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Jeffrey C. Miller

The recent colonization of Oregon by an exotic, intentionally introduced coccinellid, *Harmonia axyridis*, was the basis for lab and field research into lady beetle developmental biology and community composition. *Harmonia axyridis* was the dominant coccinellid in an arboreal habitat, and comprised over 70% of all lady beetles, and 82% of live coccinellid mass. The species has at least two generations per year, spring and fall. A parasitoid of coccinellids, *Dinocampus coccinellae*, attacks this new species at rates of less than 1%. A manipulative study focusing on the egg-development of guild members which co-occur with *H. axyridis* found a moderate adaptation to cool temperatures in this species. Another introduced predatory coccinellid, *Coccinella septempunctata*, was shown to be extremely tolerant of heat. Egg-to-adult development was found to vary significantly between three seasonal generations of *H. axyridis* in western Oregon. Overall, *H. axyridis* required 267 degree-days above a lower developmental threshold temperature of 11.2°C, and these parameters differed significantly from values reported for a population introduced into France. A study of intraspecific variation of intrinsic traits in *Calvia quatuordecimguttata*, a species which co-occurs with *H. axyridis* in Oregon, did not find differences in either lower developmental thresholds, heat-unit requirement, or pupal weight that could be correlated with elytral coloration. *C. quatuordecimguttata* was also found to express coloration independent of temperature experienced by immatures. However, this species as a whole was found to be extremely adapted to cold-temperatures. The lower threshold for egg-to-adult development of 8.2°C in *C. quatuordecimguttata* may be the lowest reported for an aphidophagous lady beetle.

Temperature-Dependent Development and Field Observations of Some Western Oregon Coccinellids (Coleoptera: Coccinellidae)

by

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Michael Louis La Mana, Author

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But that's a different story

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Chapter 1

Introduction

Lady beetles are important agents of biological control of homopteran pests, particularly aphids and scales. Introduction of these natural enemies has been the focus of biological control programs for over 100 years, especially after the introduction to California of *Rodolia cardinalis* (Mulsant) in the 1880's resulted in dramatic control of the cottony cushion scale, *Icerya purchasi* (Maskall). However, a rational basis for predicting which natural enemies offer the best chance of establishment and effective biological control of target pests has been lacking. The establishment rate of introduced coccinellids has been poor to date and release of generalist predators has created controversy because of non-target effects and possible competitive exclusion of other natural enemies. The lack of post-release studies has probably perpetuated release failures.

Why introduce generalist predators at all, especially when there are species already in the local ecosystem that appear to have many of the same biological and trophic attributes? What makes the successful species so, and might intrinsic attributes that they hold in common assist biologists in selecting future biological control agents for importation or exclusion?

One exotic species of homopterous lady beetle, *Harmonia axyridis* Pallas, has recently been introduced to the United States by USDA - APHIS, and has become established in the Pacific Northwest from Asia. Its presence in Oregon provided the opportunity to document the spread and field biology of an introduced, generalist natural enemy as it expands its range into a new habitat. The first goal of this study was to provide baseline data on the establishment and spread of *H. axyridis* as it expands its range into a new habitat (Chapter 3). Next I investigated whether patterns in one class of intrinsic biological attribute, temperature-dependent development of eggs, are correlated with patterns in species composition among coccinellids after the addition of the *H. axyridis* (Chapter 4).

The recent introduction of *H. axyridis* to the Pacific Northwest presents this species with a new habitat where natural selection on intraspecific variation might occur. To assess whether natural selection or founder effects were working on the Oregon population of *H. axyridis*, I investigated whether temperature-dependent development - a genetically controlled attribute - varied among three seasonal populations (Chapter 5). Additionally I contrasted the temperature-dependent development of the Oregon popualtion with that of a population introduced into France. Finally, I investigated whether temperature-dependent development traits vary intraspecifically in a polymorphic coccinellid, *Calvia quatuordecimguttata* (L.) (Chapter 6).

Chapter 2

Literature Review

Biological Control

Biological control is a phenomenon in which natural enemies (biotic mortality agents) regulate the population of another organism - a pest - at levels below that which would be realized in the absence of such mortality agents (DeBach and Rosen, 1991; van den Bosch et al., 1982). Based on natural trophic relationships between organisms in a community, biological control is observed in non-human dominated systems (natural biological control) (Price, 1984), as well as in human-dominated systems (applied biological control) (Altieri, 1994; DeBach and Rosen, 1991). The field of applied biological control includes both manipulation of the habitats and organisms extant in the environment to achieve this goal - for example strip-harvesting of alfalfa to retain natural enemies - as well as the addition to the environment of one or more species predicted to utilize some pest species as an energetic resource. This latter practice, the importation of natural enemies, is referred to as classical biological control (Van de Bosch et al., 1982).

The rational for the practice of classical biological control (CBC) lies largely in the fact that many pest species have been inadvertently introduced from distant areas, while their natural enemies have not (DeBach and Rosen, 1991; Sailer, 1978). The biological control program initiates with exploration of geographic areas from which the exotic pest species is thought to have originated. Here, the field entomologist searches for predators, parasites and / or pathogens that may be of use in controlling the pest in the outbreak region, and selects some for shipment back home for quarantine, mass culturing, and release. The importance of the collection phase of the classical biological control program is often understated.

