

Protection of Pandora Moth (*Coloradia pandora* Blake) Eggs
From Consumption by Golden-mantled Ground Squirrels
(*Spermophilus lateralis* Say)

by

Elizabeth Ann Gerson

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APPROVED:

Redacted for Privacy

Co-Major Professor, representing Forest Science

Redacted for Privacy

Co-Major Professor, representing Forest Science

Redacted for Privacy

Head of Department of Forest Science

Redacted for Privacy

Dean of Graduate School

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INTRODUCTION

Background

Pandora moths (*Coloradia pandora* Blake) are endemic herbivores of western pine forests. Individuals require two years to complete a life-cycle that progresses through egg, larval, pupal, and adult moth stages. The phenology of *C. pandora* populations is fairly synchronized, so visible defoliation of host trees is noticed in alternate years when populations are large. Epidemic populations ("outbreaks") have developed over the past century in Arizona, Colorado, Wyoming, Utah, California and Oregon (Patterson 1929, Wygant 1941, Furniss and Carolin 1977, Schmid and Bennett 1988) at roughly 20- to 30-year intervals (Carolin and Knopf 1968). In central Oregon, outbreaks occurred circa 1893 (Carolin and Knopf 1968), from 1918 to 1925 (Patterson 1929), from 1959 to 1966 (Carolin and Knopf 1968), and from 1988 to the present (Wickman et al. 1994). *Coloradia pandora* outbreaks generally subside within four generations, evidently in response to viral epizootics. However, a 80-ha defoliated region of ponderosa (*Pinus ponderosa* Dougl. ex Laws) and lodgepole pine (*P. contorta* Dougl. ex Loud.) first noticed in 1986 in the Deschutes National Forest in central Oregon expanded to 137,600 ha in 1994 (Eglitis, personal communication). The largest outbreak previously reported in the literature affected approximately 40,500 ha of lodgepole pine in the Arapaho National Forest in north-central Colorado (Wygant 1941).

During periods of copious moth flight in 1991 and 1993 in the Deschutes N.F., the remains of gravid females were found on the ground

and on stumps habitually used by golden-mantled ground squirrels (*Spermophilus lateralis* Say) and several species of chipmunks (*Tamias amoenus* Allen and *T. townsendii* Bachman) (Ross, personal communication; also personal observation). The eggs and wings were not consumed. *Coloradia pandora* eggs are slightly oblong, several millimeters in length, and comparable in size to lodgepole pine seeds, a common food item of *S. lateralis* and *Tamias* spp. Nutritional analysis of whole eggs confirmed their potential to supply fats, proteins, and water to these small rodents in their arid environment. Therefore, it was hypothesized that *C. pandora* eggs are chemically and/or physically protected from rodent depredation.

Objectives

My objectives were to: 1) identify a predator of *C. pandora* moths that avoids consuming eggs; 2) determine whether any of three chemical fractions of eggs, separated through a polarity gradient of solvent extractions, would elicit the avoidance behavior; and 3) determine whether the egg shell would elicit the avoidance behavior.

Significance

Insect egg defenses are a critical link in the continuity of the insect life cycle, influencing population dynamics, predator-prey relationships, host selection, and even mate selection. Reproductive success, and consequently the abundance of an insect, is determined in part by how well its eggs are defended. Some Lepidoptera are known to seek out and acquire particular compounds from host plants, using these chemicals to attract or select mates, and/or to protect their eggs (Boppré 1986, Brown 1984,

Conner et al. 1981, Dussourd et al. 1988). Therefore, an understanding of egg defense mechanisms allows a greater appreciation of the insect's ecology, and also may provide a route for managing the insect population, if desired.

Chemical egg protectants may be produced *de novo* by insects, or derived from plant sources. The foliage of *P. ponderosa*, *P. jeffreyi* Grev. and Balf., and *P. edulis* Engelm., three host species of *C. pandora* larvae, are known to contain small quantities of piperidine alkaloids (Tawara et al. 1993, Stermitz et al. 1994). Alkaloids of the pyrrolizidine class have been shown to play important roles in mate selection and egg defenses of several other lepidopteran species whose larvae sequester the chemicals from their host plants (Conner et al. 1981, Dussourd et al. 1988). Pine needles also contain other defensive compounds: terpenes, tannins, flavonoids, etc. (Tawara et al. 1993) that potentially could be utilized by *C. pandora* as egg protectants. The specificity of *C. pandora* to host trees of the genus *Pinus* suggests this lepidopteran species also may obtain defensive compounds from its host. Therefore, it seemed that this study might provide information about natural chemicals that deter feeding by small mammals. Such naturally occurring chemicals could be useful as environmentally benign protectants for seeds or other targets of rodent depredation.

LITERATURE REVIEW

Coloradia pandora Biology (Life History)

Coloradia pandora is taxonomically identified with the superfamily Bombycoidea, family Saturniidae and subfamily Hemileucinae. There are three other species of the genus *Coloradia* found in pine forests of the western U.S. and Mexico (Ferguson 1971). Ferguson (1971) recognized three subspecies of *pandora*, and distinguished the western Great Basin subspecies of Oregon and California, *C. p. lindseyi*, on the basis of size, variations in coloration and genitalia, and different behaviors reported by Patterson (1929). However, the differing behaviors (strict diurnal flight and no attraction to lights) of the subspecies *lindseyi* noted in Patterson's report of the 1918-25 outbreak in central Oregon were not seen in the current Oregon outbreak (personal observations). Variation in size and coloration of the sort described by Ferguson (1971) as distinctions between subspecies also was noted in the course of rearing hundreds of pandora moths in the lab for this study.

Pandora moths are slightly sexually dimorphic; adult males have larger, feathery yellow antennae, and females tend to be more massive. *Coloradia pandora* is a medium-sized western forest moth with a wing spread of 70 to 110 mm (Furniss and Carolin 1977). The moth has grayish-brown, cryptically marked forewings and lighter hindwings with reddish-pink hairs. The head, thorax and abdomen are covered with hairs, forming black and white stripes on the abdomen and grey elsewhere. When the abdomen is stretched or extended, a bright yellow intersegmental membrane is visible. A moth at rest on tree bark is difficult to distinguish visually. However, when disturbed, the adults typically fold their wings back and curl their abdomens, exposing red and yellow possibly aposematic coloration. They

also may eject an anal spray that does not seem particularly odorous or irritating to the skin (personal observation), but could surprise an attacker and facilitate rejection.

C. pandora deposits clusters of 3 to 20 eggs primarily on pine needles, but females will oviposit on practically anything (Schmid and Bennett 1988). The average number of eggs per female at emergence is 145 (Schmid and Bennett 1988). Egg masses tend to be deposited on middle and lower crown branches of larger trees (> 30 cm DBH) (Schmid et al. 1982) where they are vulnerable to terrestrial predators such as ground squirrels or ants. Egg deposition begins shortly after adult emergence, which usually extends from July through mid-August in central Oregon. The eggs then require an incubation period of 40 to 50 days (Carolin and Knopf 1968).

Upon hatching in mid-August through September, the first instar larvae are about 5 mm long (Furniss and Carolin 1977). They migrate to branch tips and feed together on needles until late fall when the larvae disperse to feed individually. In winter, the larvae cluster at the base of needles, overwintering mostly in the third instar (Carolin and Knopf 1968), which is about 25 mm in length (Furniss and Carolin 1977). The following spring, larvae resume feeding on pine needles of all ages, but not new buds, until the final (fifth) instar is completed (Schmid and Bennett 1988). At this time, June in central Oregon, the 60- to 80-mm long larvae descend the bole to pupate in the soil.

The pupa is formed in loose soils up to 8 cm deep (Miller and Wagner 1984), or under litter on rocky soils (Schmid et al. 1982). Female and male pupae average about 3 cm in length (Schmid and Bennett 1988) and have a hard, purplish-brown case. Pupal densities in heavily defoliated, unthinned plots in central Oregon averaged 12.7/m² but were patchily distributed (Ross, unpublished data). Spatial distributions are highly dependent on a variety of site factors including topography, canopy cover, litter depth, and

proximity to trees (Schmid and Bennett 1988). Miller and Wagner (1984) postulated that the apparent tendency toward higher pupal densities in open-canopied areas with light litter layers may be an adaptation to frequent fire intervals in the pine forest types which *C. pandora* inhabits. Outbreak populations tend to occur in pine forests with loose soils, particularly pumice soils in Oregon and California, so these physical soil characteristics may facilitate successful pupation.

The pupal stage generally lasts through one winter to the following July, but a substantial proportion of a generation may continue diapause a second year or longer (Carolin 1971). Unfortunately, the intensity and duration of cold temperatures endured by Carolin's (1971) pupae in a single winter was light compared to normal field conditions, so his conclusions regarding extended diapause in *C. pandora* may be misleading. In the laboratory, pupae require at least 8 weeks at 5 °C before emergence occurs (personal observation).

Moth emergence dates vary somewhat among years and among sites, probably because of differences in ambient temperatures and moisture. During the recent outbreak in central Oregon, moths generally began to be noticed in early July (Mitchell, personal communication). Adult emergence also varies with local site differences. Lower elevation, southerly aspect, warmer microclimate, and rain seem to contribute to earlier emergence rates (Schmid 1984, Schmid and Bennett 1988). Moths emerge earlier in thinned relative to unthinned stands of *P. ponderosa* (Ross, unpublished data) probably because of thinning effects on site microclimate.

Upon emerging, adults must crawl from their pupal site and hang from a vertical support (usually a tree) without delay to properly expand their wings. Most moths settle within 2 m of the ground and remain there until dusk (Schmid 1984). Schmid (1984) found that males substantially outnumber females during the initial days of emergence, but then equilibrate in number later in the flight period. This may help ensure that females are

rapidly mated and succeed in ovipositing fertilized eggs sooner. Female moths will deposit unfertilized eggs if isolated from males for several days (personal observation). The duration of coupling usually was at least 6 h in the lab. In the field, numerous mating pairs were observed in saplings during daylight hours (personal observations). Mating pairs are readily spotted and cannot separate quickly for escape when disturbed. Moths undoubtedly present a heavy predation risk through post-emergence activities of crawling over the ground, and hanging immobile in the lower canopy for wing expansion and mating.

Predation

Lepidopteran eggs are attacked by a variety of predators and parasites. There are about 175 genera of egg-eating arthropods, primarily ants, beetles, and flies, recorded in Hinton's (1981) review of the literature. Ants may be the most important single group of invertebrate predators of insect eggs. Of 4000 ant species, the majority are predaceous (Hinton 1981). Parasitization by hymenopteran insects can cause significant mortality to egg populations (Hinton 1981). *Trichogramma minutum* Riley and a species of *Tetrastichus* are recorded as parasitizing 5% and 17%, respectively, of a small sample of *C. pandora* eggs (Patterson 1929). Schmid and Bennett (1988) found the percentage of eggs parasitized by *Telenomus* sp. remained low (~5%) through 2 generations then increased to 56% as the population collapsed.

There is very little documentation of vertebrate predation on lepidopteran eggs. However, because eggs are small, they presumably are targeted by smaller foliage-gleaning birds, carnivorous or omnivorous rodents such as mice, or insectivores. Hinton's (1981) review of insect egg predators includes only one example of vertebrate predation on lepidopteran

eggs - the great tit (*Parus major* L.) is reported to eat the eggs of the moth *Bupalus piniarius* L. (Tichy and Kudler 1962, in Hinton 1981). In a study of bird predation on spruce budworm (*Choristoneura fumiferana* Clemens), Jennings and Crawford (1983) counted over 2000 eggs in a stomach-content analysis of a single pine siskin (*Carduelis pinus* Wilson).

