relationship to pollination and seed dispersal. *The American Naturalist,* **152,** 576-594.

Holling, C.S. (1959) The components of predation as revealed by a study of small mammal predation of the European pine sawfly. *Canadian Entomologist,* **91,** 293-320.

Holling, C.S. (1965) The functional response of predators to prey density and its role in mimicry and population regulation. *Memoirs of the Entomological Society of Canada,* **48,** 3-86.

Ims, R.A. (1990) On the adaptive value of reproductive synchrony as a predator-swamping strategy. *The American Naturalist,* **136,** 485-498.

Janzen, D.H. (1976) Why bamboos wait so long to flower. *Annual Review of Ecology and Systematics,* **7,** 347-391.

Janzen, D.H. (1978) Seeding patterns in tropical trees. *Tropical trees as living systems* (eds P.B. Tomlinson & M.H. Zimmermann), pp. 83-128. Cambridge University Press.

Juliano, S.A. (2001) Non-linear curve fitting: predation and functional response curves. *Design and analysis of ecological experiments* (eds S.M. Scheiner & J. Gurevitch), pp. 178-196. Oxford University Press, New York.

Keefover-Ring, K. & Linhart, Y.B. (2010) Variable chemistry and herbivory of ponderosa pine cones. *International Journal of Plant Sciences,* **171,** 293-302.

Kelly, D. (1994) The evolutionary ecology of mast seeding. *Trends in Ecology and Evolution,* **9,** 465-470.

Kelly, D. & Sork, V.L. (2002) Mast seeding in perennial plants: why, how, where? . *Annual Review of Ecology and Systematics,* **33,** 427-447.

Kelly, D. & Sullivan, J.J. (1997) Quantifying the benefits of mast seeding on predator satiation and wind pollination in *Chionochloa pallens* (Poaceae). *Oikos,* **78,** 143-150.

Klinger, R. & Rejmánek, M. (2009) The numerical and functional responses of a granivorous rodent and the fate of Neotropical tree seeds. *Ecology,* **90,** 1549-1563.

Koenig, W.D., Kelly, D., Sork, V.L., Duncan, R.P., Elkinton, J.S., Peltonen, M.S. & Westfall, R.D. (2003) Dissecting components of population-level variation in seed production and the evolution of masting behavior. *Oikos,* **102,** 581-591.

Koenig, W.D. & Knops, J.M.H. (1998) Scale of mast-seeding and tree-ring growth. *Nature,* **396,** 225-226.

Koenig, W.D. & Knops, J.M.H. (2000) Patterns of annual seed production by Northern Hemisphere trees: a global perspective. *The American Naturalist,* **155,** 59-69.

Koenig, W.D. & Knops, J.M.H. (2005) The mystery of masting in trees. *American Scientist,* **93,** 340-347.

Koenig, W.D. & Knops, J.M.H. (2013) Large scale spatial synchrony and cross-synchrony in acorn production by two California oaks. *Ecology,* **94,** 83-93.

Koenig, W.D., Mumme, R.L., Carmen, W.J. & Stanback, M.T. (1994) Acorn production by oaks in central coastal California: variation within and among years. *Ecology,* **75**.

Kon, H., Noda, T., Terazawa, K., Koyama, H. & Yasaka, M. (2005) Evolutionary advantages of mast seeding in *Fagus crenata*. *Journal of Ecology,* **93,** 1148-1155.

LaMontagne, J.M. & Boutin, S. (2007) Local-scale synchrony and variability in mast seed production patterns of *Picea glauca*. *Journal of Ecology,* **95,** 991-1000.

LaMontagne, J.M. & Boutin, S. (2009) Quantitative methods for defining mast-seeding years across species and studies. *Journal of Vegetation Science,* **20,** 745-753.

Liebhold, A., Koenig, W.D. & Bjørnstad, O.N. (2004) Spatial synchrony in population dynamics. *Annual Review of Ecology, Evolution, and Systematics,* **35,** 467-490.

Liebhold, A.M., Sork, V., Peltonen, M., Koenig, W., Bjørnstad, O.N., Westfall, R., Elkinton, J. & Knops, J.M.H. (2004) Within-population spatial synchrony in mast seeding of North American oaks. *Oikos,* **104,** 156-164.

Linhart, Y.B. & Mitton, J.B. (1985) Relationships among reproduction, growth rates, and protein heterozygosity in ponderosa pine. *American Journal of Botany,* **72,** 181-184.