Not all natural enemy species are created equally, and selection criteria for natural enemy introductions will depend on, among other things, intrinsic biology of the target pest, extrinsic factors such as habitat and existent fauna, and intrinsic biology of the natural enemy (Ehler, 1990; see below). Moreover, because of intraspecific variation of intrinsic attributes natural enemies of a single species may not be equally suitable for introduction. Adaptedness of exotic natural enemies to new climates is a case in point. For example, Croft (1971) found that photoperiodic cues for the onset of diapause in the predatory mite *Typhlodromus occidentalis* differed among regional strains. Similarly, a clinal variation in cues for onset and duration of diapause was found in the lady beetle *Coccinella septempunctata* L. (Hodek and Cherkasov, 1960). White et al. (1970) found that heat tolerance in *Aphytis lignanensis* Compere, a parasitoid of the California red scale *Aonidiella aurantii* (Mask.), varied within a local population, and that heat-tolerance was subject to directional selection in the lab. Another trait, temperature-dependent development, has been shown to vary within populations of the aphidophagous lady beetles *Coccinella trifasciata* LeConte (Miller, in press) and *Hippodamia convergens* Guerin-Meneville (Rodriguez-Saona, 1994). Thus, in addition to having to select the best possible species of natural enemy for introduction, the biologist may be confronted with intraspecific variation in key intrinsic traits. Genetic bottlenecks and founder effects during initial collection of individuals, when amplified in the mass-culture phase of the CBC program, may lead to the release of a population that is qualitatively different from the parental source (Hoy, 1979; Mackauer, 1976).

Coccinellidae in Classical Biological Control

In North America at least 180 species of Coccinellidae have been imported for biological control of Homoptera - especially scales - since the late 1880's, although over 91% of these species have failed to establish (Gordon, 1985). Conversely, releases that are "too successful" may result in unwanted effects on non-target species and displacement of native guild members through direct competition (Howarth, 1991) and predation (Rosenheim et al., 1993). Of a total of 27 exotic species established in North America (Gordon, 1985; Gordon and Vandenberg, 1991) only 17 are the result of intentional release, while ten are unintentional establishments

When establishment of aphidophagous species has been successful there have often been long delays between the time of release and time of establishment. For example, the seven-spot ladybird (*Coccinella septempunctata* L.) - a European native - was released into North America for 17 years before establishment was first recorded (Angelet et al., 1979). The lady beetle *Hippodamia variegata* (Goeze) is another Old World species that was intentionally released for biological control of aphids. Released in Arizona, California, Florida, and Georgia during 1957-8 (Gordon, 1985), and then again by USDA-APHIS in 1987 (Obrycki & Orr, 1990). Until 1993 *H. variegata* was known only from Quebec and Ontario (Gordon and Vandenberg, 1991), but Wheeler (1993) made the case that the species may have been present in New England south to Northern New Jersey when Gordon (1987) published that Canada was the only North American locale of this species.

Classical Biological Control and the Guild Concept

In many instances, foreign exploration for exotic natural enemies has been initiated after all chemical control proved futile and the exotic pest was at epidemic levels (DeBach and Rosen, 1991; Ehler, 1990). Thus, practitioners have been constrained in selection of natural enemies to those species easiest to obtain, quarantine, and mass rear (Waage, 1990). Ehler (1990) critiques the haphazard fashion in which CBC introductions have historically been conducted, although this is partly a function of the 'brushfire' nature of CBC programs (Waage, 1990), and cites a lack of predictive ecological theory upon which to base release and importation decisions. However, the concept of the ecological guild has provided testable hypotheses concerning best attributes of natural enemy species and assemblages of species in CBC programs.

Root (1967) defines a guild as 'a group of species that exploit the same class of environmental resources in a similar way', and this concept extends naturally to biological control (e.g. Askew and Shaw, 1986; Miller, 1977; Force 1974). A pest species may already be attacked by a number of natural enemies (although suppression may be above the economic threshold) before foreign exploration for more efficient enemies is begun, or the explorer of the pests native range may find the pest attacked by a variety of natural enemies there. Biological attributes of natural enemy species, singly and as guilds, commensurate with successful introduction have been the subject of many papers. Waage (1990) draws a dichotomy between 'reductionist' and 'holistic' approaches to biological control research. Reductionist approaches are those viewing the intrinsic biological properties of an 'ideal' natural enemy (e.g. fecundity, development rate, search ability) as a model for selection in CBC programs. A holistic approach would be one where the decision of what species to introduce is a function of the community milieu that the pest experiences in the field.

Guild-oriented theories of biological control were derived from parasitoid-dominated communities (e. g. Miller, 1977), and application to communities heavily dominated by generalist predators, such as coccinellids, may be less meaningful. For example, parasitoid species may be much more host and life-stage specific than predators. Because of this, parasitoids tend to be more efficient searchers. While direct competition and aggression does occur between larvae, adults (that are not hyper- or secondary parasites) generally do not interact with larvae. On the other hand, generalist predators by definition do not differentiate among prey species or stage to the same degree, they are comparatively poor searchers, and larvae and adults of many species co-occur and compete for the same resources (Hodek, 1973). Thus, higher niche overlap in predators may partly explain poor rates of establishment due to competitive exclusion (Ehler and Hall, 1982). One way that resources could be partitioned among generalists in the same habitat is temporally, and temperature-dependent development in temperate-zone generalists may a functional analog of ecological niche (see Chapter 4).

Life History of Aphidophagous Coccinellidae

Coccinellids are the most common and probably best studied predators of aphids (Hodek, 1973, 1967; Hagen, 1962). More than 4000 species are known worldwide (Hagen, 1967), including approximately 475 species in 57 genera in America north of Mexico (Gordon, 1985). The majority of Coccinellidae are predators of Homoptera and Acari, but most predatory species will accept a broad range of food. The subfamily Epilachninae encompasses phytophagous species such as the Mexican bean beetle, *Epilachna varivestis* Mulsant. Aphidophages are predominantly found in the subfamily Coccinellinae, but also to a limited extent in the Scymninae and Chilochorinae (Frazer 1988; Hodek 1973). The coccinelline tribe Coccinellini is exclusively homopterous and perhaps the most broadly distributed and well studied of coccinellids. Some of the aphidophagous species of this tribe are the focus of this thesis.

