Effects of mosquito larvicide on mallard ducklings and prey

A. Keith Miles, Sharon P. Lawler, Deborah Dritz, and Sarah Spring

Abstract We determined the effects of a commonly used mosquito (Culicidae) larvicide (California Golden Bear Oil®, also GB-1111) on body mass and survival of mallard (*Anas platyrhynchos*) ducklings and on target and nontarget invertebrates. Field studies conducted on natural ponds located in salt marshes in south San Francisco Bay indicated that GB-1111 had an initial impact on potential invertebrate prey of birds that dissipated rapidly 3 days post-spray. Over-spray, spray drift, or treatment of more extensive areas would likely delay recovery of nontarget prey. Ducklings held intermittently on the ponds over an 8-day period showed no significant effects of weight loss due to invertebrate prey depletion, although initial effects of exposure to GB-1111 were observed (i.e., matting of feathers and mild hypothermia). These results emphasize the importance of avoiding application of GB-1111 during cold temperatures and adherence to recommended use of this larvicide. Otherwise, GB-1111 had a short-term impact on wetland communities.

Key words ducklings, GB-1111, Golden Bear Oil, invertebrates, larvicide, mosquito

Some California Mosquito Abatement Districts (MADs) use Golden Bear Oil® (GB-1111, Clarke Mosquito Control Products, Inc., Roselle, Ill.) extensively as a larvicide (GB-1111 is the legal trade name for GB-1313, as registered under the United States Environmental Protection Agency). About 1,375,997 L were used throughout California in 1993 (last published account [Mosquito and Vector Control Association of California 1994]; estimated current annual use is 1,135,624 L), and MADs have recommended the use of GB-1111 on federal National Wildlife Refuges as a component of an integrated pest control formula for mosquito (Culicidae) suppression (T. O'Brien, United States Fish & Wildlife Service, personal communication). GB-1111 is a petroleum distillate used as a last-resort larvicide when larvae pupate before the site can be treated with other methods. Other larvicides such as *Bacillus thuringiensis* or methoprene are ineffective once mosquito larvae have pupated. GB-1111 forms a barrier at the air–water interface and suffocates air-breathing insects. GB-1111 may affect natural predators of mosquitoes, such as predatory beetles and hemipterans (Mulla and Darwazeh 1981); otherwise, there are few published reports of effects on nontarget organisms.

According to the product label, the oil is toxic to fish and other aquatic organisms. GB-1111 is classified as a hydro-treated, light naphthenic (closed-chain alkane) petroleum distillate, which implies minimal presence of known toxic polycyclic aromatic hydrocarbons. GB-1111 had a 24-hr, LC^{50} of 2,387 ppm for young sheepshead minnows (*Cyprinodon variegatus*; Tietze et al. 1995), but was not toxic to protozoa and rotifers from a sewage treatment plant after 24 hours of exposure at 2,625 ppm (Tietze et al. 1993). Because GB-1111 is composed of cycloalkanes, it might be more resistant to microbial degradation and have greater toxicity than aliphatic (open-chain alkane) hydrocarbons (Curl and O'Donnell 1977, Albers 1995).

The impact of GB-1111 on avian species or their invertebrate prey has not been tested. Thermoregulation is critical to the survival of ducklings,

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particularly during the first few weeks after hatching (Harun et al. 1997). Young ducklings are less sensitive to cold than gallinaceous chicks, but the young of the most cold-sensitive species (e.g., mallard [*Anas platyrhynchos*] and green-wing teal [*A. crecca*]) require metabolic rates of about 5 times the basal level to maintain their heat balance at an air temperature of 10°C (Koskimies and Lahti 1964). These metabolic requirements compel ducklings to consume large quantities of invertebrates to thermoregulate, with mosquito larvae forming a large component of their diet when available (Meyer and Swanson 1982). If GB-1111 is toxic to nontarget insects, the prey base may be suppressed sufficiently to affect survival of ducklings if applied during critical weeks post-hatching. We evaluated the effects of GB-1111 on 1) survival of hand-reared mallard ducklings exposed under field conditions and 2) potential aquatic invertebrate prey of waterfowl and other migratory birds.