Unfortunately, few of the 142 birds collected were taken while egg masses were present in the field, and only this individual had consumed spruce budworm eggs. In an experimental study, Torgersen and Mason (1987) placed Douglas-fir tussock moth (DFTM) (*Orygia pseudotsugata* McD.) egg masses in lower crown branches at 9 sites and measured predation for up to 5 years. They concluded that partial predation and complete removal of egg masses reduces overall egg survival 43% to 71%, or about 52% on average. Red-breasted nuthatches (*Sitta canadensis* L.), mountain chickadees (*Parus gambeli* Ridgway), dark-eyed juncos (*Junco hyemalis* L.), and Nashville warblers (*Vermivora ruficapilla* Wilson), were implicated as DFTM egg predators.

Documentation of predation on any of the four life stages of *C. pandora* is limited to casual observation. Patterson (1929) reported that Steller's jays (*Cyanocitta stelleri* Gmelin) and vireos (*Vireo* spp.) fed sparingly on larvae, and brown creepers (*Certhia familiaris* Bonaparte) and nuthatches (*Sitta* spp.) fed on eggs on the Klamath Indian Reservation in central Oregon. He also reported that ground squirrels and chipmunks (unidentified species) prey on pupae. In Colorado, Wygant (1941) assumed that squirrels (unidentified species) and bears (*Ursus americana* Pallus) destroy many pupae, but saw no birds feeding on larvae and noted minimal parasitization. Some animal feces contained eggs. Carolin and Knopf (1968) mentioned that American robins (*Turdus migratorius* L.) were observed feeding on female moth abdomens and that viable eggs were recovered from robin feces in the Rocky Mountains. It is unlikely, however, that eggs ingested from moth abdomens would be viable since eggs are fertilized during

deposition. Schmid and Bennett (1988) also reported seeing robins capturing moths, and Kaibab squirrels [*Sciurus aberti kaibabensis* (Merriam)] feeding on pupae in the Kaibab National Forest, Arizona. Contrary to Patterson's observations, captive feeding tests with *S. lateralis*, *T. amoenus* and *T. townsendii* from central Oregon indicated neither species of chipmunk eats the pupal stage, but *S. lateralis* does consume pupae (personal observation).

Lepidopteran Egg Defenses

Eggs typically are immobile and usually not tended by the parents so the egg stage is a vulnerable part of the lepidopteran life cycle. Because eggs provide resources for developing larvae, they also are valuable nutritionally to predators and parasites. Eggs are a substantial biological investment by the parents, especially the female, so the successful progenitor provides the eggs some form of defense, concealment, and/or produces eggs in abundance to offset depredation. Egg defenses may be chemical, physical, and/or visual in nature; a combination of these strategies may be used for protection against different predators. Eggs that are conspicuous and deposited in clusters are likely to be protected by deterrents or toxins. Eggs deposited in clusters frequently have a longer incubation period (> 10 days), and so are more vulnerable to predation (Stamp 1980). Pandora moth eggs may or may not be conspicuous, depending on the type of predator and the oviposition site, but they usually are deposited in clusters and they do have a 40- to 50-day incubation period (Schmid and Bennett 1988).

Howard et al. (1982) noted that:

"rigorous examples of chemically protected eggs are curiously sparse...In the majority of investigations of insect defensive chemistry, the eggs are simply ignored. Even when they are

chemically analyzed, eggs are usually tested only for compounds previously identified from larvae or adults, and bioassays against appropriate predators or parasites are generally omitted. With these limitations, it is likely that the chemical attributes of insect eggs have been underrated."

Some investigators have examined lepidopteran egg defensive chemistry. Conner et al. (1981) elucidated an elegant plant-derived chemical egg defense in the bella moth [*Utetheisa ornatrix bella* (L.)]. Bella moth larvae feed on legumes containing pyrrolizidine alkaloids (PA's). The alkaloids are retained through the pupal stage and used by both male and female moths. The prenuptial alkaloid content of male moths was linearly proportional to the male pheromone titer, which in turn was proportional to the amount of alkaloid transferred to females during mating. Females incorporated this "nuptial gift" along with their own sequestered alkaloid defenses into a protective egg coating (Conner et al. 1981). The degree of bella moth egg predation by a coccinellid beetle was inversely proportionate to the amount of alkaloid in experimentally manipulated larval diets (Dussourd et al. 1988).

The persistence of host plant PA's from larval through egg stages also was reported by Aplin and Rothschild (1972) for *Arctia caja* L., and by Benn et al. (1978) for *Nyctemera annulata* Boisduval. Both species of arctiid larvae fed on *Senecio* spp. Eggs were tested for alkaloid content but were not bioassayed with predators. Brown (1984) reported that primarily males of several species of adult ithomiine butterflies collect PA's from various plant sources, transferring the alkaloids to females via spermatophores, presumably for egg protection.

The effectiveness of PA's as defensive agents toward a variety of predators was reviewed by Boppré (1986):

"In addition to chemical studies, feeding experiments on predators were carried out with some Lepidoptera, and they

demonstrated that insects are rejected due to stored PAs by predators such as spiders and thrushes [62-64]. Tests with a variety of animals (including mice, toads, frogs, lizards, titmice, ants, cockroaches, locusts, and various lepidopteran larvae) have also demonstrated that PA-contaminated food is rejected by taste [67]. Humans find PAs bitter, and when vertebrates taste PAs, signs of discomfort are often observed. PAs are thus definitely unpalatable to a very diverse range of animals and the mode of action of PAs as defensive chemicals is not their noxious long-term effects, but stimulation of taste receptors responsible for rejection behavior; PAs are predator deterrents. Since PAs do not cause instant harm, sensitivity to avoiding PA-containing food is relative, depending, for example, on the degree of hunger. It is also especially interesting that insects are generally deterred by PAs."

Physical or mechanical egg defenses include hard shells, scales and hairs. Hard shells make it difficult for parasitic wasps to insert their ovipositors. Eelsey (1972) found that the protective capability provided by the serosal cuticle of hawk moth [*Manduca sexta* (L.)] eggs increased with larval development. Hawk moth eggs are readily eaten by a hemipteran predator immediately after being laid. Predation begins to be inhibited 14 to 19 hours later, and is almost totally prevented midway through the incubation period. The cuticle effectively prevents stylet penetration by the time the pharate larvae are developed. The tortricid moth *Aesiocopa patulana* Walk. constructs a fairly elaborate "palisade of scales", a sort of fortress with sharp tips around the eggs for protection against crawling predators (Hinton 1981). Hairs from the parent moth also may be used for protection. For example, many Lepidoptera use a spumaline substance to glue scales or setae from the end of the abdomen to the egg-mass; lymantriids sometimes use poisonous setae (Hinton 1981). *Orygia pseudotsugata* covers its egg masses with a frothy gelatinous substance intermixed with body hairs (Furniss and Carolin 1977), and the gypsy moth (*Lymantria dispar* L.) embeds its eggs in a mass of hairs that presumably are distasteful to birds and small mammals (Campbell 1974). The "glue" used

to attach eggs to foliage may be a defense in itself if it prevents a forager such as an ant from carrying the egg off to be stored for later consumption. *Coloradia pandora* eggs are very difficult to dislodge from their substrate.

Visual egg defenses take the form of camouflage, mimicry, and aposematic (warning) coloration. Eggs may be hidden by blending with background colors, or with patterns such as the disruptive coloration of several lappet moth species [*Gastropacha quercifolia* (L.) and *Epicnaptera americana* (Harr.)] (Hinton 1981). Mimicry occurs when eggs are made to look like something else, such as an unacceptable food to the predator. For example, the eggs of a Mexican cossid moth (*Langsdorfia franckii* Hübn.) closely resemble the seeds of an umbellifer (Hinton 1981). Aposematic coloration is the strategic opposite of camouflage. The use of bright colors for protection requires that the predator be capable of learning to associate color with the presence of a toxic or distasteful substance on or in the egg. The egg-laying parent also must provide the egg with an aversive chemical that is effective against its particular predators, or use a color that mimics an unacceptable food. The green color of *C. pandora* eggs probably camouflages them when deposited on pine needles, as many are, from predators such as *S. lateralis* and foliage-gleaning birds. Eggs deposited on bark are conspicuous, however. *Coloradia pandora* eggs fluoresce under short-wave UV light and appear quite distinct from pine needle foliage (personal observation). This could be an aposematic signal to predators visually capable of detecting these wavelengths: possibly parasitic hymenoptera or some avian predators.

Defoliation Effects on Nutrient Cycling

Herbivore epidemics can result in substantial loss of chemicals from forest plants. Kimmons (1987) calculated that "a single complete defoliation

of a 36-year-old Douglas-fir stand would remove about 70 kg/ha of K, 82 kg/ha of Ca, 115 kg/ha of N, and 32 kg/ha of P, representing 28, 22, 32, and 43% of the total stand capital of these nutrients, respectively." The effect of complete *C. pandora* defoliation, in terms of total stand capital, would probably not be as extreme because, unlike most forest defoliators, only old foliage is consumed. Scarce nutrients in old foliage may be recycled into the stem and buds for new needle formation before the March through May larval feeding period. Also, in contrast to Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco], critical levels of foliar nitrogen and phosphorus are lower in *P. ponderosa*, a species capable of satisfying its nutritional needs on relatively infertile soils (Oliver and Ryker 1990).

Insect herbivore activity is said to stimulate nitrification, nitrogen-fixation, litter decomposition and/or plant root growth rates within the litter-soil complex via insect feces (frass), litterfall, and leachate (Schowalter 1981). The bulk of *C. pandora* frass is generated in late May in central Oregon, so it is likely to remain at the soil surface through a hot, dry summer before autumn rains and decomposition under snow provide a pulse of nutrients the following Spring. "Because frass contains higher concentrations of nutrients and lower concentrations of various decomposition-inhibiting organic chemicals (such as tannins) than does leaf litter, heavy inputs of frass to the forest floor will stimulate decomposition and mineralization of the litter" (Kimmons 1987). Rates of nutrient transfer from foliage to soil through frass additions to the forest floor are mediated by the hydrologic regime of the system. Mass transfer into the root system is also dependent on water availability. Whether or not this flux of nutrients actually occurs in central Oregon stands defoliated by *C. pandora* remains to be demonstrated.

Wickman et al. (1994) quantified the nutrient content of larval frass collected from fertilized and control plots in the central Oregon outbreak area. Control frass contained 0.595% N, 0.131% P, 0.042% S, and

0.267% K; available N was 94 ppm. These amounts are fairly low so a very large quantity of frass would have to be generated to add a significant amount of nutrients to the soil. This is a distinct possibility. *Coloradia pandora* larvae can generate frass in considerable quantity: "the constant dropping of excrement made a noise like a sleet storm..." (Englehardt 1924). Fowler and Walter (1985) mentioned that the Paiute people, in the process of collecting larvae for food, gauged numbers of larvae per tree by masses of frass at the base of the trees. However, the impact of frass production in the cycling of nutrients is confounded by the fact that the understory of these stands is dominated by the nitrogen-fixing shrub *Purshia tridentata*.

Stand Level Defoliation Effects

The current outbreak in central Oregon has affected ponderosa and lodgepole pine stands of varying structure, density, and age class. No particular stand type has been documented as being especially vulnerable. Saplings and old-growth pines alike are subject to defoliation (Schmid and Bennett 1988; Eglitis, personal communication). A characteristic pattern Schmid and Bennett (1988) noted was heavier defoliation on ridgetops and southerly aspects.