Coccinellidae exhibit many combinations of voltinism and diapause (Hodek, 1973; Hagen, 1962). Aphidophagous North American species tend to be univoltine, and spend late-summer and winter in a state of reproductive diapause. This state is characterized by increased fat reserves in the abdomen and, in females, atrophy of the ovaries. Most aphidophagous species spend the hibernating period in some degree of aggregation. An extreme example of this is that of *H. convergens* in California where millions aggregate to overwinter in the foothills of the Sierra Nevada (Hagen, 1962). Many species mate before dispersal and thus this behavior facilitates gene flow in what may be very dispersed populations

Adult lady beetles are highly vagile creatures, within and between habitats. In one extreme case, Kieckhefer & Olsen (1974) released three million marked *Hippodamia convergens*, and recaptured only 15 of them one day later. Populations of *Coccinella trifasciata* LeConte and *Coccinella californica* Mannerheim were reduced 6-16% per day due to emigration (Ives 1981). Because of the searching behavior of adults and the ephemeral nature of aphid resources, high vagility is probably most adaptive (Frazer 1988).

Searching behavior of coccinellid larvae and adults is analogous, although adults are generally less active. Duration of activity and searching have increased with hunger for larval *Adalia bipunctata* (Mills 1982) and *C. septempunctata* (Carter & Dixon 1992), and adult *Coccinella undecimpunctata* L. and *C. trifasciata* (Frazer & Gilbert 1976). During local searching, coccinellids are positively phototactic and negatively geotactic and this results in species tending toward the actively growing extremities of host plants (Frazer 1988). Coccinellid adults are not believed especially efficient at prey location (Hodek 1973, Dixon 1959). It is most likely that these species rely heavily, if not exclusively, on tactile cues acting on the maxilliary palps and associated chemosensory structures, as prey capture is usually initiated only after mouth parts contact prey organisms (Frazer 1988). However, some species can detect prey at short distances. *Anatis ocellata* (L.) has been observed to stop random search in the vicinity of its prey (*Choristoneura* sp.) before contact has been made (Frazer, 1988). Stubbs (1980) demonstrated that adult *Coccinella septempunctata* L. located crushed pea aphids faster than would be expected by random motion when the aphids remains were within 1 cm.

Coccinellids are oviparous, and eggs are usually laid in clusters attached to a substrate. First instars rupture the chorion using an egg-bursting appendage on the thorax, and remain huddled on the cluster consuming unhatched eggs, dead larvae, and occasionally each other. Depending on species and ambient temperatures, first instars leave the egg mass in 12 to 48 hours and go in search of food (Agarwala & Dixon 1992). Larvae pass through four instars, occasionally five, the last of which attaches its anal organ to the substrate prior to molting to the pupal stage. Pupae are capable of an anterior 'flicking motion' in response to tactile stimulation which is known to repel some predators and parasites (Hodek 1973). The newly emerged adult is opaque-white and very soft. It grasps onto the pupal exuvium until the exoskeleton has chemically tanned to rigidity. During this tanning process color patterns appear on the elytra and thorax.

Many species of coccinelline lady beetles are polymorphic for adult coloration. In these instances, color of elytra, thorax, sternites, and head may differ between forms, and color-determination has been shown to be both entirely genetic as well as a function of genotype / temperature interaction. In *H. axyridis*, degree of adult melanisation of many genotypes increases with decreasing temperatures experienced by immatures, particularly pupa (Sakai et al., 1974). However, such environmental influence on coccinellid melanization is uncommon. For example, Houston and Hales (1980) demonstrated that nine alleles controlled the number, position, and fusion of black spots on the yellow elytra of *Coelophora inaequalis* (F.). In many species, such as *H. convergens,* variation in spot position and size has been shown to be polygenic (Komai, 1956). However, truly melanic forms of many species exist in which the ground color of elytra and thorax is black, usually with some red spotting present, and these color forms are usually conferred by one (often dominant) allele as is true of *H. axyridis* (Komai, 1956). For other species, there is little variation within any of the color patterns, but instead dramatic differences between them. An example is *Calvia guttata* which expresses three distinct morphs that appear to be inherited in strict Mendelian fashion (Lusis, 1971).

The adaptive significance of color polymorphism is unclear, but several hypotheses have been raised. Industrial pollution was shown to correlate with distribution of melanic *A. bipunctata* in the United Kingdom (Creed, 1970). Differential fitness of color forms in response to predation pressure (Marples et al., 1989), as well as due to the effect of 'Mullerian mimicry rings' among similarly colored, chemically defended beetles (Brakefield, 1985), has been suggested. Fitness differences between color forms due mating preferences for some forms have been demonstrated for *H. axyridis* (Osawa and Nishida, 1992), *Aphidecta obliterata* (L.) (Parry and Peddie, 1981) and *A. bipunctata* (O'Donald and Majerus, 1992; Kearns et al., 1992; Kearns et al., 1990). Some evidence for a climatic association with distribution of melanic forms has been presented by Dobzhansky (1933), and this led to investigations of correlates of polymorphism and temperature-dependent biology. Thermal advantage of melanism coloration in coccinellids (due to black body effects and increased ability to reach thermal optima) has been demonstrated at cool ambient temperatures in *A. bipunctata* and *C. septempunctata* (Stewart and Dixon, 1989; Brakefield and Willmer, 1985; Brakefield, 1984).

Temperature dependent development in Coccinellidae

Temperature-dependent development rates of insects have been the subject of evolutionary (e.g. Taylor 1981), ecological/energetic (e.g. Gutierrez et al. 1981), and pest management research (e.g. Pruess 1983). Insects, as ectotherms, are constrained in their metabolic rates by ambient temperatures. The amount of chronological time required to complete a given life-stage is governed by biochemical-kinetics, which are thermal-energy constrained (Ratte, 1985; Hodek, 1973). These biochemical processes are typified by an accelerating component that operates at temperatures below the thermal optimum, and a retarding component that predominates at temperatures above the optimum. Thus, at temperatures above a lower threshold for development, growth and development require less chronological time as the optimum temperature is approached (Fig. 2.1).

