



## Deer Browsing and Population Viability of a Forest Understory Plant

James B. McGraw, *et al.*  
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## Deer Browsing and Population Viability of a Forest Understory Plant

James B. McGraw\* and Mary Ann Furedi

American ginseng is the premier medicinal plant harvested from the wild in the United States. In this study, seven populations of ginseng plants were censused every 3 weeks during the growing season over 5 years to monitor deer browse and harvest and to project population growth and viability. The minimum viable population size was ~800 plants, a value greater than that of all populations currently being monitored. When simulated deer browsing rates were reduced 50% or more, population viability rose sharply. Without more effective deer population control, ginseng and many other valuable understory herbs are likely to become extinct in the coming century.

American ginseng (*Panax quinquefolius* L.) is a widespread but uncommon herbaceous understory plant of the U.S. eastern deciduous forest (1–3). The harvest of wild ginseng to supply the Asian market is economically and culturally important, particularly in central Appalachia. Though poorly quantified, many lines of evidence suggest that ginseng was more abundant in the presettlement forest than at present (1, 4). Harvest figures from the 1800s suggest a three- to fourfold greater export of ginseng than currently occurs (5). Herbarium specimens show that the size of plants collected by botanists has shrunk significantly in the past century (4). Permanent land use conversion from forest due to farming, mining, and development has reduced the number of populations. Concern about the rarity of ginseng led to its listing on Appendix II of CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) in 1973 (6).

The conservation of rare species benefits from demographic modeling based on accurate field data (7). Formal demographic analyses of ginseng were first performed for four populations at the northern margin of the distribution in southern Quebec (8, 9). The resulting models showed that ginseng populations grow or decline slowly, with larger plants contributing most to population growth rate. The minimum viable population

size (defined as having a 95% chance of persisting for 100 years) was estimated at 172 plants, a figure that raised serious concerns about the long-term future for ginseng, because most populations are smaller than this threshold. The extinction risk for ginseng near the range center in Appalachia may differ from that in Quebec, because populations are subject to the potential negative effects of harvest and deer browsing; however, there is also a greater frequency of near-optimal habitat.

In the present study, carried out over a period of 5 years (2000–2004), we censused seven natural populations of American ginseng in West Virginia in order to evaluate population viability (10). These populations varied in size and occurred over a range of elevations, aspects, and forest community types representative of the range center of ginseng (1). Transition matrices summarizing the fates of stage classes were formed from the census data for each pair of years (10, 11).

Early in the censusing procedure, we noted that plants were being browsed by

white-tailed deer (*Odocoileus virginianus* Zimm.); all foliage, and frequently all flowers and fruits, were removed (10). Annual mean browse rates varied from 19 to 42% across 4 years. This rate varied more widely across populations (10 to 63%) than among years. The browse rate was greater for adult plants (11 to 100%; weighted mean, 45%) than for seedlings and juveniles (2 to 64%; weighted mean, 21%). We also documented occasional legal and illegal harvest in the seven populations. Over four growing seasons, two of seven populations experienced harvest, and 0.45 to 3.04% of all monitored plants were harvested, a rate that is within the range of the overall rate observed in 36 monitored populations.

The dominant eigenvalue of the transition matrix yields the finite rate of increase ( $\lambda$ ), a measure of annual growth rate for each population (11).  $\lambda$  varied from population to population and year to year. The geometric mean eigenvalue of the transition matrix for all populations (across years) was 0.973, representing an annual rate of decline of 2.7%. To examine the impact that deer were having on  $\lambda$ , we constructed equivalent matrices minus the deer-browsed plants.  $\lambda$  was estimated to be 1.021 for this “no browse” matrix, suggesting that populations would in fact grow slightly (2.1% per year) when the effect of browsing was removed. The increase in  $\lambda$  for the unbrowsed population was statistically significant (one-tailed paired *t* test;  $P = 0.034$ ,  $n = 4$  years). To further test the relationship of browse to  $\lambda$ , we regressed the deviation of  $\lambda$  from the annual mean  $\lambda$  versus browse rate for all population/year combinations. A significant negative relationship demonstrated that among-population variation in  $\lambda$  was explained in part by browse rate variation (slope =  $-0.318$ ,  $P = 0.022$ ,  $r^2 = 0.19$ ).