Methods

Study area

We conducted this study from late spring to early summer 1998. Experimental sites were established at 2 adjacent high intertidal, saltmarsh wetlands that were physically separated by a road and railroad levee. The marshes were located at the Don Edwards National Wildlife Refuge, San Francisco Bay, Alameda County, California (Figure 1). These marshes had a well-developed invertebrate fauna (W. Maffaei, Napa County MAD, personal communication) that were heavily used by waterfowl and shorebirds. Although mosquitoes occasionally bred at both marshes, they were infrequently treated to limit breeding. Invertebrates sensitive to GB-1111 were more likely to occur in these rarely treated sites than on more frequently treated sites. The marshes contained numerous small ponds that were separated during summer, low-precipitation years, or periods of average to low high-tide cycles. Invertebrate numbers and diversity were highest during the summer (S. Lawler, unpublished data).

We established replicates of 5 treatment and 5 control ponds within the Hetch-Hetchy and West Vaco-Newark Slough marshes of the Refuge (Figure 1). The selected ponds were <1.0 m deep and ranged in size from 430–1,300 m^2. Assignment of treatment and control was random. Ponds were unvegetated with standing water and separated by a minimum of 20 m of moist ground and vegetation (primarily pickleweed, *Salicornia* spp.) during the study.

Invertebrates

We reared larval mosquitoes in predator-exclusion cages on each site. The cylindrical plastic cages (15 cm diameter × 12 cm deep) had top and side panels screened with plankton netting to expose organisms to the oil and were suspended in the water by styrofoam floats to provide an air space for pupating larvae. We removed cage tops during GB-1111 application. We placed two cages at each pond, each of which contained 15 second-stage mosquito (*Ochlerotatus dorsalis*) larvae. The most abundant invertebrates in the ponds were water boatmen (*Trichocorixa reticulata),* and we

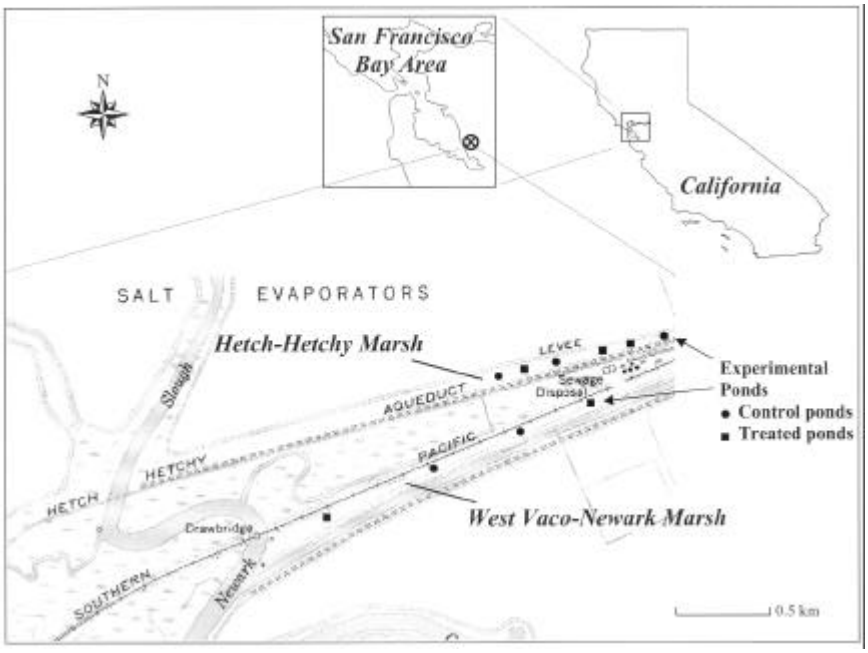

Figure 1. Location of experimental ponds in south San Francisco Bay, Alameda County, California used for study of effects of Golden Bear Oil® (GB-1111) on mallard ducklings and their invertebrate prey, June 1998.

GB-1111 being applied to experimental ponds at the West Vaco-Newark Marsh.

used these as nontarget "sentinels" to monitor the effects of GB-1111 on their survival. We placed 2 predator-exclusion cages that each held 10 *T. reticulata* on every site. We replaced sentinels on days 3 and 15 after GB-1111 application to measure the persistence of any effect of the oil on invertebrate survival. This was necessary because the initially high mortality rates in treated sites would have made it difficult to detect residual oil effects on populations recovering through immigration or breeding.