Because thermal energy is very difficult to measure in practice, a simple formula for approximating thermal energy required for discrete development is based on the assumption that completion of a given life-stage requires a fixed amount of thermal energy is often used. This is the general equation of an equilateral hyperbola (Fig 2.1; 'days') and is given by $D(T- D_{th}) = C$, where D = chronological time required to complete a life stage at some temperature (T), D_{th} = the lower developmental-threshold temperature, and C = a constant (Hodek, 1973). The rate of development is then expressed as the reciprocal of days, and approximates a linear relationship to temperature over a wide range (Fig 2.1;'1/days'). The point at which this rate line

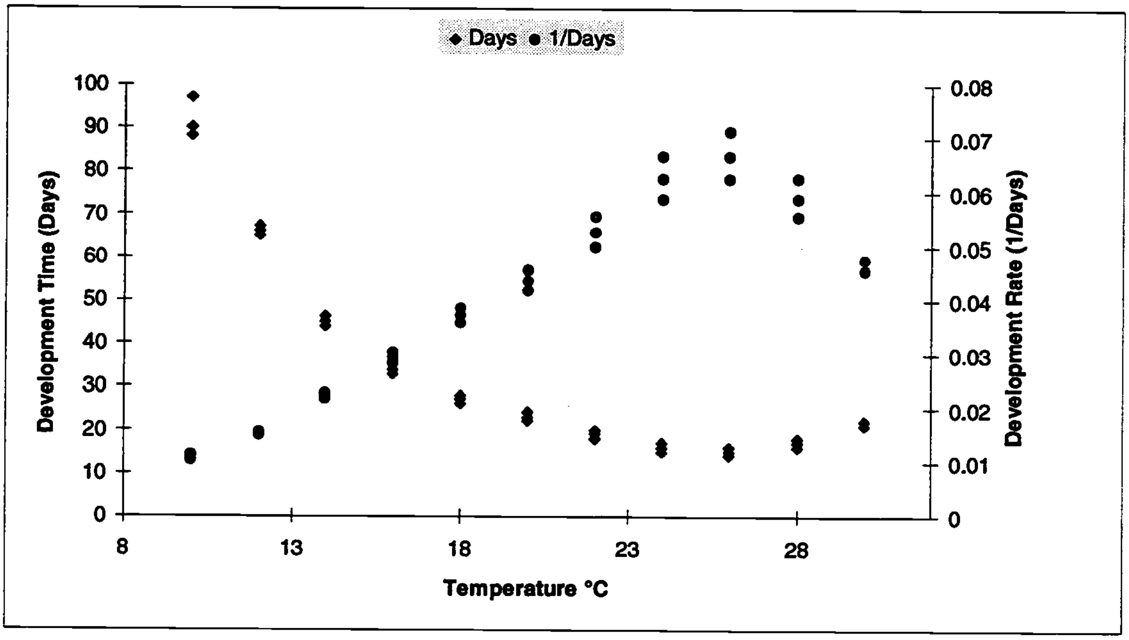

Figure 2.1. Distribution of insect development times and rates among temperatures.

($y = mx + b$) is projected to cross the x-axis (temperature) is interpreted as the lower developmental threshold for development ($D_{th} = -b/m$) and the total thermal energy required to complete the given stage is interpreted as degree-days, given by ($DD° = 1/m$).(Campbell et al., 1974).

These parameters are a species specific measures of the rate at which beetles develop at constant temperatures. Published lower thresholds egg-to-adult development in aphidophagous coccinellids vary from approximately 7°C for *C. quatuordecimguttata* (see Chapter 6) to 13°C for *Coccinella trifasciata* LeConte (Miller, in press), beetles require from approximately 197 to 340 DD° in *Coccinella septempunctata* (L.) (Obrycki and Tauber, 1981) and *Hippodamia sinuata* Mulsant (Michels and Behle, 1991), respectively. Thus, quantification temperature-dependent development parameters provides one basis for making testable predictions about both suitability of species for introduction in terms of prey phenology and local climates, as well as about possible competitive implications for existing fauna.

Chapter 3

Field observations on *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae) in western Oregon

Michael L. LaMana and Jeffrey C. Miller

Submitted to *Biological Control*

Chapter 3

Field observations on *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae) in western Oregon

Abstract

Ten years elapsed between the intentional release of *Harmonia axyridis* Pallas into the Pacific Northwest of North America and the first observation of an individual on July 18, 1991, in Kings Co., Washington. The beetle was extremely abundant in western Washington and Oregon throughout 1993-94, where the species currently ranges from mid-eastern Washington, to just north of the California / Oregon border, east to 1371 m in the Cascade Mtns. and west to the Pacific Coast. Field sampling confirmed the habits and habitats of *H. axyridis* as a generalist, semi-arboreal, aphidophagous predator. Our records associate *H. axyridis* with 16 aphid prey species on 16 plant hosts. In western Oregon, *H. axyridis* co-occurs with 11 species of native and 2 species of exotic aphidophagous coccinellids on trees and shrubs. In this habitat, 70% of individual beetles and 82% of live adult beetle mass was represented by *H. axyridis*, while comprising only 4% of the coccinellids in alfalfa, clover, and peppermint. Adult *H. axyridis* dispersed during March to feeding sites from overwintering aggregations formed the preceding October. At least two generations, with a partial to complete third generation, occur per year. Parasitism of field-collected adult beetles by *Dinocampus coccinellae* (Schrank) was less than 1%.

Introduction

Recovery of exotic coccinellids in successful classical biological control programs has often been delayed from the time of release (Gordon and Vandenberg, 1991). For example, *Coccinella septempunctata* L. was released into the United States beginning in 1956 before a resident population was discovered in 1973 (Angelet *et al.*, 1979). Similarly, *Hippodamia (Adonia) variegata* (Goeze) was released in Canada in the 1950's but not recovered until 1984 in Montreal, Quebec (Gordon, 1987) and 1992 in New York and Vermont (Wheeler, 1993). Both of these species dominated the aphidophagous coccinellid guild in their recovery sites, with *C. septempunctata* and *H. variegata* comprising over 80% and 36% of coccinellids, respectively (Wheeler, 1993; Angelet *et al.*, 1979).