Mooney, K.A., Linhart, Y.B. & Snyder, M.A. (2011) Masting in ponderosa pine: comparisons of pollen and seed over space and time. *Oecologia,* **165,** 651-661.

Nilsson, S.G. & Wästljung, U. (1987) Seed predation and cross-pollination in mast-seeding beech (*Fagus sylvatica*) patches. *Ecology,* **68,** 260-265.

Norton, D.A. & Kelly, D. (1988) Mast seeding over 33 years by *Dacrydium cupressinum* Lamb. (rimu) (Podocarpaceae) in New Zealand: the importance of economies of scale. *Functional Ecology,* **2,** 399-408.

Ostfeld, R.S. & Keesing, F. (2000) Pulsed resources and community dynamics of consumers in terrestrial ecosystems. *Trends in Ecology and Evolution,* **15,** 232-237.

Pasek, J.E. & Dix, M.E. (1988) Insect damage to conelets. second-year cones, and seeds of ponderosa pine in Southeastern Nebraska. *Journal of Economic Entomology,* **81**, 1681-1690.

Richardson, D.M. (1998) *Ecology and biogeography of Pinus*. Cambridge University Press, Cambridge.

Ruscoe, W.A., Elkinton, J.S., Choquenot, D. & Allen, R.B. (2005) Predation of beech seed by mice: effects of numerical and functional responses. *Journal of Animal Ecology,* **74,** 1005-1019.

Sala, A., Hopping, K., McIntire, E.J.B., Delzon, S. & Crone, E.E. (2012) Masting in whitebark pine (*Pinus albicaulis*) depletes stored nutrients. *New Phytologist,* **196,** 189-199.

Silvertown, J.W. (1980) The evolutionary ecology of mast-seeding in trees. *Biological Journal of the Linnean Society,* **14,** 235-250.

Schmid, J.M., Mitchell, J.C. & Mata, S.A. (1986) Ponderosa pine conelet and cone mortality in central Arizona. *Great Basin Naturalist*, **46**, 445–448.

Smith, C.C., Hamrick, J.L. & Kramer, C.L. (1990) The advantage of mast years for wind pollination. *The American Naturalist* **136,** 154-166.

Solomon, M.E. (1949) The natural control of animal populations. *Journal of Animal Ecology,* **18,** 1-35.

Sork, V.L., Bramble, J. & Sexton, O. (1993) Ecology of mast-fruiting in three species of North American deciduous oaks. *Ecology,* **74,** 528-541.

Trexler, J.C., McCulloch, C.E. & Travis, J. (1988) How can the functional response best be determined? *Oecologia,* **76,** 206-214.

Visser, M.D., Jongejans, E., van Breugel, M., Zuidema, P.A., Chen, Y.-Y., Kassim, A.R. & de Kroon, H. (2011) Strict mast fruiting for a tropical dipterocarp tree: a demographic cost–benefit analysis of delayed reproduction and seed predation. *Journal of Ecology,* **99,** 1033–1044.

Wolgast, L.J. (1978) Effects of site quality and genetics on bear oak mast production. *American Journal of Botany,* **65,** 487-489.

Table 1. Form of the functional response of insect seed predators to available seed cones at a) population level (n=29 years), b) individual level (n=217 trees) in both mast and non-mast years and c) individual level (n=217 trees) in mast years. Logistic regressions were used to examine the linear and quadratic effects of $\log_{10}$-transformed available cones on the proportion of attacked cones. Significant *P*-values are shown in bold.

Effects	**df**	**Parameter**	**SE**	χ^2	***P***
a) Population level					
Available cones	1, 23	-20.34	5.85	12.08	**<0.001**
Available cones^2	1, 23	9.29	2.82	10.85	**0.001**
b) Individual level (across both mast and non-mast years)[1]					
Available cones	1, 211	-17.25	0.99	301.11	**<0.001**
Available cones^2	1, 211	8.27	0.49	280.12	**<0.001**
c) Individual level (mast years)					
Available cones	1, 211	-19.05	2.97	41.22	**<0.001**
Available cones^2	1, 211	9.36	1.48	40.05	**<0.001**

[1] Each tree was included during both mast and non-mast years and tree identity was included in the analysis as a fixed effect.