Coloradia pandora larvae complete their final instar and pupate before the current year's needles elongate in June. Thus, although 100% defoliation may occur in the Spring, the host pine is able to partially re-leaf in the same year. This timing of defoliation prior to budbreak, along with the alternate year pattern, tends to minimize the impact of *C. pandora* herbivory. Another notable herbivore, the pine butterfly (*Neophasia menapia* Felder and Felder), is considered one of the most destructive defoliators of *P. ponderosa* in the Northwest (Furniss and Carolin 1977). It has a one-year life cycle with a larval stage that causes defoliation of old and new needles each year,

sometimes resulting in primary mortality on a large scale. The pandora moth, in contrast, rarely kills its host but does affect tree growth and susceptibility to other mortality agents.

Bennett et al. (1987) estimated severe defoliation by *C. pandora* reduced volume growth of *P. ponderosa* 10% to 15%, but these figures do not account for missing annual rings caused by defoliation. With tree ring dating, Miller and Wagner (1989) found high percentages of missing rings associated with defoliated trees. Growth response to moderate and heavy defoliation was mixed. Reductions in basal area growth were not directly proportionate to defoliation intensity, or compensatory at lighter defoliation levels as Mattson and Addy's (1975) review suggests is typical.

Precipitation can be a significant environmental moderator of defoliation effects. Miller and Wagner (1989) considered water the most limiting factor affecting growth in *P. ponderosa* forests of northern Arizona. Differences in basal area growth response were attributed in part to variations in annual precipitation (Miller and Wagner 1989). Miller and Wagner (1989) reasoned, "Heavily defoliated trees that produce a lower volume of new foliage may require much less moisture than moderately defoliated trees with higher foliage volumes. Furthermore, trees with primarily new foliage may begin growth more rapidly when moisture levels increase in July."

The timing of precipitation is an important consideration. In northern Arizona, the melting snowpack from winter precipitation provides moisture early in the growing season. Additional precipitation typically occurs as summer rain in July and August. Significantly greater BA growth observed following heavy defoliation in a year of relatively high drought stress probably reflects compensatory growth (Miller and Wagner 1989). In effect, the heavily defoliated trees experienced less drought stress because their much reduced needle biomass limited evapotranspiration until later in the season when more water was available. The findings of Miller and Wagner's

(1989) study should not be directly extrapolated to central Oregon because the annual precipitation profile differs somewhat from that of northern Arizona. In central Oregon very little rainfall occurs from the peak of defoliation (May) until the following autumn (October-November). Most precipitation accumulates as snow. From 1985 through 1992, excepting 1987, the region received below average precipitation (Wickman et al. 1994), however, stand growth responses specific to this region have not been documented. The interaction of drought and *C. pandora* outbreak dynamics also has not been studied.

Defoliation may alter water availability to *P. ponderosa* by shifting the competitive balance between overstory and understory. *Purshia tridentata* comprises at least 90% of existing understory vegetation in *C. pandora* affected areas of central Oregon. Even though full canopy pine leaf area is low (relative to west-side forests), shrub density is lower in stands with higher canopy leaf area. Diameter growth of central Oregon *P. ponderosa* was 9 cm per decade where completely surrounded by understory shrubs, and 12 cm per decade with no competitive ground cover (Oliver and Ryker 1990). Defoliation approaching 100% by the month of May enables *P. tridentata* to claim more leaf area, as well as a higher than usual proportion of the winter snowmelt. This undoubtedly is a competitive disadvantage to the overstory in this water-restricted system, however, fairly rapid refoliation with new pine needles in June, and the alternate year cycle of defoliation probably mitigates these defoliation effects.

Stand level growth and mortality in *C. pandora* outbreak areas may be mediated by interactions with dwarf mistletoe (Wagner and Mathiasen 1985) and bark beetles (Patterson 1929, Schmid and Bennett 1988). Wagner and Mathiasen (1985) found that tree mortality in heavily defoliated sample areas of the Kaibab N.F. was associated with heavy Southwestern dwarf mistletoe (*Arceuthobium vaginatum* subsp. *cryptopodum* Engelm.) infection. Neither pandora moth nor dwarf mistletoe normally are primary mortality

agents, but the combination may be lethal and may ultimately reduce stand mistletoe infestation levels in unmanaged forests. The interaction of *C. pandora* defoliation and bark beetle attacks have not been studied directly, but Patterson (1929) maintained that a bark beetle (*Dendroctonus brevicomis* LeConte and *D. ponderosae* Hopkins) infestation developed 5 years into the pandora moth outbreak of the 1920's in central Oregon. He reported a 15-fold increase over pre-outbreak conditions in numbers of trees killed by *Dendroctonus* spp. Schmid and Bennett (1988) however, stated that bark beetle-caused mortality was not significantly different during or after the pandora moth outbreak on the Kaibab N.F. in the early 1980's. They surmised that above average summer rains moderated the "weakened state" of the defoliated *P. ponderosa* thus reducing their susceptibility to beetle attack.

Coloradia pandora defoliation affects the visual quality of a stand, sometimes drastically. Totally defoliated trees appear dead for several months (May-July) causing concern among landowners and tourists. The current outbreak on the Deschutes N.F. involves several visitor centers, recreational areas and a heavily travelled highway but effective control options are limited for *C. pandora*. Spray application of acephate during early larval instars may help protect high value trees (Schmid and Bennett 1988) but is not an option on federal lands. Prescribed burning as a tool for controlling *C. pandora* pupal densities was determined to be largely ineffective unless a substantial amount of litter is uniformly distributed throughout the stand (Schmid et al. 1981, Miller and Wagner 1984).

Fertilization also has been examined as a method of reducing the impact of *C. pandora* defoliation (Wickman et al. 1994). Wickman et al. (1994) applied a large single dose of urea on a young stand of *P. ponderosa* in the Deschutes N.F. and followed the growth response through 2 generations of *C. pandora* defoliation. They found significantly higher radial growth on fertilized plots but similar visual defoliation ratings on treated and

control plots. Larval weights dropped significantly in fertilized plots for the first generation following fertilization, but further data collection was aborted because a local population collapse occurred on both treated and control plots in the second generation. Pupal weights were unaffected by the fertilization. Wickman et al. (1994) concluded that, aside from the predictable boost to stem growth, fertilization effects on host tree chemical defenses and *C. pandora* populations remain unclear.

MATERIALS AND METHODS

Study Area Description

The general area of the Descutes N.F. affected by *C. pandora* is within the pumice region of the High Plains Province of central Oregon. The loose pumice soils of this region tend to support relatively low total plant cover and few herbaceous plants. The edaphic plant communities are *Pinus ponderosa*/*Purshia tridentata* (PUTR) with a minor component of *Pinus contorta* in the canopy and *Arctostaphylos patula* (ARPA) and *Ceanothus velutinus* (CEVE) in the understory. With higher elevation and moisture, CEVE replaces ARPA; with lower elevation and moisture, the sclerophyllous shrubs disappear (Franklin and Dyrness 1988). Much of the pine overstory in the region was selectively harvested (high-graded) in the late 1800's and clearcut earlier this century. A history of fire suppression in the region has favored the proliferation of smaller pine regeneration and of PUTR over ARPA and CEVE in the understory.

The 1992 U.S. Forest Service aerial defoliation survey (Appendix A) showed the heaviest levels of *C. pandora* defoliation near the Lava Butte Geological Area on the Fort Rock Ranger District south of Bend, Oregon. *Coloradia pandora* moths, pupae and *S. lateralis* were sampled from this area.

Spermophilus lateralis Trap Sites and Care

A total of 33 *S. lateralis* were live-trapped in the Fort Rock Ranger District from eight sites separated by at least 0.8 km. The eight 1993 trap sites were in areas classified as medium or heavily defoliated in 1992

(Appendix A). These sites were near the epicenter of the outbreak so the ground squirrels were likely to have experience with *C. pandora* as a food item.

Eighteen *S. lateralis* were trapped on 30 June and 1 July 1993 from Sites A-D: 5 males from Site A (T19S,R11E,S35); 4 males from Site B (T20S,R11E,S3); 4 males from Site C (T19S,R11E,S13); and, 5 males from Site D (T19S,R12E,S29). These individuals were tested in the whole moth, chemical fractions, and initial physical fractions bioassays. They all were at least yearlings when trapped.

Fifteen *S. lateralis* were trapped on 27-30 October 1993 from Sites E-H: 1 male(M) and 2 females (F) from Site E (T19S,R11E,S26); 3M, 1F from Site F (T19S,R11E,S16); 1M, 3F from Site G (T19S,R11E,S33); and, 1M, 3F from Site H (T20S,R11E,S5). These individuals were tested in the physical fractions follow-up bioassay. Because of the time of year, mostly juvenile males and females were trapped; older individuals with sufficient fat reserves had begun hibernation.

The *S. lateralis* were housed in individual cages at 21°C with a 12:12 h light:dark photoperiod at Oregon State University's Laboratory Animal Resources facility. Water, sunflower seeds, Harlan Teklad rodent diet, and occasional fruit or peanuts were provided *ad libitum* when the animals were not engaged in feeding trials. The animals were transferred to separate cages (32 x 36 x 23 cm) with no bedding, food (other than testants), or water for all of the bioassays. Pre-trial periods of food withdrawal had little effect in minimizing *S. lateralis* feeding variability and were not included in the bioassay procedures.

Bioassay of Whole Moths

Male and female moths were reared in the lab from pupae collected in May of 1993 at T20S, R10E, S1 in the Deschutes N.F. Female moths were

unmated and had not begun to oviposit. All moths were within 3 days of eclosion.

On one afternoon each ground squirrel was offered 5 preweighed, live male moths. After 2.5 h the moth remains were collected and reweighed. Three days later each subject was offered 4 preweighed, live female moths. After 2.0 h the moth remains were collected and reweighed. An additional 30 gravid moths were weighed then dissected to estimate the weight percent of eggs in live females. A randomized bioassay of male and female moths was not conducted because most male moths emerged several days earlier than most females. Therefore, sufficient numbers of similarly-aged male and female moths were not available concurrently.

Bioassay of Chemical Egg Fractions

Egg Source

Adult moths were light trapped from three sites on the Deschutes N.F. (T19S,R11E,S23; T20S,R10E,S1 and T21S,R09E,S23) on 18 July 1993. After transport to the Corvallis Forestry Sciences Laboratory, females and males in good condition were held in screen cages in a rearing room at 25°C with a 16L:8D photoperiod while pairs mated and females laid eggs on the screen. A gender ratio in each cage of at least 2 males per female ensured most eggs were fertilized. After 5 days, eggs were collected from the cages, sifted clean and weighed (162.0 g). Fertilized eggs were used to ensure that the potential transfer of defensive chemicals through copulation would not be overlooked, even though undeposited eggs of unfertilized females were not consumed in the whole moth feeding tests. Deposited, rather than dissected, eggs were used because dissection of sufficient

quantities of eggs was not practicable, and because bioassays of deposited eggs more directly test potential consumption of the egg stage in the field.

Egg Fraction Preparation

Hexane surface extraction: eggs were swirled in 5 ml hexane per gram of egg for 5 min, the solvent was decanted through sharkskin filter paper, and the eggs rinsed with 2 ml hexane per gram of egg. The eggs were dried overnight in a fume hood, and the hexane extract roto-evaporated dry in a 30°C water bath.

Methanol extraction: the hexane extracted whole eggs were macerated in a blender in 5 ml methanol per g egg. Egg shells were separated from the extract by rinsing through glass wool with an additional 2 ml methanol per g egg. The filtrate was centrifuged at 3000 rpm and the supernatant was roto-evaporated in a 30°C water bath until the methanol had vaporized. Insoluble egg tissue including some egg shells (hereafter, egg residue) was recovered non-quantitatively from the glass wool filter and centrifuge tubes, air dried, and ground with mortar and pestle.