The phenomenon of delayed post-release recovery accompanied by numerical dominance of the aphidophagous coccinellid guild has been repeated in western Oregon by the multicolored Asian lady beetle, *Harmonia axyridis* Pallas. A color-polymorphic, semi-arboreal predator of certain Homoptera/Psocoptera and native to Western Asia (Hukusima & Kamei, 1970), *H. axyridis* was imported to the United States for biological control of pear psylla, pecan aphid and other arboreal Homoptera. Releases of *H. axyridis* date back to 1916 in California and in many states by USDA between 1978 and 1982 (Chapin & Brou,1991). Establishment in North America was first recorded in Louisiana and Mississippi (Chapin & Brou, 1991). Mass releases of *H. axyridis* occurred in three counties of the state of Washington in 1981-1982. A total of 37,852 adult beetles were released in Chelan, Klickitat, and Yakima counties. Yakima Co. was also the release site of 14,376 larvae in 1978 (Coulson, 1992). On 18 July, 1991, an adult was collected in King Co., Washington. No known releases of *H. axyridis* were made in Oregon, but presence of the species was recorded in Oregon for the first time in October, 1991, when one larva was recovered in Kaiser, Marion Co. (R. Westcott, Oregon Department of Agriculture, personal communication). By early spring of 1993 this species was very abundant on aphid-infested trees and shrubs in Corvallis, Benton Co., OR (personal observation).

The release of generalist predators for biological control programs has been the subject of debate based on: 1) inconsistent and long-delays between release and establishment (e.g. Angelet, 1979), 2) possible displacement of other guild members (see Rosenheim et al., 1994), and 3) impacts on nontarget species (Howarth, 1991). The occurrence of *H. axyridis* in Oregon provided an opportunity to record baseline data on certain aspects of the behavior and ecology of such a biological control agent during a phase of population expansion into a new environment. The objectives of this study were to: 1) document the occurrence and distribution of *H. axyridis* in Oregon in 1993 and 1994; 2) record the types of elytral color-morphs; 3) assess guild composition of semi-arboreal, aphidophagous coccinellids between March and October of 1994; 4) document the phenology of all life-stages and overwintering; 5) record host plant and prey associations, and; 6) document parasitism of larvae, pupae, and adults.

Materials and Methods

Distribution and color forms. Field surveys to assess presence/absence of *H. axyridis* were conducted on 18 September 1993, 16 April 1994, and 9 July 1994. Ranging from Corvallis, Benton Co., south to Ashland, Jackson Co., Oregon 19.3 km north of California, this survey covered approximately 373 km. Additionally, surveys of the central region of the Cascade Mountains (Linn, Jefferson, and Deschutes Counties) and Pacific coast areas (Lincoln, Lane, Douglas, Coos and Curry Counties) were conducted during the springs and summers of 1993 and 1994. A 75cm X 75cm beating sheet was used to collect adult and larval lady beetles from various trees and shrubs. All adult and larval (excluding first instars) aphidophagous coccinellids were identified to species. The occurrence of all color forms of *H. axyridis* (after Komai, 1956) was documented. A proportion of the sampling effort was timed to provide a measure of abundance based on 100 s of sampling effort. Voucher specimens of *H. axyridis* have been placed in the Systematic Entomology Laboratory, Department of Entomology, Oregon State University, Corvallis, Oregon.

Aphidophagous coccinellid guild composition. Shrubs and trees were sampled in September, 1993, and then from 10 April to 30 October 1994, using a beating sheet. Individuals representing each coccinellid species were counted and weighed in the laboratory to compare relative abundance and live mass within the guild. All samples for the guild composition analysis occurred in the vicinity of Corvallis. Samples were blocked by host plant and conducted on windless-days in the cool morning or evening hours to minimize flight response of adult beetles. Although sampling was conducted at least twice per month, no individual host plant was sampled twice per four-week interval. Concurrently, a survey of aphidophagous coccinellids was conducted in fields of alfalfa, clover, and peppermint in the same vicinity[1>] . Each of these crops was sampled by conducting 100, 180°-arc sweeps with a 40.6 cm diameter net. Six fields (two per crop) were sampled twice per month from June through September of 1993 and 1994. All adult coccinelline and chilocorine lady beetles were identified to species. Larvae were not counted.

Sample data were analyzed to assess community composition of semi-arboreal, homopterous coccinellids by month and host plant. The analysis included (Table 3.1): (1) abundance of each species per sample (ρ: Eq. 1) and species richness (s: Eq. 2); (2) a

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[1>] Data for alfalfa, clover, and peppermint are from Dr. J. C. Miller, unpublished, and are incorporated here for comparison among habitats.

species diversity index ($e^{H'}$: Eq. 5) after Ludwig and Reynolds (1988); (3) a variance estimate for the species diversity index (var H': Eq. 4) after Poole (1974), and; (4) the Berger-Parker index of dominance (d: Eq. 6) (Southwood, 1978).

Table 3.1. Indices used to compare composition of aphidophagous coccinellid guilds among months, habitats, and plants.

Statistic	Equation	Equation Number
Sampling abundance (ρ)	$\rho = \frac{n}{\text{sample time}} * 100$	[1]
Species richness (s)	s = species count	[2]
Shannon-Weaver Index (H')	$H' = -\sum_{i=1}^{s}(\frac{ni}{n}\ln\frac{ni}{n})$	[3]
Variance about H'	$\text{var (H')} = \frac{(\sum_{i=1}^{s} p_i \ln^2 p_i) - (\sum_{i=1}^{s} p_i \ln p_i)^2}{N} + \frac{(s-1)}{2N^2}$	[4]
Species diversity (N1)	$N1 = e^{H'}$	[5]
Dominance Index (d)	$d = n_{\text{dominant}} / n_{total}$	[6]

Phenology, prey-species, and parasitism. Phenology of *H. axyridis* was documented in the vicinity of Corvallis each week from 10 April to 9 November 1994. Presence of immature and adult *H. axyridis* was noted according to host plant and date. Relative abundance of larvae and adults from the field sampling (see above) was also used to assess voltinism. To document prey exploitation patterns, Aphidoidea associated with *H. axyridis* on respective host plants were retained for identification. Incidence of parasitism of *H. axyridis* was determined by maintaining field collected larvae, pupae, and adults. Immatures were reared to adults, and all adults were kept until death.