Table 2. Multiple logistic regressions testing the effects of individual synchrony in seed production (r_i), the variation at individual level in seed cone production (CV_i), the total cone production for each tree (T_i) upon the individual proportion of attacked seed cones (P_i).

Effects	**df**	**Parameter**	**SE**	χ^2	***P***
Total cones	1, 211	-0.000	0.000	1680.03	**<0.001**
Variability	1,211	-0.517	0.024	479.36	**<0.001**
Synchrony	1, 211	-0.376	0.071	28.46	**<0.001**

Table 3. Multiple logistic regressions testing the effects of total cone production for a tree within a year and the mean number of cones produced by all other trees within the population for that year upon the proportion of cones attacked on a single tree within a single year. With this approach, the partial regression coefficients compare the influences of variation in individual vs. population-wide cone production for the rate of seed predator attack. Individual tree was included as a fixed effect in the statistical model.

Effects	**df**	**Parameter**	**SE**	χ^2	***P***
Individual cones	1, 2996	-0.0026	0.000	4092.94	**<0.001**
Population-wide cones	1, 2996	-0.0001	0.000	12775.90	**<0.001**
Individual tree	1, 2996	-0.0004	0.001	17.41	**<0.001**

FIGURE LEGENDS

Fig 1. (a) Types of functional responses of predators to available cones. See Materials and methods section for specific details about the forms of the functional responses. (b) Relationship between the number of available seed cones and the proportion of attacked seed cones by insect seed predators at population level (white dots for non-mast years, grey dots for intermediate years and black dots for mast years, $r = -0.44$, $P = 0.017$). Each point represents a year ($N = 29$). (c) Relationship between the number of available seed cones and the proportion of attacked seed cones by insect seed predators at individual level in non-mast years (white dots, $r = -0.11$, $P = 0.117$) and mast years (black dots, $r = -0.20$, $P = 0.003$). Each point represents an individual ponderosa pine tree ($N = 217$). The single line shows the functional response across all trees in both mast and non-mast years.

Fig 2. Annual estimates (from 1979 to 2008, except 2004) of the total number of seed cones per tree (white dots, dashed line and left axis) and proportion of attacked seed cones by specialist insect seed predators per tree (black dots, solid line and right axis). Each point represents the average of 217 ponderosa pine trees. Error bars are omitted for clarity.