Water/dichloromethane fractionation: the evaporated methanol extract was redissolved in 2.5 mL water and 2.5 mL dichloromethane per g egg, swirled vigorously, then allowed to settle in a separatory funnel. After 20 min the dichloromethane and water layers were separated and roto-evaporated at 30°C until almost dry.

Feed Preparation

Solvent fractions: the hexane, dichloromethane, and water fractions were applied topically to shelled, unroasted sunflower seeds. The evaporated fractions were redissolved in 0.5 mL solvent (hexane,

dichloromethane, or methanol in the case of the water fraction) per g egg (81 mL). These solutions were sprayed onto 162 g seeds (1 g seed per g of egg extracted) with Preval nebulizers in a fume hood. The seeds were coated evenly by drying and stirring the seeds between 6-10 repeated spray applications. The coated seeds were dried 2.5 h under rapid air flow to dissipate residual solvent vapors. Control seeds were prepared to accompany each treatment by spraying 162 g of seeds with solvent only. Except for minimal loss of extract to overspray, this procedure resulted in application of extracted egg constituents at concentrations equivalent to that of whole eggs.

Egg residue: the dried egg residue was incorporated into gelatinous feed blocks for bioassay. Seventy percent of the total residue recovered (22.0 g) was used in treatment blocks prepared by volume as: 6 parts egg residue: 1 part oats: 1 part cracked corn: 1 part creamy peanut butter: 1 part unflavored gelatin: 4 parts water. Control feed blocks were prepared by increasing the proportions of oats and cracked corn to 4 parts oats and 4 parts cracked corn. A double batch of control feed was prepared to provide dehydration controls for the bioassay.

Feeding Test Procedure

Solvent fractions: the hexane, dichloromethane, and water egg fractions were bioassayed in a series of two-choice tests during which each ground squirrel was given one of the 3 egg fractions and the corresponding solvent control per day. Treatments were allocated randomly with the constraint that each squirrel receive each solvent extract once, thus the bioassay was conducted over 3 days.

About 9 g of treated and 9 g of control seed was weighed into feed cups taped to opposite sides of individual bioassay cages. Relative positions of treatment and control cups were selected randomly each day. The 18

ground squirrels were given 6 h to feed at will. At the end of the test any spilled seed was returned to the nearest feed cup and its contents reweighed.

Egg residue: the egg residue bioassay was a one day two-choice test. Treatment and control feed blocks were weighed into separate cups taped to randomly chosen opposite sides of bioassay cages. Initial feed block weights ranged from 4.8-8.1 g. Eighteen additional control feed blocks were weighed into cups and placed in empty cages for the duration of the 6 h feeding test. Remaining feed blocks and evaporation control blocks were reweighed immediately following the test, and calculations of feed block consumption were adjusted for evaporation.

Analysis

Treatment response was expressed as a preference index (PI) equal to control minus treated seed consumption divided by total consumption for each ground squirrel. These indices range from -1 (when only treatment is consumed) to +1 (when only control is consumed). The PI is 0 when treatment and control consumption are equal, which is convenient for testing the null hypothesis. Since the bioassay was conducted over 3 consecutive days, a correlation analysis of PI by day was done to examine the potential influence of prior experience. The analysis suggested day was not generally correlated with consumption, however, the power of the analysis was low because of small sample sizes ($N = 18$). Also, scatterplots of PI versus day of bioassay and order of presentation of treatments displayed no trends in consumption with time. Consequently, observations were assumed to be independent.

A split-plot analysis of variance (ANOVA) with trap site as the whole plot factor, ground squirrels as replicates nested within trap sites, and egg fractions as subplot treatments was used to test the hypothesis that PI's did

not differ among treatments. Least squares means of effects with p-values less than 0.10 were tested against the null hypothesis that treatment and control consumption were equal (i.e. $PI = 0$) using Bonferroni adjusted critical p-values given $\alpha = 0.10$. Analyses were conducted using the SAS General Linear Models Procedure (Proc GLM). The ANOVA was weighted by total consumption to ensure that PI 's from squirrels that consumed relatively more food were more influential than PI 's from those that consumed relatively less food. Residuals were normal but heteroscedastic, and angular transformations failed to correct the nonconstant variance, so a separate ANOVA on ranked PI 's was conducted to verify p-values. The egg residue bioassay data was analyzed similarly, except that the experimental design was reduced to a one-way ANOVA with trap site as the main factor.

Initial Bioassay of Physical Egg Fractions

Egg Source

Adult moths were light trapped during the nights of 2 Aug and 3 Aug 1993 from the same locations and by the same procedure as for the chemical egg fractions bioassay, except that fertilized eggs were collected from screen cages after allowing 9 days for mating and egg-laying. Egg yield was 141.0 g.

Egg Fraction Preparation

Four physically distinct egg fractions were prepared: whole eggs, "whole" egg shells, ground egg shells, and egg contents. Nearly whole shells were separated from egg contents by breaking 36.0 g of whole eggs open with a mortar and pestle. Contents were rinsed from the shells with

excess methanol through a screen filter. The suspension of egg contents in methanol was roto-evaporated to dryness in a 30°C water bath. The "whole" egg shells, which remained fairly intact and round, were dried in a fume hood, yielding about 10% of the initial whole egg weight. The ground egg shell treatment was prepared by grinding "whole" egg shells from an additional 36.0 g of eggs in a Wiley mill to pass a 40-mesh screen.

Feed Preparation

The egg fractions were incorporated into gelatinous feed blocks for the initial bioassay of the physical egg fractions. For each treatment, nine grams of unflavored gelatin and 2.7 g peanut butter were dissolved in 90 mL boiling water, and the mixture was cooled until it began to thicken. Then, either 36.0 g of whole eggs, or "whole" shells, ground shells, or egg contents from an equivalent weight of whole eggs, were suspended in the peanut butter flavored gelatin. The mixtures were poured into 11 x 11-cm plastic molds, allowed to solidify, and cut into 18 blocks. A double batch of control feed blocks were prepared by the same procedure to provide evaporation controls for the bioassay. The cut blocks were air dried 5 h then refrigerated in sealed containers until needed.

Feeding Test Procedure

The initial physical egg fractions bioassay was a series of two-choice tests where each ground squirrel was given a control feed block and either a whole egg, "whole" egg shell, or egg contents treatment block per day. Treatments were allocated randomly with the constraint that each squirrel receive each treatment once. The ground egg shell treatment was prepared and bioassayed 10 days after the initial bioassay.

One control and one treatment feed block (2.5-5.5 g each) were weighed into cups taped to opposite sides of individual bioassay cages. Relative positions of treatment and control were selected randomly each day. Eighteen additional control feed blocks were weighed into cups and placed in empty cages for the duration of the feeding test. The 18 *S. lateralis* were allowed 4 h to feed at will, then remaining feed blocks and evaporation control blocks were reweighed immediately following the test, and calculations of feed block consumption were adjusted for evaporation.

Analysis

Consumption was expressed as a preference index (PI) for each treatment/control pair as defined previously. The ground egg shell data were combined with the initial bioassay data for the other 3 physical egg fractions and analyzed as described for the bioassay of the chemical egg fractions.

Follow-up Bioassay of Physical Egg Fractions

Egg Source

Moths or eggs were no longer available from the field for the November follow-up bioassay so moths were reared from pupae collected 14 May 1993 from a heavily defoliated area of the Deschutes N.F. (T20S,R10E,S1). Following collection, pupae were stored at 5°C for 17-18 weeks and incubated at 25°C for 4 weeks prior to eclosion. Females were isolated in large screen cages in a 25°C rearing room with a 16L:8D photoperiod until a sufficient quantity (> 60 g) of unfertilized eggs were deposited.

Egg Fraction Preparation

Separation of the 4 physical egg fractions (whole eggs, "whole" egg shells, ground egg shells, and egg contents) was the same as for the initial bioassay of the physical egg fractions.

Feed Preparation

Feed block preparation for the follow-up physical egg fractions bioassay differed from the initial bioassay in that 21.7 g of whole, unfertilized eggs were suspended in 9.0 g unflavored gelatin, 3.0 g peanut butter, and 3.0 g sunflower butter dissolved in 56.25 mL water. The shell and egg contents treatments also were prepared from 21.7 g of whole eggs for each treatment. Consumption was calculated on a dry weight basis so evaporation controls were not prepared.

Feeding Test Procedure

The follow-up bioassay procedure was like the initial bioassay except that the 4 treatments were tested in 4 consecutive days; the treatment and control feed blocks were presented together at the center of the cage; and the duration of the choice tests was variable. Each ground squirrel was allowed to feed until at least 1/3 of either the control or treatment feed blocks was eaten. Consumption was monitored hourly and a maximum of 10 h was allowed before terminating the test.

Instead of maintaining a separate set of controls to estimate weight loss caused by water evaporation, a slice of each feed block was sampled just prior to the initial weight measurement. The samples were weighed, oven dried at 105°C for 16 h, and reweighed. Sample water contents were

used to calculate the initial feed block dry weights. Final feed block dry weights were measured by oven drying remains of the feed blocks following the bioassay, and consumption was quantified as the difference from the initial dry weight estimate.

Analysis

The follow-up bioassay dataset, consisting of all 4 physical egg fraction treatments, was analyzed by the methods described for the bioassay of the chemical egg fractions. One female *S. lateralis* from Site H consumed zero treatment and control in 3 of the 4 choice tests and so was dropped from the analysis.

Nutritional Analyses of Moths and Eggs

Sample Preparation

Male and female moths, whole eggs, egg contents, and *P. tridentata* seed were submitted for analysis. Moths were reared in the lab from pupae collected 14 May 1993. Ten female and 17 male moths were frozen 24 hrs post-emergence. Wings were removed prior to analysis, but eggs were not removed from the female moths. Unfertilized eggs were collected from screen cages with isolated female moths reared in the lab to produce eggs for the follow-up physical egg fractions bioassay. The egg contents were separated from shells by the method described for the physical egg fractions bioassay. Semi-dried egg contents were analyzed and the concentrations corrected using the actual water content measured in whole eggs. *Purshia tridentata* seeds were collected 2 years prior to analysis in Idaho and were stored dry in the lab.

Analysis

The samples were analyzed by the Forage Analytical Service of the OSU Department of Agricultural Chemistry. Crude fat content was quantified by ether extraction of dried sample material (direct method) [Association of Official Analytical Chemists (AOAC) 1990]. Crude protein content was quantified using an automated Brinkman Buchi-Kjeldahl Nitrogen Analyzing System (AOAC 1990). Ash content was calculated by weight after combustion (AOAC 1990). Carbohydrates were estimated by subtraction.