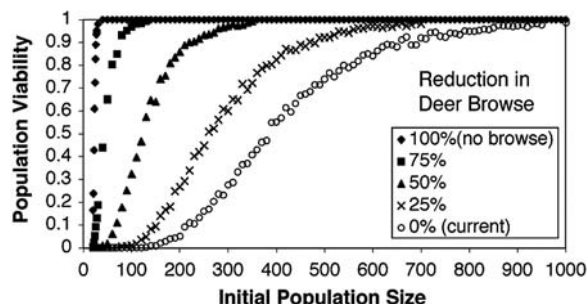


Fig. 1. Population viability as a function of initial population size at five levels of simulated browse.

Department of Biology, Post Office Box 6057, West Virginia University, Morgantown, WV 26506–6057, USA.

\*To whom correspondence should be addressed.  
 E-mail: jmcgraw@wvu.edu

Population viability analysis (PVA) provides a formal structure for making prognoses concerning extinction probabilities for populations (10–12). The procedure involves making stochastic population projections for a time frame of interest and determining whether the population goes extinct during that time (10). This process is repeated until an accurate estimate of viability (the chance of surviving that time frame) is reached. For our simulations, we chose a time frame of 100 years as the length of a simulation run, and we completed 1000 replicate runs to estimate the probability that a population would be viable. Population viability estimates vary with initial population size. Within a PVA, we can therefore also determine the minimum viable population (MVP) (10), in our case defined as the initial  $N$  corresponding to a 95% probability of persisting for 100 years. Although this is a liberal MVP criterion (in that it allows a 5% extinction risk), the existence of many ginseng populations in the wild means that more risk is likely to be acceptable in conserving any one population.

Population viability at current browse rates followed a sigmoidal curve (Fig. 1). Spline fitting of the curve suggested that second-order polynomial regression fits were adequate to describe the function locally. Therefore, to find the initial  $N$  corresponding to 95% population viability, we fit a second-order polynomial to the portion of the curve between viabilities of 0.92 and 0.98, solving the resulting quadratic equation for initial  $N$  at a viability of 0.95. We found an MVP of ~800 individuals (95% confidence limits; ~780 to 820) (10).

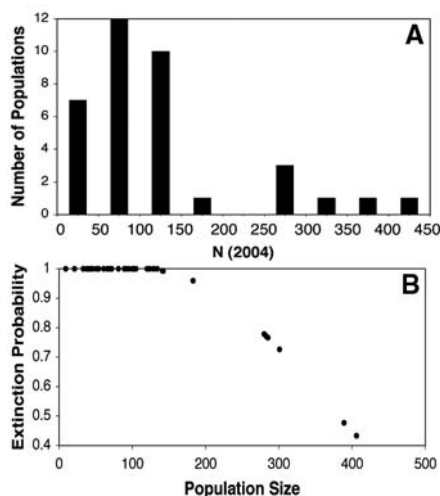


Fig. 2. (A) Frequency distribution of monitored population sizes in an eight-state region near the center of the range of ginseng and (B) estimated extinction probabilities for these same populations based on local polynomial fits to the viability versus  $N$  function in Fig. 1.

To put this new MVP estimate into perspective, Fig. 2A shows the distribution of population sizes we observed in an eight-state (Indiana, Kentucky, Maryland, New York, Ohio, Pennsylvania, Virginia, and West Virginia) demography study of 36 populations containing a total of 4448 plants. The median population size was 93 individuals, well below the MVP estimate. The maximum population size we observed was 406 individuals, which is only about half of the MVP, suggesting that by this relatively liberal definition, none of the 36 populations was viable. We know of only two natural populations in existence that exceed the MVP.

A plot of the chance of extinction within a century (1 minus viability) versus population size of these existing natural populations (Fig. 2B) shows that at current rates of browsing, all populations with  $N < 143$  (29 of 36 of these natural populations) are projected to go extinct within a century (probability of extinction > 99%). The stochastic simulations showed that even the largest population had a 43.3% chance of going extinct before 100 years, a level considered unacceptable for conservation purposes. Acceptable risk is typically defined as 5% or less (12).

Because we observed high rates of deer browsing in the seven intensively studied populations described here, we investigated through simulation how reducing browse rates would affect population viability. This was done by randomly removing browsed individuals from the data set until the desired browse rate was achieved, then forming a new population projection matrix. After repeating this process 10 times for each year and finding the mean resulting matrix for each year, the entire PVA was repeated, and the resulting population viability functions are shown in Fig. 1 for simulated browse rates corresponding to browse rates reduced by 25, 50, 75, and 100% (no browse).