We counted surviving mosquitoes and water boatmen on each sampling day, which were 2 days and 1 day before the oil was applied and on days 1, 2, 3, 5, 7, 14, and 21 post-treatment. We also collected aquatic invertebrates from ponds on these days, using 4 replicated 1-m sweeps with a "d-ring" net (1-mm mesh) per site. We subsampled collections by wet weight, enumerated subsamples of at least 500 insects per sample, and calculated total abundances. Insects were identified to family, genus, or species, and other taxa were identified to order.

On 15 June 1998, Alameda County, California MAD personnel applied GB-1111 at the maximum label rate of 47 L/ha by backpack sprayer (Chapin Handcan Sprayer # 1 53-09, R. E. Chapin Manufacturing Works, Inc., Batavia, N.Y.).

Ducklings

We obtained 74 1-day-old mallard ducklings from Metzer Farms (Gonzales, Calif.) on 4 June 1998. Hens were not used in this study because of the possibility of parental rejection. Ducklings hatched in incubators and reared without hens lack waterproof oil and usually cannot swim or thermoregulate in water for 2–3 weeks (Raethel 1988). Therefore, our ducklings were maintained *ad libitum* on commercial feed supplemented with live brine shrimp (*Artemia franciscana*) for 12 days. During this time, we identified each duckling by a coded web punch system and evaluated each for condition and its ability to recognize and consume live food. We weighed the ducklings at age days 2 (received the day after hatching), 6, 8, and 12 to establish a growth curve before treatment. Ducklings exhibiting abnormal behavior (e.g., not eating or preening, weakness), injury, or pretreatment growth (i.e., no weight gain) different from that reported by Sugden et al. (1981) were excluded from the study.

On 15 June 1998 at 2 hr post-spray, we placed 5 randomly selected, 12-day-old ducklings (at least 2 males and 2 females) in each of 10 4.3-m-diameter, fully enclosed cages. Six cages were constructed at the Hetch-Hetchy marsh and 4 at West Vaco-Newark Slough marsh. The cages were constructed of plastic netting (0.64 cm^2) with wood stakes. A fence constructed of chicken wire encircled each cage to deter predators. We placed each cage on ponds so that about two-thirds of the inner area was in water and one-third on land during mean low-low tide. We tethered a styrofoam box in each cage to provide shelter and a floating platform in the event tidal action completely inundated the cages. The dimensions of the duckling cages were suggested as the minimal area necessary to support 5 ducklings each (M. Tome and D. Johnson, United States Geological Survey [USGS], personal communication) but the maximum area feasible to conduct the experiment under field conditions.

The ducklings were removed from the cages every other evening before sunset, allowed to settle in warm shelter overnight, weighed starting at 0900 the next morning, and returned to the cages usually by late morning. This was done primarily to

Researcher Dritz examines mosquito larvae for post-spray mortality.

Mallard ducklings foraging in enclosure pens at the Hetch-Hetchy Marsh.

obtain routine weights, but also for humane reasons to allow time for the caged areas to potentially replenish with mobile invertebrate prey from outside the cage and because a hen was not used to shelter the ducklings (the nightly low temperature was cool, about 13°C). The ducklings were provided only with fresh water during the sheltering period, about 18 hours.

Statistical analyses

We analyzed all data using Systat™ (Systat 1992) or JMP™ (SAS 2000). We transformed invertebrate sentinel survival data using arcsine-square root and analyzed them with an analysis of variance (ANOVA). However, when parametric assumptions of normality and homogeneous variances could not be met, we used Kruskal-Wallis tests with the χ^2 approximation as the test statistic. Parametric methods are more powerful and were therefore used wherever possible to maximize the probability of detecting effects; however, the nonparametric method was necessary in some cases. Invertebrate abundance data from sweep-net samples were log-transformed and analyzed with repeated measures analysis of variance (RANOVA). We used the average abundances of each two adjacent samples in this analysis because Systat can only analyze up to 8 dates.

We made comparisons between treatment and control mass of mallard ducklings over time using a multivariate approach to a repeated measures univariate ANOVA. The statistical null hypothesis was that invertebrate prey of ducklings were not affected by GB-1111, resulting in comparable weight gains of ducklings on treated and control ponds over time. The power of this experiment was determined a priori as the number of ducklings required to detect a difference due to treatment (Zar 1996). We estimated that a minimum of a 50-g change in weight due to treatment was necessary to detect a difference, based on variability of growing duckling weights observed by Hunter et al. (1984). Using 25 ducklings each per treatment and control ($\upsilon_1 = 1$, $\Phi = 1.61$, $\alpha = 0.05$, $\upsilon_2 = 46$), the power of analysis was 0.96.