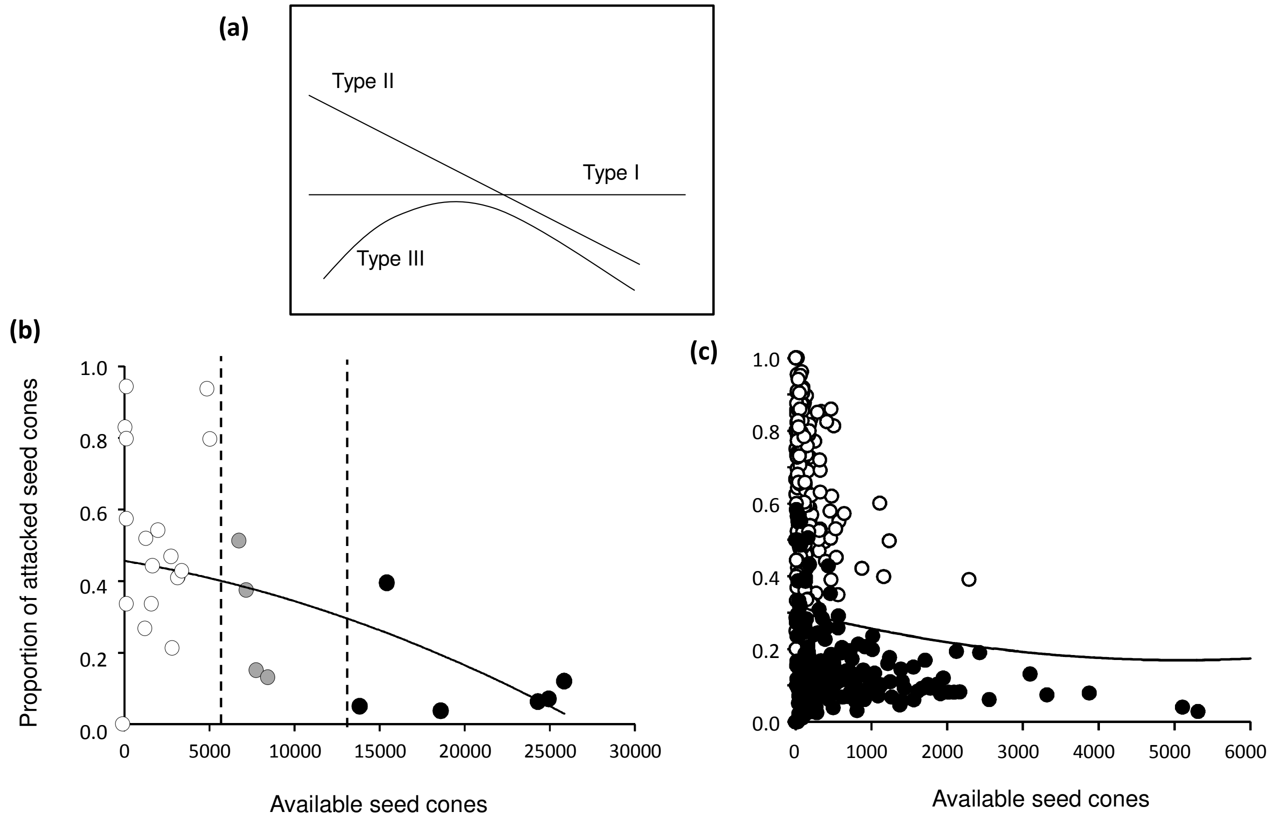

Fig 1. Linhart *et al.*

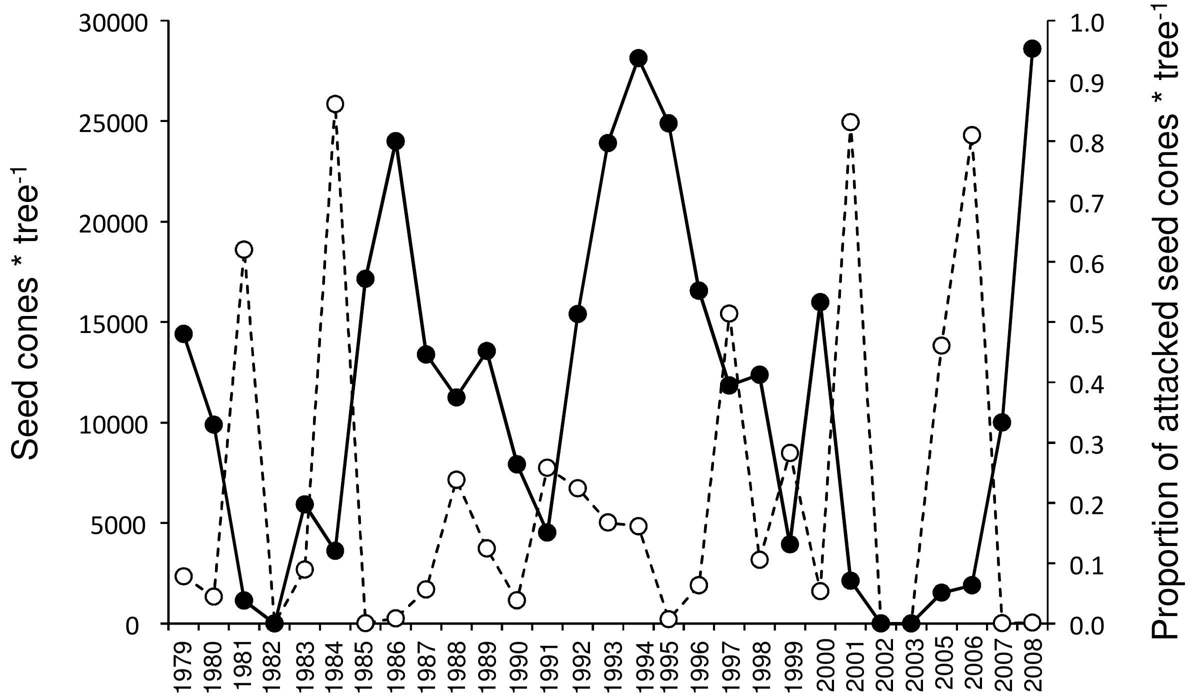

Fig 2. Linhart *et al.*

Plate 1. Example of healthy and aborted seed cones in our *Pinus ponderosa* population. Later in summer and early fall, healthy cones open to release the seeds, while aborted cones stay closed. Credits: Ken Keefover-Ring.