Deer reduced population viability and increased extinction risk. However, population viability increased rapidly with increasing initial  $N$  when browse rates were reduced in the simulations (Fig. 1). Concomitantly, MVP decreased as browse rates were reduced below

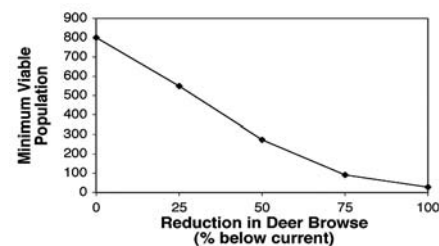


Fig. 3. Reduction in minimum viable population size as simulated deer browse rate is reduced.

current levels (Fig. 3). To place these results in the context of real populations, Fig. 4 shows the percent of populations we monitored in 2004 that would meet the MVP criteria as a function of deer browsing rate. A 50% reduction in deer browsing was required to achieve viability of any of the 36 populations we have censused, and viability monotonically increased as simulated herbivory rates were further reduced.

Any PVA should be cautiously interpreted and is only as good as the data used (12). Fortunately, ginseng's life cycle is amenable to accurate censusing, and its transition through biologically meaningful stages leads to a logical Lefkovich matrix model formulation (13). Though never abundant, the existence of many natural ginseng populations justifies the liberal definition of population viability we adopted. By pooling data across populations, we may have underestimated the annual within-population variation in  $\lambda$ , which would in turn be likely to result in underestimating the risk of extinction. Therefore, in this respect, we believe that our analysis does not exaggerate the risks of extinction. Deforestation, the expansion of invasive species effects, further increases in deer populations, the introduction of disease from cultivated plants, and increases in the price of wild root could all worsen the prognosis. Alternatively, unpredictable positive effects might include the reintroduction of top carnivores, the spread of ungulate diseases, forest maturation, and the substitution in the marketplace of wild simulated ginseng (cultivated seeds grown untended under natural conditions) for plants from natural populations.

We conclude that current deer population densities in central Appalachia jeopardize the future of ginseng, as well as the culture of harvest and trade surrounding this important herb. Although demographic analyses of understory herbs are few, those that do exist suggest that deer represent a similar threat to many understory species (14, 15), as well as tree seedlings and saplings (16–18). Indeed, deer have been identified as a keystone species that can have far-reaching direct and indirect effects on plant and animal communities (16, 18, 19).

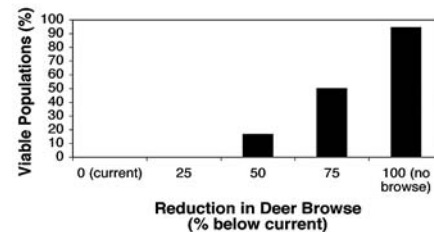


Fig. 4. Proportion of 36 monitored populations predicted to be viable as simulated deer browse rate is reduced from the current rate to no browse.

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# Glycolipids as Receptors for *Bacillus thuringiensis* Crystal Toxin

Joel S. Griffiths,<sup>1</sup> Stuart M. Haslam,<sup>2</sup> Tinglu Yang,<sup>3</sup> Stephan F. Garczynski,<sup>4</sup> Barbara Mulloy,<sup>5</sup> Howard Morris,<sup>6</sup> Paul S. Cremer,<sup>3</sup> Anne Dell,<sup>2</sup> Michael J. Adang,<sup>4</sup> Raffi V. Aroian<sup>1\*</sup>

The development of pest resistance threatens the effectiveness of *Bacillus thuringiensis* (Bt) toxins used in transgenic and organic farming. Here, we demonstrate that (i) the major mechanism for Bt toxin resistance in *Caenorhabditis elegans* entails a loss of glycolipid carbohydrates; (ii) Bt toxin directly and specifically binds glycolipids; and (iii) this binding is carbohydrate-dependent and relevant for toxin action in vivo. These carbohydrates contain the arthroseries core conserved in insects and nematodes but lacking in vertebrates. We present evidence that insect glycolipids are also receptors for Bt toxin.