Results

Invertebrates

Nearly all of the first set of sentinel mosquitoes and water boatmen died in treated sites, but survival was consistently high in control sites, demonstrating that the pesticide could harm some nontarget insects while it controlled mosquitoes (Figure 2, for each species χ^2_1 approximation > 7, $P < 0.01$; see also Mulla and Darwazeh 1981). GB-1111 did not cause detectable mortality in the next 2 sets (i.e., days 3–15 and 15–22) of sentinel mosquitoes

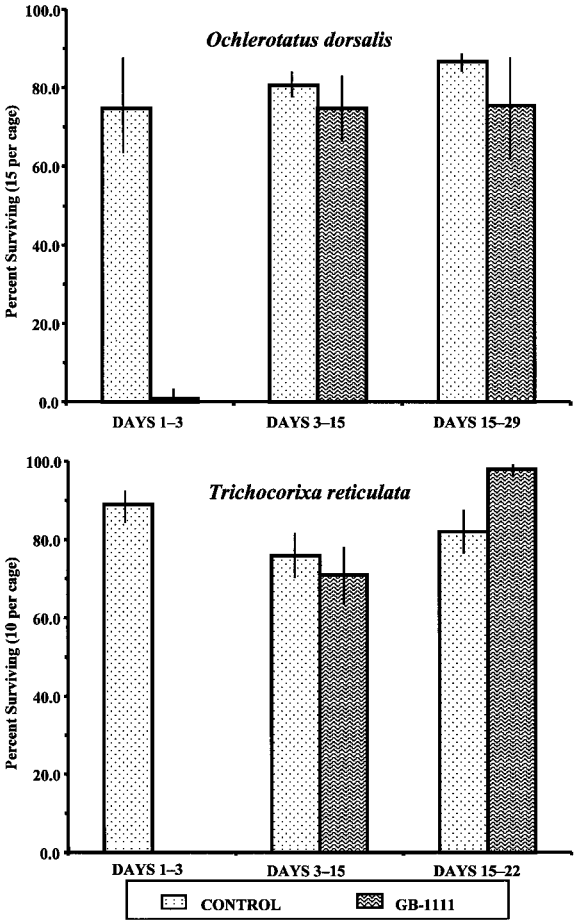

Figure 2. Survival of sentinel mosquito larvae (*Ochlerotatus dorsalis*) and water boatmen (*Trichocorixa reticulata*) enclosed in 2 cages at each of 5 control sites and 5 sites treated with GB-1111 in a saltmarsh. Each bar represents the mean and standard deviation.

(set 2: $F_{1,8}=0.319$, $P=0.59$; set 3: $\chi^2_1= 0.1$, $P=0.75$). There was a slight trend toward a negative effect of GB-1111 on the second set of water boatmen ($F_{1,8}=3.804$, $P=0.087$), but clearly no negative effect on the third set, in which average survival was higher in the treated sites ($F_{1,8}=5.926$, $P=0.04$, Figure 2). The brief activity of GB-1111 was consistent with some of our informal observations during the study. The oil was somewhat volatile and we did not see or smell it after day 3. Wind swept most of the oil from the water surface by 24 hr post-spray.

Sweep-net collections yielded approximately 1,400 invertebrates per site per day, over 90% of which were water boatmen. Other taxa included marine worms (Annelida), beetle adults and larvae (Coleoptera), fly larvae (Diptera), and amphipods (Amphipoda); however, these were either too scarce or too patchy among sites for meaningful statistical analysis. In comparison to the predator-exclusion cages, the sweep-net collections of water boatmen were more variable and indicated a lower level of mortality. Water boatmen abundance over the entire time series did not differ by treatment ($F_{1,8}=1.60$, $P=0.24$) or by time and treatment interaction ($F_{4,32}=1.10$, $P=0.37$). However, the number of water boatmen decreased over time ($F_{4,32}=6.08$, $P=0.001$). The high variance of a long series could obscure differences that occurred shortly after treatment, when the largest differences were expected *a priori.* We therefore analyzed a truncated data set consisting of the 2 pretreatment samples and the first 2 post treatment samples. This analysis suggested a decrease in water boatmen in treated sites relative to those in control sites immediately after treatment (time × treatment interaction $F_{1,8}=9.45$, $P=0.015$).