Results and Discussion

Distribution. I observed an increase in the geographic range of *H. axyridis* in western Oregon during approximately 15 months of study. On 18 September 1993, *H. axyridis* was found from Corvallis south to Myrtle Creek, Douglas Co. (Fig. 3.1). No *H. axyridis* were recovered further south. Similarly, the first survey of the following spring (16 April 1994) showed no further southerly extension. However, during the final survey on 9 July 1994, we found *H. axyridis* as far south as Medford, Jackson Co., where one adult and one larva were collected from an ornamental birch (*Betula pendula* L.) (Table 3.2). In counties of southwestern Oregon, *H. axyridis* was found associated with *Adalia bipunctata* (L.), *Cycloneda polita* Casey, *Olla v-nigrum* (Mulsant), and *C. septempunctata*, but did not dominate the aphidophagous guild as it did in Benton Co. (see below). In all 1994 collections, relative abundance among aphidophagous lady beetles of *H. axyridis*

Table 3.2. Locality records and relative abundance among arboreal coccinellids of *Harmonia axyridis* Pallas in western Oregon, 1993 - 1994.

County	Year	Larvae	Adults	Rel. Abundance[1>]	N
Benton	1993	Yes	Yes	N/A[2>]	N/A
	1994	Yes	Yes	70.4%	3427
Lane	1993	Yes	Yes	N/A	N/A
	1994	Yes	Yes	58.5%	82
Douglas	1993	Yes	Yes	N/A	N/A
	1994	Yes	Yes	30.4%	112
Josephine	1993	No	No		0
	1994	No	Yes	2.2%	134
Jackson	1993	No	No		0
	1994	Yes	Yes	2.3%	86

1> Relative abundance equals percent of all adult aphidophagous coccinellids in samples comprised by *H. axyridis*.
2> Data for 1993 were presence/absence only

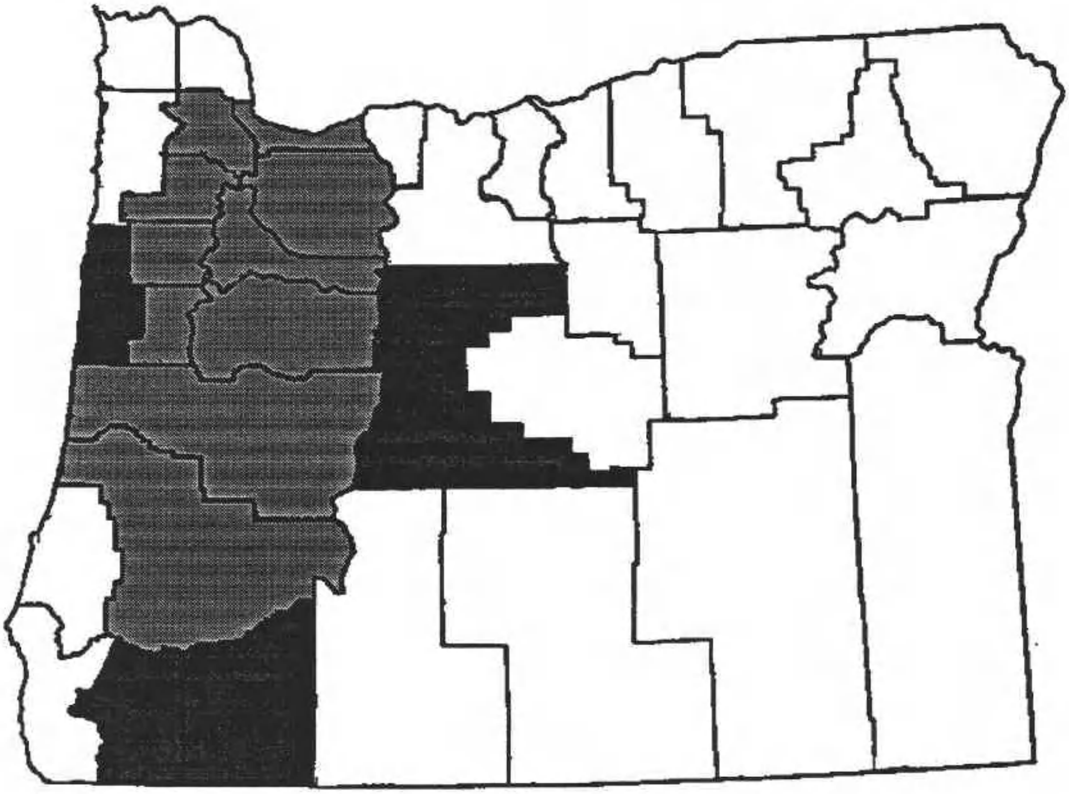

Figure 3.1. Oregon County distributions for *Harmonia axyridis* during 1993 and 1994 (grey) and 1994 only (black).

decreased south of Benton Co. and was 30.4%, 2.2%, and 2.3% in Douglas, Josephine, and Jackson Counties, respectively (Table 3.2). Thus, by 9 July 1994, *H. axyridis* was found in most western Oregon counties, indicating a contiguous distribution from Washington State to California. Collections of *H. axyridis* in this region spanned 1371 m in elevation at the Deschutes Co./ Jefferson Co. border just east of the crest of the Cascade Mountains, to sea level in Newport, Lincoln Co., in the west.