RESULTS AND DISCUSSION

Only 11 of 90 male moths offered in the whole moth bioassay remained intact. Consumption of male moths by individual *S. lateralis* ranged from 25% to 84% of initial moth weights with an overall mean of 67.2% (SD = 17.2%). Consumption of female moths ranged from 3% to 46% of initial moth weights with an overall mean of 30.6% (SD = 12.4%) (Figure 1). Several *S. lateralis* left only eggs and wings. The weight percent

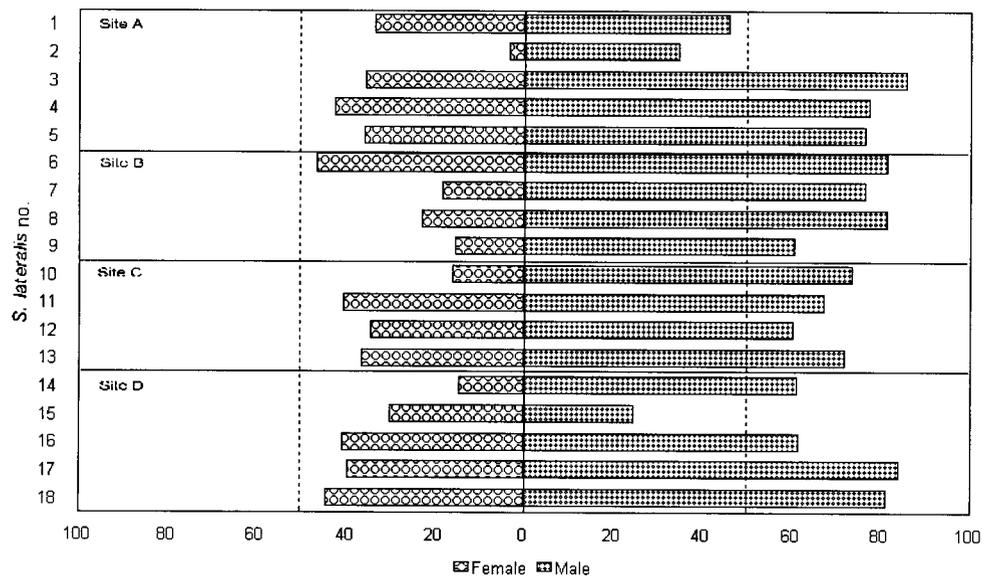


Figure 1. Percent of total moth weight consumed in whole moth feeding tests.

of eggs dissected from 30 gravid females was 39.5% (SD = 6.7%). The difference in consumption of male versus female moths (27.7%) was slightly less than the mean weight percent of eggs in the 30 gravid females. Generally, the ground squirrels readily ate entire male moth abdomens, leaving wings, eyes, antennae, legs and often the head-thorax section. All

18 *S. lateralis* left 2.6 to 3.6 g of eggs constituting 49.7% to 73.7% of the remaining female moth weights. These results provided strong evidence that *S. lateralis* does not eat *C. pandora* eggs, and in fact, rejects eggs in hand, while consuming female pandora moths.

Results of the nutritional analyses for female and male moths, whole eggs, egg contents, and *P. tridentata* seeds are presented for comparison in Table 1. The fat and protein content of *C. pandora* eggs is high enough that a ground squirrel eating a female moth should not reject the eggs from the abdomen for lack of caloric value. In fact, the percentages of fat and protein in egg contents and female moths (including eggs) are nearly the same, so from a nutritional standpoint, they ought to be equally valuable food items. Egg tissue constitutes about 40% of the female moth weight so discarding this amount of a potential food item is a waste of foraging energy. Also, the high moisture content (~50-65%) of pandora moths and eggs should be quite valuable as they are abundant in the field from mid-July through mid-September, the driest part of the year.

Table 1. Nutritional analyses of *C. pandora* eggs and moths, and *P. tridentata* seeds.

Sample	Moisture %	Fat %	Protein %	Carbohydrate %	Ash %
whole eggs	65	7	22	2	4
egg contents	65	13	18	2	2
female moths	66	11	21	2	1
male moths	48	32	15	4	1
bitterbrush seeds	6	15	14	63	2

Personal observations of *S. lateralis* feeding stumps indicate *P. tridentata* seeds, pine cone seeds, and *C. pandora* pupae and moths are

primary foods. *Purshia tridentata* seeds are a very abundant and sought after food in the study area. Nutritionally, the seeds complement *C. pandora* eggs and moths by being very high in carbohydrates but very low in water content. Hypogeous fungi and grubs, other insects and carrion complete *S. lateralis*' diet (O'Neil 1992). There is no reason to suspect that an omnivorous rodent such as *S. lateralis* would not utilize an undefended food resource such as a cluster of large insect eggs. Pandora moth eggs may be somewhat camouflaged on pine foliage but lower to mid-crown branches are accessible to *S. lateralis*. And the adeptness with which this ground squirrel forages for pupae and fungi underground indicates an ability to disclose hidden food items. The fact that *S. lateralis* does not consume eggs encountered in gravid female moths, even though the eggs are nutritionally comparable to other food items, strongly suggests the eggs possess some defensive quality.

The bioassay of the chemical egg fractions was designed to determine whether a chemically-based defense mechanism was operative in defending *C. pandora* eggs from *S. lateralis* depredation. Bioassay results indicated this is not the case. There were no significant differences in PI among chemical egg fractions or among trap sites, however, the p-value for the subplot interaction SITE*FRACTION was small enough ($p = 0.064$) (Table 2) to warrant examination of the least squares (LS) means (Figure 2). Treatment and control consumption were found to be unequal only for the Site D *S. lateralis* response to the water egg fraction ($p = 0.009$, Bonferroni critical p-value = 0.008). These animals ate significantly more of the water egg fraction than the control feed (LS mean PI = -0.73)(Figure 2). None of the group means indicated significantly reduced consumption of any of the egg chemical fractions.

Table 2. ANOVA table of consumption preference index (PI) for chemical egg fractions bioassay.

Source	df	MS	p
Site	3	0.577	0.553
error a	14	0.793	
Fraction	2	0.274	0.722
Fraction x site	6	1.898	0.064
error b	28	0.829	

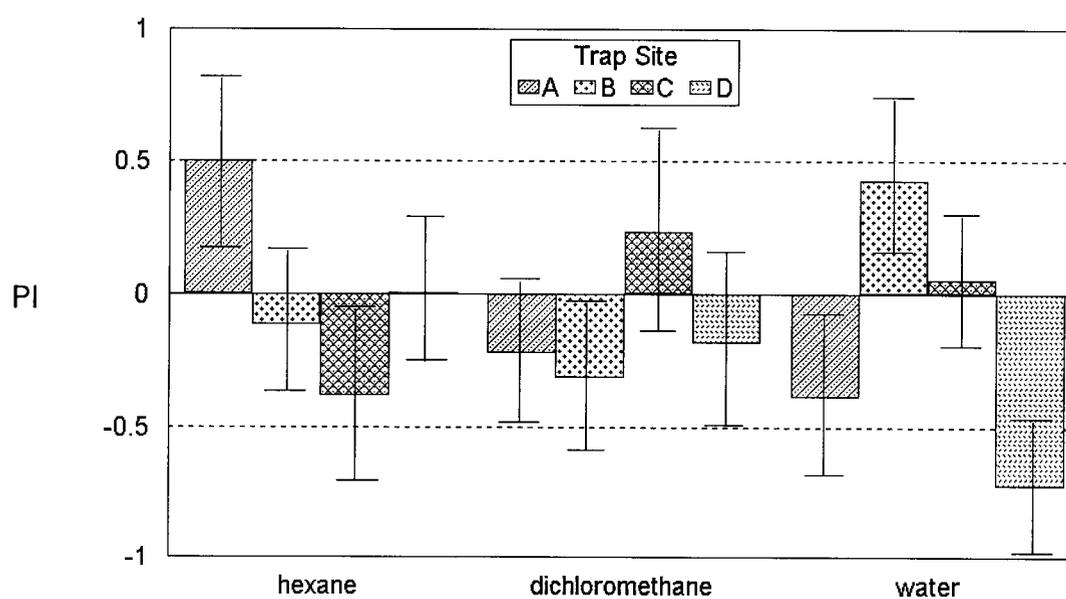


Figure 2. LS mean preference indices for chemical egg fractions by site. Positive PI values indicate higher control consumption; negative PI values indicate higher treatment consumption. Bars indicate \pm standard error of the LS mean.

A power analysis was conducted to determine how large a difference in consumption was necessary to detect treatment effects, given the number of ground squirrels available and the observed variance. This analysis indicated that treatment consumption would have to be less than 25% of control consumption in order to detect a significant reduction in treatment consumption, given $\alpha = 0.10$ and $\beta = 0.20$. This corresponds to a mean PI greater than 0.60. Conversely, mean PI's less than -0.60 indicate significant preference for the treatment over the control. Only the Site D *S. lateralis* response to the water egg fraction met this criterion.

The residue of egg tissue left after solvent extraction was bioassayed to ensure that any non-extracted chemical constituents were not overlooked. The one-way ANOVA of the egg residue indicated a highly significant difference among trap sites ($p = 0.001$). Subjects from Sites A and C ate more of the control than the treatment, while subjects from Site D ate more of the egg residue treatment ($p = 0.017, 0.050$ and 0.003 , respectively, Bonferroni critical p -value = 0.025) (Figure 3). This result indicated that different subpopulations of *S. lateralis* may have different feeding preferences, but it does not explain the aversion toward consumption of *C. pandora* eggs that appeared consistent among trap sites in the whole moth bioassay. The lack of a consistent inhibitory response to any of the chemical egg fractions or the egg tissue residue leads to the conclusion that *C. pandora* eggs are not chemically defended with respect to consumption by *S. lateralis*. Therefore, the physical attributes of *C. pandora* eggs were examined to provide an explanation for the aversive behavior.

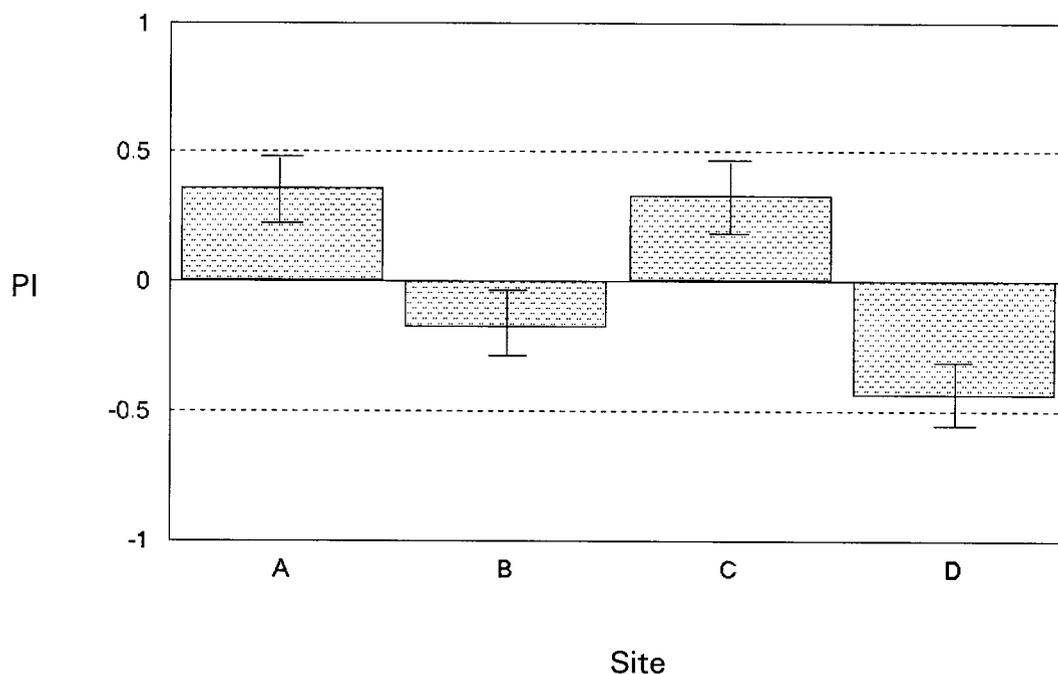


Figure 3. LS mean preference indices for the egg residue feeding test. Positive PI values indicate control consumption higher than treatment consumption. Negative PI values indicate relatively greater treatment consumption. Bars indicate \pm standard error of the LS mean.