Water boatmen survived poorly in treated sites relative to controls. We compared percent population change of water boatmen in saltmarsh ponds 2 days before versus 2 days after the date of pesticide application. We calculated population as ([number after application date – number before] / number before). Juvenile losses were 78.5% (SE=17.5) and 34.7% (SE=15.3) in treated and control ponds, respectively. Adult loss was 73.0% (SE=9.1) in treated ponds, compared with a population gain of 12.7% (SE=9.1) in control ponds.

Ducklings

Post-treatment gain or changes in duckling mass did not differ between treatment and control sites during the study ($F_{1,31}=0.103$, $P=0.75$, Figure 3). We placed ducklings in the caged wetlands 2 hr post-spray on the afternoon of 15 June, with an average mass of 208.5 g (SE=3.6) and 209.0 g (SE=2.7) in control and treatment ponds, respectively. We noted that treatment ducklings were exposed immediately to an oily sheen of GB-1111. The ducklings preened continuously, huddled, and appeared agitated in response to this exposure, raising concern about the possibility of hypothermia. However, all ducklings survived overnight and appeared healthy and active the following morning. Duckling mass increased 4-fold while fed turkey (*Meleagris* sp.) starter diet prior to the study, but weight gain in both treatments was static for 7 days following introduction into the cages. Mass of ducklings in this study was between that shown for wild and captive ducklings from other studies, although those ducklings gained mass during a similar growth period (Figure 3). On the eighth day, duckling mass in treatment and control cages was 9% lower than those on the previous day, and 11% (control)–14% (treatment) lower than those on the start day. In general, ducklings held in cages at the Hetch-Hetchy site fared better than those from the Vaco-Newark Slough site, possibly due to the intermittent occurrence of brine flies (*Ephydra* spp.) from a salt pond close to the Hetch-Hetchy site ($F_{1,31}=0.001$, $P=13.24$). Ducklings at the Hetch-Hetchy site maintained mass, but those at the Vaco-Newark Slough site began to lose mass by day 4 in the field.

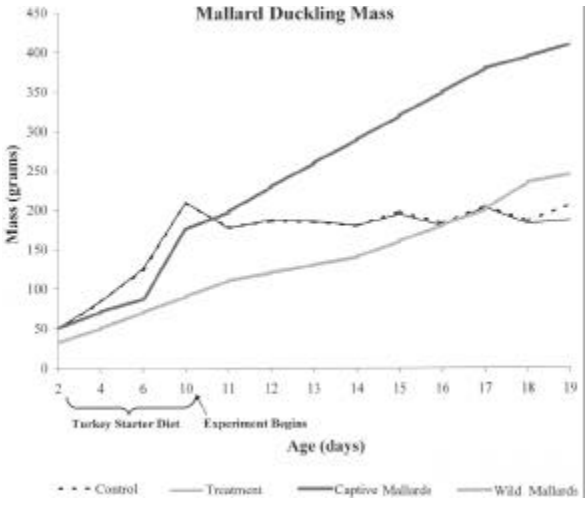

Figure 3. Pre- and post-treatment and control changes in mass of mallard ducklings held on experimental ponds at the Don Edwards National Wildlife Refuge, San Francisco Bay, California, 5–23 June 1998. Treatment ponds were sprayed with the larvicide GB-1111 on 15 June 1998. Wild mallard growth curve adapted from Lokemoen et al. (1990). Captive mallard growth curve adapted from Sugden et al. (1981).

Discussion

Proper diet to sustain thermoregulation is critical during the first few weeks of development of Anatinae (Koskimies and Lahti 1964, Sedinger 1992, Harun et al. 1997, Cox et al. 1998). Aquatic invertebrates comprise 50 to 100% of the diets of young ducklings from age day 1 to day 25, tapering to 10% of the diet by the class IIb stage (day 36–45), and then to about 1% by class III (day 44–55, Chura 1961). Any reduction in forage probably increases mortality, especially during the first few weeks (Street 1978, Cox et al. 1998). Mosquito larvae can comprise a large part of the diet of developing ducklings (Meyer and Swanson 1982); without this component, the remaining prey base becomes more important.