Color polymorphism. Three main adult elytral color-forms of *H. axyridis* were recovered in western Oregon. Most of the adults encountered during the western Oregon distribution survey were of the orange-elytra / black spot morph (*f. succinea*). A black morph with two red elytral spots (*f. conspicua*) was uncommon in southwestern Oregon, comprising 1.4% (N = 414) of *H. axyridis* collected in Lane, Douglas, Jackson, and Josephine Counties. A single specimen of the black form with four red elytral spots (*f. spectabilis*) was encountered only once in the intensive guild sampling in Benton Co (N = 2984).

Relative proportion of adult beetles expressing the *conspicua* (2-red spot) color morph in Benton Co. increased significantly among months from 1.88 % during September, 1993, to 7.69 % during October, 1994 (χ^2 = 17.38, df = 7, P = 0.015). Because the *conspicua* allele is dominant over all others coding for elytral coloration (Komai, 1956), this change in frequencies concurrent with a drastic range expansion following colonization is not surprising if the initial frequency of the *conspicua* allele was very low. Relative abundances of these elytral color-forms in western Oregon are extremely dissimilar to those in Japan, the source area for cultures used in the Washington releases (Coulson, 1992). In Japan, the forms *conspicua* and *spectabilis* predominate, comprising over 60% of individuals in one study (Komai, 1956). Conversely, in the vicinity of Vladivostok, Russia, melanic forms constituted only ca. 10% of adults (Kholin, 1991).

Aphidophagous coccinellid guild composition. A total of 13 coccinellid species in the subfamilies Coccinellinae and Chilocorinae were collected in Benton Co. In timed arboreal samples, *H. axyridis* accounted for 70.4% of all individuals (adults and larvae) and 82.0% of all adult live mass in the overall sampling effort of 14,357 s (Table 3.3). The four most abundant species, *H. axyridis*, *A. bipunctata*, *C. septempunctata*, and *C. polita*, comprised 91.8% of all adult individuals and 96.2% of all adult live mass. These four species comprised 87.3% of all larvae, accounting for 73.8%, 3.5%, 8.4%, and 1.6% of larvae, respectively. The abundance (ρ) of all species differed by month (Table 3.4). Sampling abundance of beetles was highest in May, July, and October, with over 24 beetles per 100 s of sampling effort. Beetles were least abundant in June and August

Table 3.3. Species, number of individuals, and live mass within the guild of arboreal aphidophagous coccinellids in Benton Co., Oregon: April-October 1994

	Trees and Shrubs					Alfalfa, Peppermint, and Clover	
	Adults			Larvae		Adults	
Species	n	% Adults	% Adult Mass	n	% Larvae	n	% Adults
Harmonia axyridis	2079	69.6	81.6	333	75.2	29	3.6
Adalia bipunctata	396	13.3	6.3	15	4.5	4	0.5
Coccinella septempunctata	149	5.0	7.0	4	0.9	214	26.4
Cycloneda polita	116	3.9	1.4	37	8.4	14	1.4
Exochomus quadripustulatus	110	3.7	1.4	25	5.6	0	0.0
Mulsantina picta	35	1.2	0.3	16	3.6	3	0.4
Calvia quatuordecimguttata	17	0.6	0.4	9	2.0	0	0.0
Coccinella trifasciata	25	0.8	0.4	3	0.7	376	46.4
Hippodamia sinuata	29	1.0	0.5	0	0.0	55	6.8
Hippodamia convergens	18	0.6	0.4	1	0.2	73	9.0
Coccinella californica	6	0.2	0.2	0	0.0	14	1.7
Myzia subvittata	2	0.1	0.1	0	0.0	0	0.0
Chilocorus sp.	2	0.1	0.0	0	0.0	0	0.0
Coccinella undecimpunctata	0	0.0	0.0	0	0.0	1	3.6
Total	2984			443			

Table 3.4. Indices describing various attributes of the arboreal coccinellid guild by month. Benton Co., Oregon, April-October, 1994

	Index[1>]					
Month	n	s	ρ	d[2>]	$e^{H'}$	95% C. I. for $e^{H'}$
April	304	9	29.4	0.674	3.1ac[3>]	2.70 - 3.55
May	196	8	24.0	0.735	2.5a	2.14 - 2.98
June	420	8	18.6	0.498	4.2b	3.83 - 4.50
July	1052	10	33.4	0.557	4.0bc	3.42 - 4.75
Aug.	722	11	18.5	0.787	2.5a	2.30 - 2.79
Sept.	148	8	19.7	0.944	1.3d	1.24 - 1.45
Oct.	484	8	24.9	0.963	1.2d	1.14 - 1.35
Apr-Oct	3426	13	23.9	0.704	3.1	2.95 - 3.22

[1>] s = species richness
ρ = sample abundance
d = dominance
$e^{H'}$ = species diversity
n = All adults and larvae in samples in given month.

[2>] Dominant species was *H. axyridis* in each month.

[3>] Estimates in a column followed by the same letter are not significantly different ($\alpha = .05$)

when ca. 18.5 beetles per 100 s were collected. Numerical dominance of larvae by *H. axyridis* was nearly complete during September and October (Table 3.4).

In arboreal samples, guild dominance by *H. axyridis* was weaker on conifers (d = 0.48) than on angiosperms (d = 0.73), and is reflected in the higher species diversity observed on conifers. Species diversity was higher on conifers ($e^{H'} = 3.4$) than on angiosperms ($e^{H'} = 2.9$), however absolute coccinellid abundance was much lower on conifers (13.7 beetles/ 100 s) than on angiosperms (25.9 beetles/ 100 s) (Table 3.5). Species diversity differed significantly by month. It was highest in June and July, moderate in April, May, and August, and very low in September and October because of the near complete dominance of *H. axyridis* in the fall (Table 3.4). Although the arboreal coccinellid guild was dominated by *H. axyridis* each month, this dominance ranged from 49.8% of all coccinellids in the June sampling to 96.3% in October. Sample abundance of adult *H. axyridis* was relatively

Table 3.5. Indices describing various attributes of the arboreal coccinellid guild by host plant. Benton Co., Oregon, April-October, 1994