The crystal (Cry) proteins produced by Bt are pore-forming toxins lethal to insects and nematodes but nontoxic to vertebrates (1, 2). In 2002, more than 14 million hectares of transgenic corn and cotton crops that express Cry proteins were planted worldwide, making these crops safe from specific insect pests and simultaneously resulting in substantial decreases in hazardous chemical pesticide use (3, 4). Cry proteins have now been shown to target nematodes as well, including the intestinal parasite *Nippostrongylus brasiliensis*, suggesting that Cry proteins may be used in the future to control parasitic nematodes of animals and plants (5). In the face of the enormous selective pressure generated by widespread use of Cry proteins in crops and organic farming, development of Cry toxin resistance among target populations is considered the major threat to their long-term

use (6). The ability to detect resistance in the field, which is important for monitoring current resistance-management programs and making corrections before the resistance becomes a widespread problem, relies on molecular and genetic knowledge of the genes and pathways that give rise to resistance. Resistance can be mediated by multiple loci, the identities of which have remained largely elusive. To date, only insect cadherins, which serve as toxin receptors, have been definitively demonstrated to mutate to Cry toxin resistance (7, 8). Other candidates for resistance alleles include a second Bt toxin-binding protein, aminopeptidase N, and a host protease required to process the Bt toxin (9, 10). There are also a number of as yet unidentified loci that can mutate to Cry toxin resistance, including ones important for toxin binding (11, 12).

Using forward genetics, we identified four genes (called *bre* genes for Bt toxin resistant) that mutate to Bt toxin resistance in the nematode *C. elegans* (13–15). Loss-of-function mutants in this pathway resist at least two Cry proteins, Cry5B, which targets nematodes (Fig. 1A), and Cry14A, which targets nematodes and insects (13, 14). Cry5B and Cry14A are members of the main family of three-domain Bt toxins, which includes the commercially used Cry1, Cry2, and Cry3 toxins (16). The *bre* genes encode four glycosyltransferase proteins, act in a single pathway, and are required for the uptake of toxin into intestinal cells, suggesting that they might make a Bt toxin host cell

receptor (13, 14). Based on their in vitro activities, the BRE-3 and BRE-5 counterparts in *Drosophila*—EGGHEAD and BRAINIAC, respectively—have been suggested to synthesize the carbohydrate chains present on glycosphingolipids (14). We therefore hypothesized that the BRE enzymes might be involved in the biosynthesis of glycosphingolipids and that glycosphingolipids might be heretofore-unrecognized host cell receptors for Bt toxins.

To investigate these possibilities, lipids from wild-type and *bre* mutant animals were extracted, partitioned into two phases, resolved by thin-layer chromatography (TLC), and visualized with the orcinol reagent that stains carbohydrates (Fig. 1B). Wild-type animals contain multiple high-polarity glycolipid species (Fig. 1B, upper phase, components B to F). These glycolipids are ceramide-based (and hence glycosphingolipids) because the carbohydrates can be removed with leech ceramide glycanase (17). These upper phase glycolipids are completely absent in *bre-3*, *bre-4*, and *bre-5* mutant animals. In *bre-2* mutant animals, most (B, C, and F) but not all (D and E) upper phase components are missing. In contrast to what was seen in the upper phase, analysis of lower phase (presumably less complex) glycolipids from *bre-4* and *bre-5* mutant animals revealed the appearance of new glycolipid species (Fig. 1B), presumably each representing a different precursor that accumulates as a result of deficiencies in the biosynthetic pathway. Genetic epistasis allows us to infer that the BRE enzymes act in the following order in the synthesis of glycolipids: BRE-3, BRE-5, BRE-4, and lastly BRE-2 [supporting online material (SOM) text], in agreement with the known or proposed activities of these enzymes and the structures of their products. These data demonstrate that BRE enzymes are required to synthesize the carbohydrate chain of glycolipids. The lack of observable defects in protein-linked carbohydrates based on mass spectrometry analysis of N- and O-linked glycans from *bre-3* animals suggests that BRE-3 is not involved in the synthesis of glycoproteins (fig. S5 and table S5). These data and the fact that linkages dependent on *bre-3* and *bre-5* have been found only in glycolipids indicate that glycolipids and not

<sup>1</sup>Section of Cell and Developmental Biology, University of California, San Diego, La Jolla, CA 92093–0349, USA. <sup>2</sup>Department of Biological Sciences, Imperial College London, London, SW7 2AZ, UK. <sup>3</sup>Department of Chemistry, Texas A&M University, College Station, TX 77843, USA. <sup>4</sup>Department of Entomology, University of Georgia, Athens, GA 30602–2603, USA. <sup>5</sup>Laboratory for Molecular Structure, National Institute for Biological Standards and Control, Blanche Lane, South Mimms, Potters Bar, Hertfordshire EN6 3QG, UK. <sup>6</sup>M-SCAN Mass Spectrometry Research and Training Centre, Silwood Park, Ascot, Berkshire SL5 7PZ, UK.

\*To whom correspondence should be addressed. E-mail: raroian@ucsd.edu