GB-1111 was very effective in controlling caged mosquito larvae. However, it also had an adverse impact on both caged and free-ranging water boatmen, which were abundant at the marshes. The effect of GB-1111 was more pronounced on caged water boatmen because the cages reduced their ability to avoid the oil. Uncaged water boatmen may have reduced their contact with the oil by climbing out on emergent vegetation and grooming; mosquito larvae were restricted to the water column. In addition, the impact of the oil may have seemed smaller on uncaged water boatmen if others immigrated from untreated areas. However, immature water boatmen were unlikely to move across land, and only 80% of immatures disappeared from treated sites in the first days post-spray, in contrast to 100% mortality of caged. Loss of adults could be caused by either death or emigration from treated ponds because adults of this species have wings, whereas loss of immatures was likely to reflect only mortality. Mortality caused at least some loss of both life stages because we observed many dead adult and immature water boatmen floating on the surface of treated sites and virtually none in untreated sites.

The only significant mortality of caged and uncaged invertebrates occurred within the first 3 days, and we did not detect differences between treated and control sites by 1 week post-spray. The rapid recovery of uncaged invertebrates may have resulted from immigration of adult insects from untreated areas or recruitment from within sites.

Recommended applications of GB-1111 did not affect duckling survival and mass, despite the initial impact on prey mosquitoes or water boatmen. Ducklings on the treated ponds may have fared well immediately after the application of GB-1111 because of the abundance of dead or floating insects available for consumption, which was also observed by Hunter et al. (1984). We recognized a potential problem of oiling of duckling feathers immediately following application of GB-1111. Oiled, matted feathers impede the ability of water birds to thermoregulate and can result in poor condition or mortality in cold weather (Stephenson 1997). The ducklings in our study were in good condition (e.g., visually healthy with good weight gain) at the time of exposure and daytime ambient conditions were fairly mild (>15°C), but younger or less robust ducklings might not have survived direct exposure to GB-1111.

Class Ic (days 13–18) ducklings consume about 75% invertebrates (Chura 1961); thus, the experimental period was sufficient to determine an effect of GB-1111 on their prey. Organophosphate pesticide studies have reported a difference in mass of ducklings on treated and untreated areas 2–3 days post-treatment (Hunter et al. 1984, McCarthy 1995). Class IIa (days 19–25) ducklings consume about 50% invertebrates and 50% plant material, and conceivably the ducklings at this point could switch more to plant material for sustenance. The area of cages was probably sufficient for duckling maintenance, but the confinement of the cages might have inhibited effective capture of highly mobile prey, resulting in static mass. Brine flies or water boatmen were capable of avoiding capture because the cage had water inside and outside, thus allowing the prey to move beyond the reach of the ducklings. Further, water boatmen were abundant, but apparently were not the main prey of mallards, possibly because of their quick response to predator avoidance (Batzer et al. 1993).

Management implications

Human resource managers are confronted with controlling mosquitoes for both nuisance and health concerns, whereas wildlife managers face the dilemma of an impaired prey base for fish and wildlife and potentially toxic effects of chemical mosquito controls. Our results indicated that GB-1111, at recommended field application rates, caused no significant or substantial effects on young ducklings. Recovery of invertebrate fauna from the initial effects was rapid, and exposure effects on ducklings were apparently temporary.

However, field application of GB-1111 should be avoided in early spring and during peak hatching of waterfowl in wetland situations, particularly if daily low temperatures are below about 15°C, based on metabolic studies by Koskimies and Lahti (1964).

The effects of GB-1111 on invertebrates attenuated rapidly over time. While the spatial scale of GB-1111 application was typical of some operational pest control activities, GB-1111 drift or overlap spray could result in a higher than recommended application or application to a larger area than planned, and in these instances community recovery could be slower than we observed. Strict adherence to recommended use and rates for field applications of GB-1111 is important to ensure the survival of avian wetland species. Mallard ducklings and potential prey populations of aquatic invertebrates (genera Ades and Trichocorixa) or similar species are typical of brackish or saltwater marshes in the northern and southern hemispheres. When used properly, GB-1111 appeared to have a minimal or short-term impact on these species.

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