The bioassays of the physical egg fractions were designed to elucidate potential feeding inhibitions caused by the hard egg shell. Four treatments were prepared that separated the egg shell from the egg contents to varying degrees: whole eggs, "whole" egg shells, ground egg shells, and egg contents. These treatments were bioassayed initially with the 18 *S. lateralis* that were tested in the whole moth and chemical egg fractions bioassays, then again with a novel group of 15 younger *S. lateralis* from different trap sites.

The egg physical fractions main effect was highly significant ($p=0.0004$) in the initial bioassay and marginally significant in the follow-up bioassay ($p=0.061$). Differences among sites were not significant (Tables 3 and 4). LS mean PI's for the initial bioassay treatments were significantly

greater than zero for the whole egg and ground egg shell fractions ($p=0.001$ and 0.029 , respectively) and marginally greater than zero for the "whole" egg shell treatment ($p=0.078$, Bonferroni critical p -value = 0.025) (Figure 4). LS mean PI's for the same treatments in the follow-up bioassay were significantly greater than zero for the whole egg and "whole" egg shell fractions ($p=0.012$ and 0.002 , respectively) (Figure 5). Preference indices for the ground egg shell fraction in the follow-up bioassay, and for the egg contents in both initial and follow-up bioassays, were not statistically different from zero.

Table 3. ANOVA table of consumption preference index (PI) for initial physical egg fractions bioassay.

Source	df	MS	p
Site	3	0.186	0.885
error a	14	0.866	
Fraction	3	3.950	0.0004
Fraction x Site	9	0.842	0.136
error b	36	0.508	

Table 4. ANOVA table of consumption preference index (PI) for follow-up physical egg fractions bioassay.

Source	df	MS	p
Site	3	0.142	0.325
error a	10	0.109	
Fraction	3	0.384	0.061
Fraction x Site	9	0.174	0.292
error b	21	0.132	

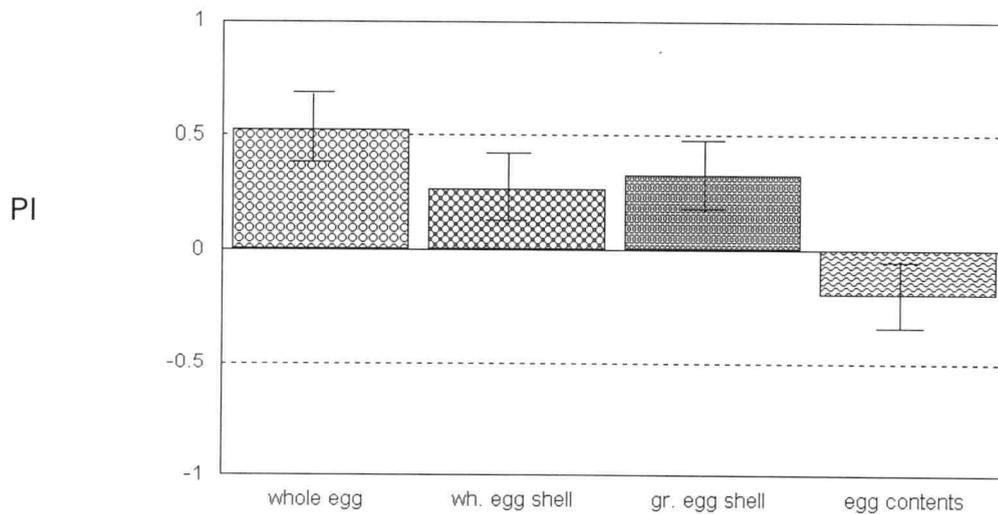


Figure 4. LS mean preference indices for initial bioassay of physical egg fractions. Positive PI values indicate relatively higher control consumption; negative PI values indicate relatively higher treatment consumption. Bars indicate \pm standard error of the LS mean.

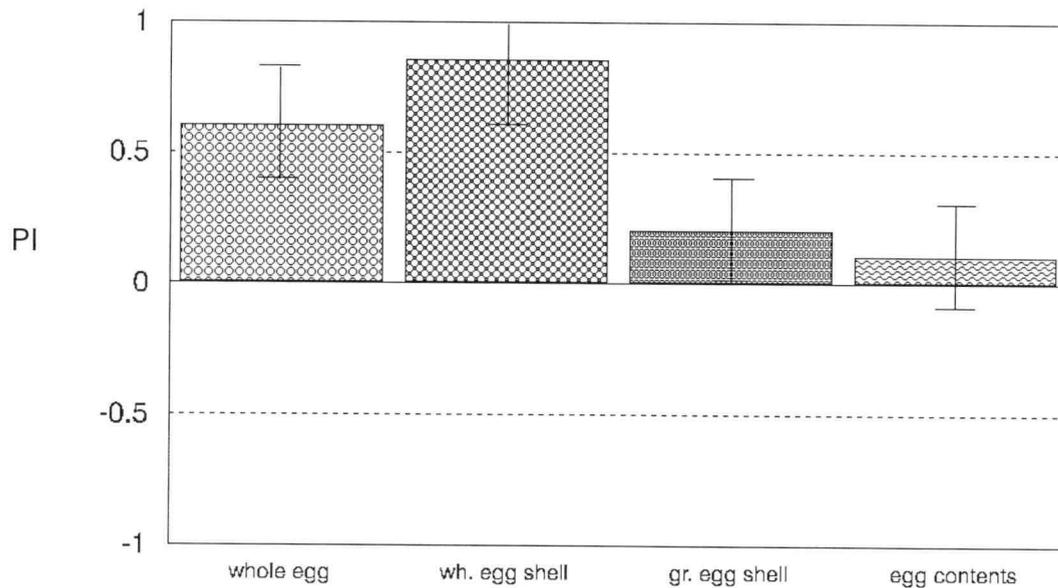


Figure 5. LS mean preference indices for follow-up bioassay of physical egg fractions. Positive PI values indicate control consumption greater than treatment consumption. Negative PI values indicate relatively higher treatment consumption. Bars indicate \pm standard error of the LS mean.

The initial and follow-up bioassays of the physical egg fractions clearly show that *S. lateralis* select against whole eggs and egg shells. Consumption of the whole egg treatment was about 1/3 of control consumption for *S. lateralis* from all 8 trap sites. Consumption of "whole" egg shells was lower than controls for *S. lateralis* from all sites, and especially so for the juveniles from sites E-H. The weaker response to the "whole" egg shell treatment in the initial bioassay may be a function of age, since *S. lateralis* trapped from sites A-D for the initial bioassay tended to be older than those trapped from sites E-H. Responses to the ground egg shell treatments varied between the initial and follow-up bioassays in that aversion toward the ground egg shell treatment was statistically significant for *S. lateralis* from trap sites A-D, but not for those from trap sites E-H. The ground egg shell treatment had a "gritty" texture that apparently was

less acceptable to the older ground squirrels. Consumption of the egg contents was not significantly different from that of controls, however the subjects in the initial bioassay showed a slight preference for the egg contents. This again, may be an age or learning effect. Individual *S. lateralis* may learn to accept the egg shell, but in general this species seems to regard the egg shell as unpalatable enough to deter consumption of *C. pandora* eggs.

It is probable that the hard shell of *C. pandora* eggs did not evolve specifically for defense against consumption by *S. lateralis*, but rather as a primary defense against penetration by parasitic wasps or flies, against desiccation during a long incubation period in a dry environment, and/or against consumption by small foliage-gleaning birds and foraging ants. These mortality agents are more likely to exert a limiting influence on *C. pandora* egg abundance. With respect to *S. lateralis* depredation, the hard egg shell probably ought to be considered a secondary or incidental defense mechanism. The eggs that *S. lateralis* discard while consuming gravid female moths are not viable; they become fertilized only as the egg is passed through the oviduct during deposition. It is possible that *S. lateralis* learns to avoid deposited eggs through predatory encounters with gravid female moths because the moth stage, often in great abundance, precedes the egg stage. Consumption of one gravid female moth is equivalent to preventing deposition of 100 eggs. Therefore, eggs carried by female moths would be defended more efficiently by a mechanism that prevented depredation of gravid females.

Spermophilus lateralis consumes the pupal and moth stages of *C. pandora*, but does not consume the egg and probably not the larval stages (personal observation). Prior to this study, a 2-day choice test with 10 *S. lateralis* resulted in an average of 6.5 pupae eaten or damaged per squirrel per day. With no alternate food available, the 10 ground squirrels ate or damaged 15.9 pupae in one day, on average. A conservative estimate of

the predation impact of an individual *S. lateralis* would be 1170 pupae per year, based on active foraging 6 months of the year at a rate of 6.5 pupae per day. Also, the whole moth feeding tests indicated *S. lateralis* is capable of destroying at least 5 adult moths in several hours.

About 14 months of *C. pandora*'s 2-year life cycle is spent in the vulnerable pupal and moth stages. Conventional biocontrol theory maintains that vertebrate predation on insect pests can limit insect populations to low levels until some external factor(s) reduce the vertebrate population or otherwise allow the insect population to "escape" (Campbell 1974).

Spermophilus lateralis is not highly specialized to a particular habitat type, it is fairly short-lived (less than 10 years under predation) and has good reproductive and dispersal capacity. Therefore, local populations can be responsive to fluctuations of resources. Published density estimates for *S. lateralis* range from 0.4 to 1.2 individuals per acre (O'Neil 1992). I have noted higher densities where food is especially abundant, e.g. tourist parking lots. Given this species' appetite for *C. pandora* pupae and moths, its foraging abilities, and its tolerance of high densities, it probably functions as a control agent on endemic *C. pandora* populations.

The influence of a natural enemy in an ecosystem is a function of the predator's ability to restrict prey population levels. With respect to vertebrate predation on insects, this influence is often limited by the predator's ability to consume a sufficiently large proportion of the insect population. Vertebrate predation efficiency may be restricted by foraging abilities (e.g. prey detection, energetics, locomotion) and by relatively low predator densities caused by territorial behaviors or environmental limitations (e.g. nest site availability, alternate food availability). *Spermophilus lateralis* seems to be a thorough forager that cohabits with conspecifics and *Tamias* spp. at fairly high densities where sufficient food is available. For example, it is difficult to find an intact bitterbrush seed in areas with high rodent populations. Therefore, if *S. lateralis* were not deterred by *C. pandora* egg

defenses, it potentially could be more influential as a natural enemy of *C. pandora*.

Spermophilus lateralis occupy a variety of forest types along the Cascade Range. In addition to providing food, certain components of these forested environments provide cover for hiding, sleeping, hibernation, thermoregulation, and reproduction. A required habitat component is ground cover such as rocks, shrubs, logs and/or stumps (O'Neil 1992). These structures probably function as burrow supports in the loose pumice soils, and as protection against digging predators such as coyotes. Heavy litter layers and grass cover may discourage burrowing. Taller structures (rocks, stumps, rootwads) are utilized for observation and feeding posts (personal observation). *Spermophilus lateralis* will also feed in shrubs and trees (O'Neil 1992) and is a good climber (personal observation). Degradation in quality or quantity of these habitat components may limit *S. lateralis* population levels, thereby decreasing the influence of *S. lateralis* predation on *C. pandora* populations.

SUMMARY AND CONCLUSIONS

Captive feeding trials with *S. lateralis* verified field observations that members of this rodent species feed on adult *C. pandora* moths but eschew consumption of eggs from gravid females. Therefore, *Spermophilus lateralis* are unlikely to forage for *C. pandora* eggs under natural conditions, despite the nutritional value of eggs in terms of fat and protein content. The bioassay series of chemical and physical fractions of *C. pandora* eggs demonstrates that pandora moths do not produce a chemical defense for their eggs that is effective against *S. lateralis*, but that the egg shell inhibits consumption. The repeated bioassay of the physical egg fractions confirms the initial results and extends the scope of inference to 8 trap sites, and juvenile as well as adult-aged *S. lateralis*.

Chemically-based defenses are often invoked to explain vertebrate avoidance behaviors but physical, behavioral and/or combined mechanisms should be considered as well. With respect to *S. lateralis* predation on *C. pandora* in central Oregon, the egg stage is protected by its hard shell. This defense may be of secondary importance relative to other functions of the hard shell, such as protection against desiccation or other more aggressive egg predators. Also, the results of this study do not preclude the possibility that *C. pandora* eggs are chemically defended against potential egg predators other than *S. lateralis*.

As a natural enemy, *S. lateralis* preys on the pupal and moth stages of *C. pandora*, but not the eggs and probably not the larval stage. Forest resource managers should consider preserving and/or providing structural components that meet the habitat requirements of *S. lateralis* in order to maximize their value as predators.

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APPENDICES

Appendix A
Map of Defoliation and Trap Site Locations

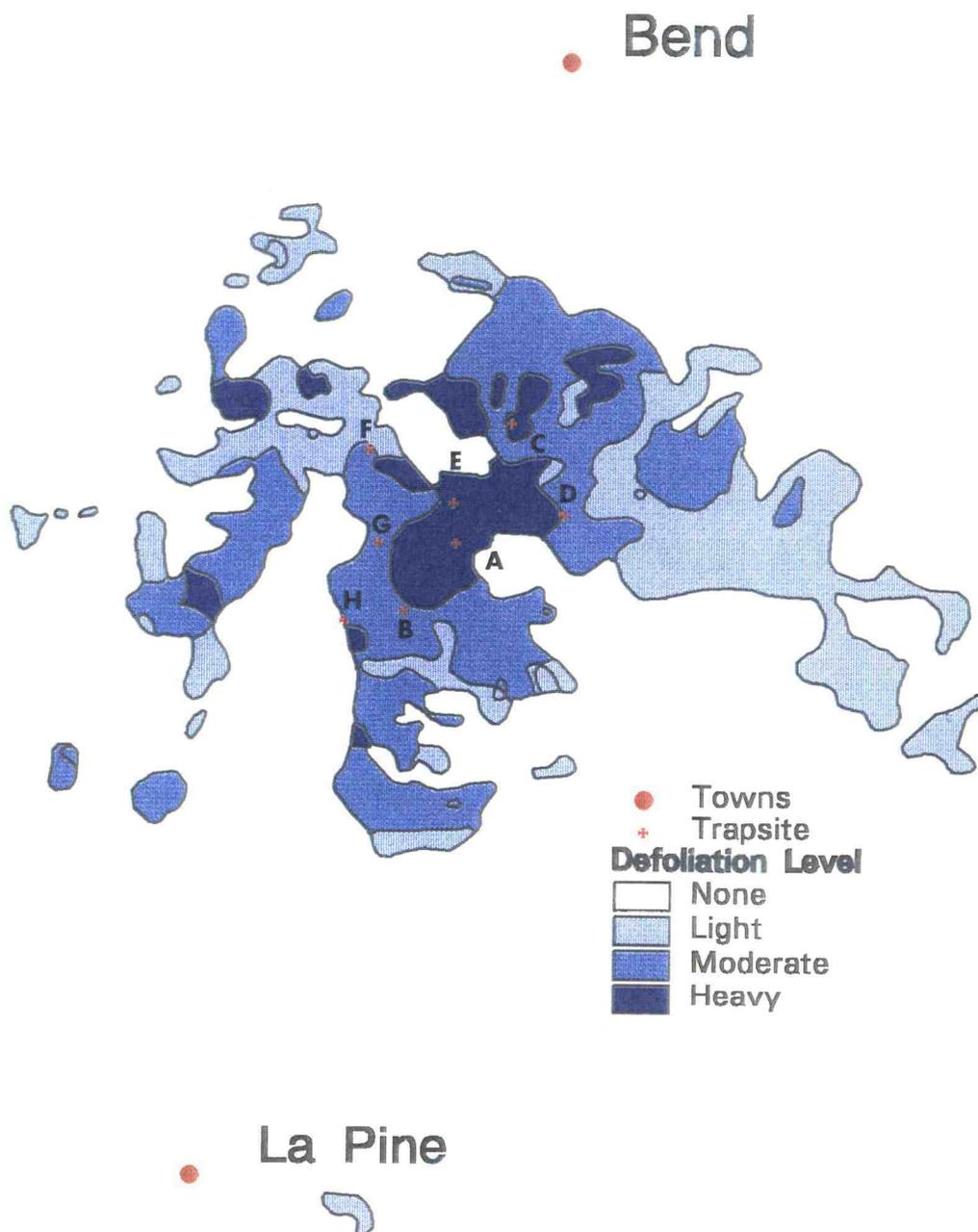


Figure A.1 Map of 1992 *C. pandora* defoliation and 1993 *S. lateralis* trap site locations. U.S. Forest Service aerial survey of the Deschutes N.F., Oregon. Distance from Bend to LaPine is 48 km.

Appendix B Consumption of Chemical Egg Fractions

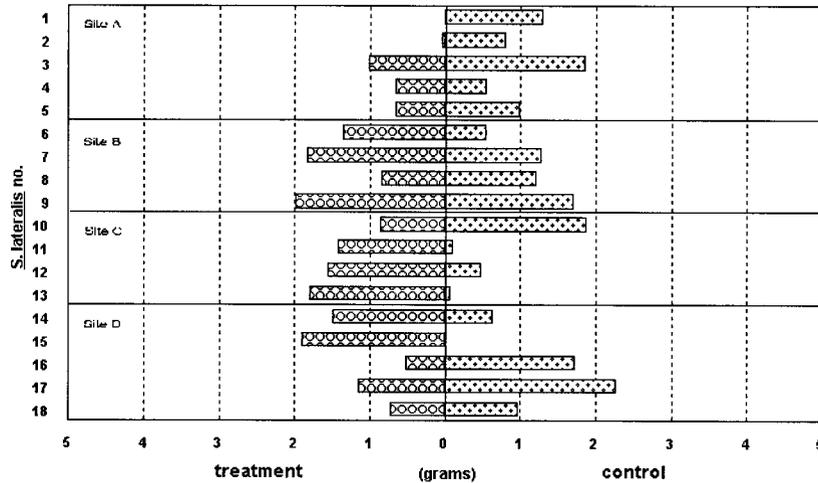


Figure B.1. Consumption of treated and control sunflower seeds in bioassay of chemical egg fractions. Treatment is hexane extract of eggs.

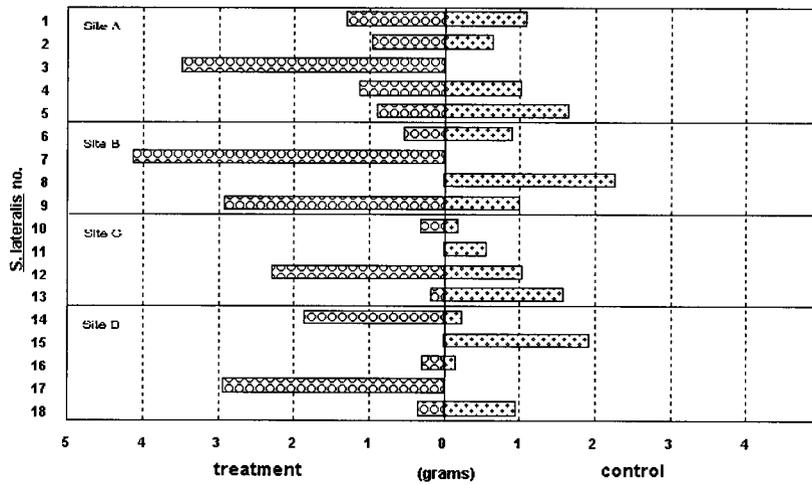


Figure B.2. Consumption of treated and control sunflower seeds in bioassay of chemical egg fractions. Treatment is dichloromethane fraction of egg tissue.

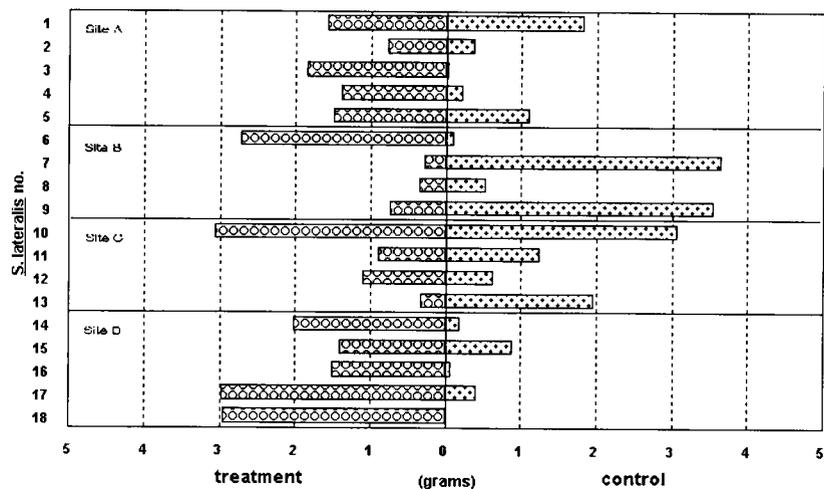


Figure B.3. Consumption of treated and control sunflower seeds in bioassay of chemical egg fractions. Treatment is water fraction of egg tissue.

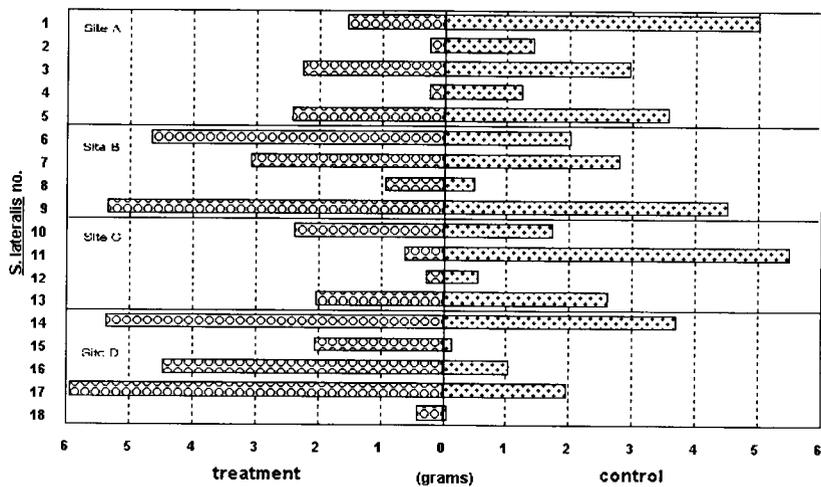


Figure B.4. Consumption of treated and control feed blocks in bioassay of chemical egg fractions. Treatment is egg tissue fractionation residue.

Appendix C Consumption of Physical Egg Fractions, Initial Bioassay

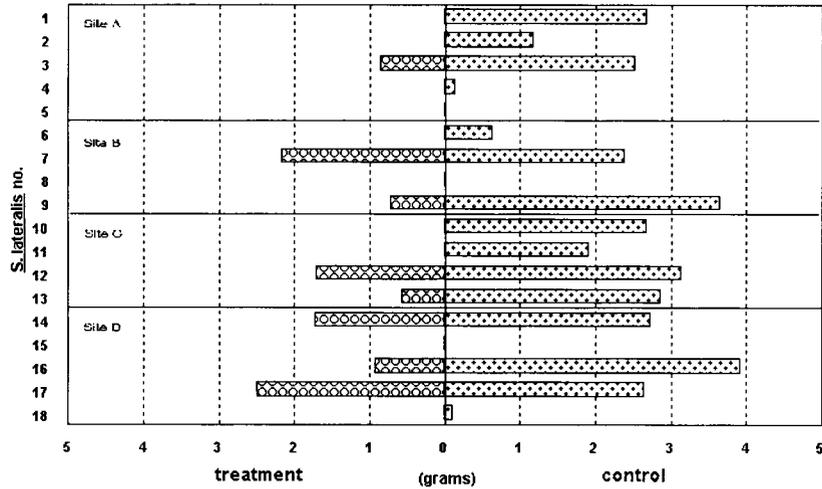


Figure C.1. Consumption of treated and control feed blocks in initial bioassay of physical egg fractions. Treatment is whole eggs.

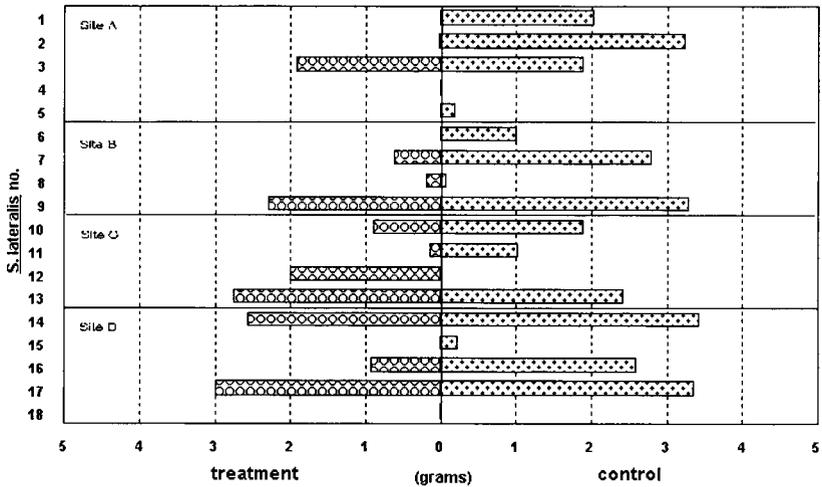


Figure C.2. Consumption of treated and control feed blocks in initial bioassay of physical egg fractions. Treatment is "whole" egg shells.

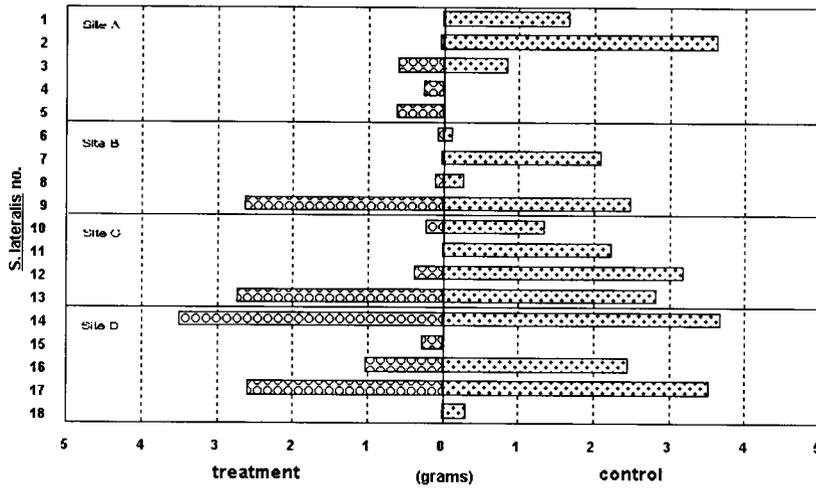


Figure C.3. Consumption of treated and control feed blocks in initial bioassay of physical egg fractions. Treatment is ground egg shells.

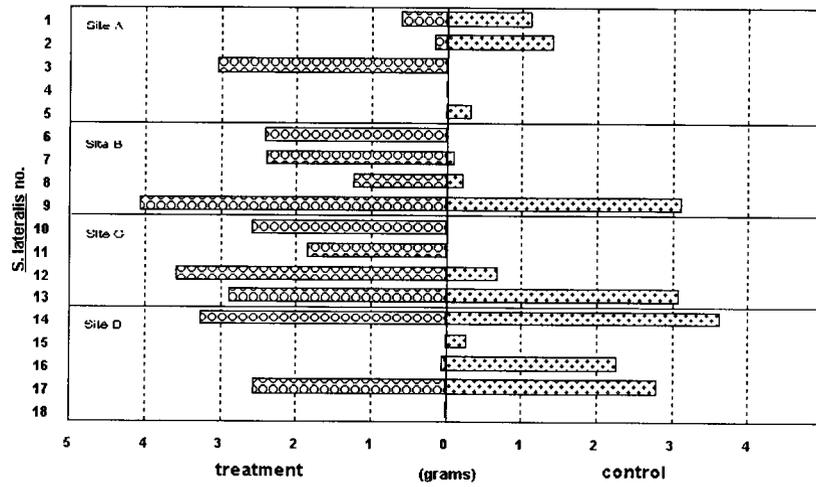


Figure C.4. Consumption of treated and control feed blocks in initial bioassay of physical egg fractions. Treatment is egg contents.

Appendix D

Consumption of Physical Egg Fractions, Follow-up Bioassay

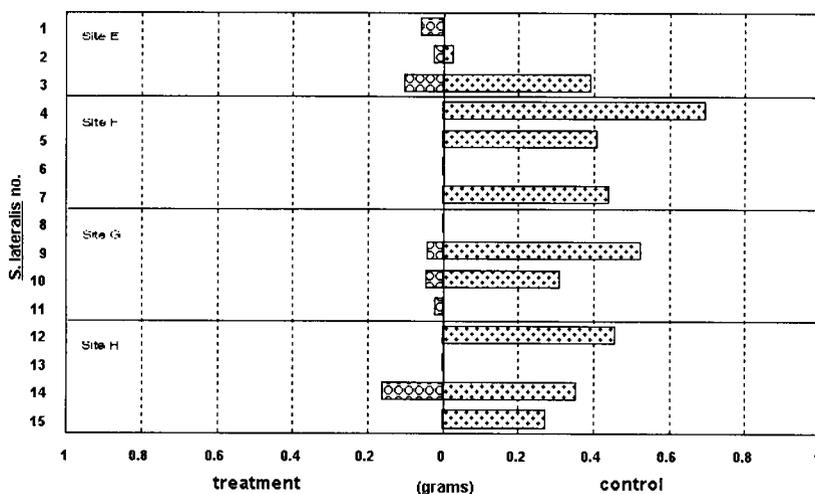


Figure D.1. Consumption of treated and control feed blocks in follow-up bioassay of physical egg fractions. Treatment is whole eggs.

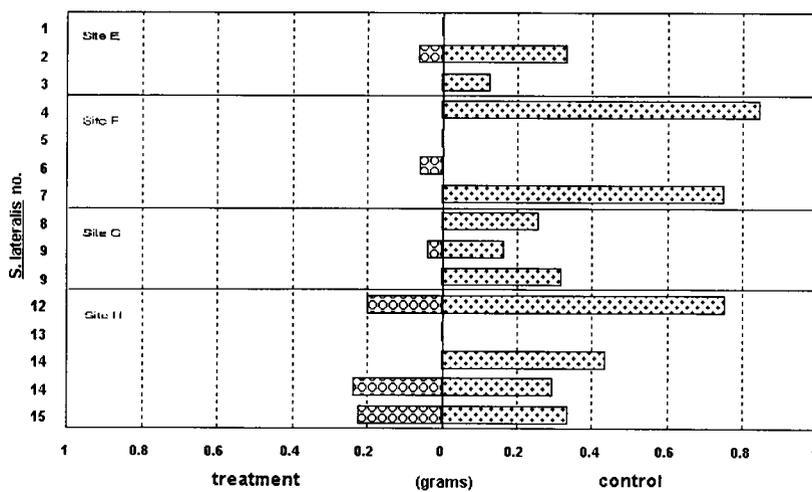


Figure D.2. Consumption of treated and control feed blocks in follow-up bioassay of physical egg fractions.. Treatment is "whole" egg shells.¹

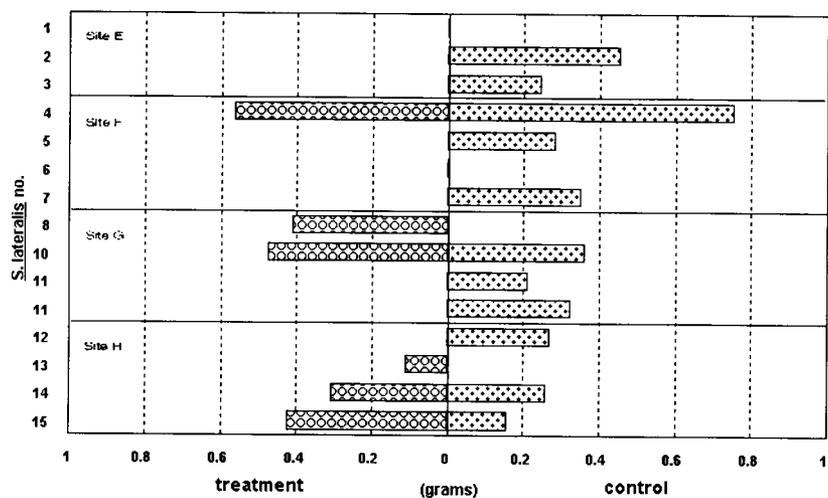


Figure D.3. Consumption of treated and control feed blocks in follow-up bioassay of physical egg fractions. Treatment is ground egg shells.¹

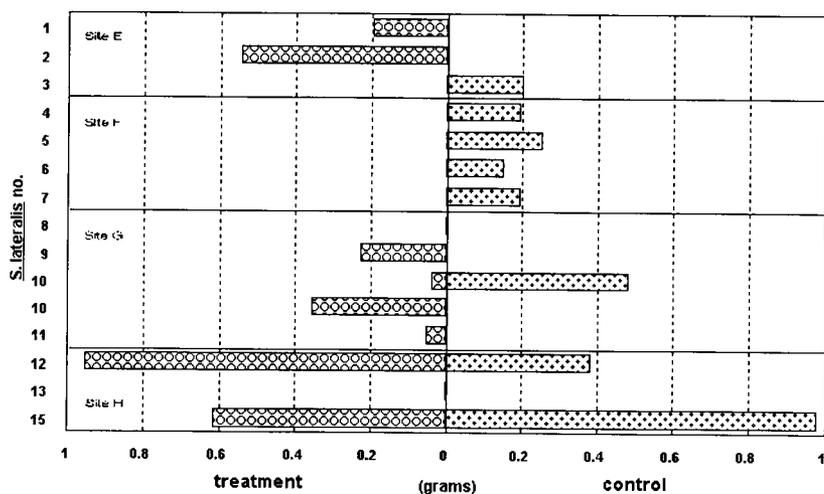


Figure D.4. Consumption of treated and control feed blocks in follow-up bioassay of physical egg fractions. Treatment is egg contents.¹

¹ Some *S. lateralis* numbers on the y-axes are non-consecutive due to errors in assigning treatments. Duplicate consumption data was not included in statistical analyses.