	Index[1>]				
Plant	Time (sec)	n	s	ρ	$e^{H'}$
Acer platanoides L.	803	111	6	13.8	2.4
Acer saccharum Marsh	638	49	5	7.6	2.9
Betula pendula L.	2579	664	9	25.7	2.2
Humulus lupulus L.	462	119	9	25.8	4.5
Juglans nigra L.	296	84	8	28.4	4.4
Liriodendron tulipifera L.	2307	977	10	42.5	2.2
Picea sitchensis Carr.	136	32	4	23.5	3.3
Pinus contorta Dougl.	195	15	3	7.7	1.6
Pinus mugo L.	383	116	7	30.3	3.1
Pinus sylvestris L.	465	35	6	7.5	4.0
Prunus persica Batsch.	243	75	6	30.9	3.7
Prunus sp.	139	31	4	22.3	3.3
Pseudotsuga menzeisii Franco	573	83	7	14.5	4.0
Quercus garryana Dougl.	95	15	4	15.8	2.0
Quercus palustris Muench.	258	19	4	7.4	2.8
Quercus rubra L.	905	221	6	24.4	2.2
Tilia americana L.	1133	383	8	33.8	2.5
Ulmus americana L.	841	210	9	25.0	2.2

[1>] n = number of larvae and adults, s = species richness, ρ = sample abundance, $e^{H'}$ = species diversity

constant, ranging from 12.8 to 19.8 beetles per 100 s, for all months except May when only 6.8 *H. axyridis* per 100 s were observed.

Twelve species of aphidophagous Coccinellidae were collected in sweep-net sampling of alfalfa, clover, and peppermint. In contrast to its predominance in arboreal samples, *H. axyridis* was a minor guild component in these herbaceous crops, comprising only 3.7% of adult coccinellids (N = 810) and 5.5% of adult mass (Table 3.3). The four most abundant coccinellids in these three crops were *Coccinella trifasciata* LeConte, *C. septempunctata*, *Hippodamia convergens* Guerin-Meneville, and *Hippodamia sinuata* Mulsant, which comprised 46.4%, 26.4%, 9.0%, and 6.8% of adults, respectively. In contrast to the numerical domination of the arboreal guild by *H. axyridis*, the alfalfa, clover, and peppermint systems were dominated each month by *C. trifasciata* ($d = 0.32$ to 0.68), except during June and July when the aphidophagous coccinellid guild in these three crops was co-dominated by *C. trifasciata* and *C. septempunctata*. Because of different sampling methods comparison of the absolute abundance of *H. axyridis* in trees/shrubs versus the crop plants was not possible, but species diversity ($e^{H'}$) of coccinellids was higher in the herbaceous habitat ($e^{H'} = 4.7$) than the arboreal habitat ($e^{H'} = 3.1$).

Phenology. In Benton Co., adult *H. axyridis* dispersed from overwintering aggregations in March of 1994, and comprised 76% of coccinellid adults in timed samples during the first two weeks of April. Adult copulation was documented on 27 March, sporadically through the season and last recorded on 5 October. Oviposition by *H. axyridis* was first observed on 7 April 1994, and continued into October. Larvae were first detected on 9 May, when larvae of *H. axyridis, A. bipunctata*, and *Calvia quatuordecimguttata* L. were collected simultaneously. Abundance of *H. axyridis* larvae was high in May and June (6.5 and 5.9 larvae/100 s, respectively), and then decreased to a low of 0.6 larvae/100 s in August. Larval abundance then increased dramatically through September to 6.1 larvae/100 s in October. The abundance of larvae in late fall was particularly notable because no other species but *H. axyridis* were present as larvae after August (see Table 3.4). The bimodality in larval abundance evident in peaks in April and October suggests bivoltinism, but we observed what appears to be at least a partial third generation in 1993 and 1994. Pupae and larvae were observed in the field as late as 11 November. These late pupae were quite often small in size and very dark in color, indicating larvae were exposed to low temperature (Sakai et al. 1974). Small pupal size was likely a result of low prey availability due to coincidence of leaf fall with presence of third and fourth instars. *Harmonia axyridis* is reportedly multivoltine in Japan.

Annual aggregations were first observed in Benton Co. in late October during 1993 and 1994. Adult *H. axyridis* were observed aggregating on buildings and natural edifices that were prominent, exposed and, often, light in color. All color forms, *succinea, spectabilis,* and *conspicua*, were observed to aggregate together. Some beetles of the orange *succinea* morph were very light in color, a condition that often indicates recent adult emergence (Authors, personal observation), and supports the hypothesis that the final generation proceeds directly to overwintering sites. No other species of coccinellid was observed in these aggregations.

Table 3.6. Host plant and prey records for *Harmonia axyridis* in Benton County, Oregon, 1992-1994.

Host Plant	Prey	*H. axyridis* Life Stage
Abies procera Rehder	*Cinara* sp.	L, A
Acer saccharum Marsh	*Periphyllus testudinaceae* (Fernie)	A
A. saccharum	*Drepanaphis idahoensis* Smith & Dilley	E, L, P, A
A. saccharum	*Drepanosiphum platanoides* (Schrank)	E, L, P, A
Betula pendula Roth	*Euceraphis betulae* (Kalterbach)	E, L, P, A
B. pendula	*Callipterinella calipterus* (Hartig)	L, P, A
Cirsium arvense (L.) Scop.	*Aphis fabae* Scopoli	L, A
Dipsacus sylvestris Huds .	*Macrosiphum rosae* (L.)	L, A
Fagus sylvatica L.	*Phyllaphis fagi* (L.)	P, A
Humulus lupulus L.	*Phorodon humuli* (Schrank)	L, P, A
Liriodendron tulipifera L.	*Illinoia liriodendri* (Monell)	E, L, P, A
Medicago sativa L.	*Acyrthosiphon pisum* (Harris)	A
Mentha piperita L.	*Ovatus crataegarius* (Walker)	A
Nasturtium sp.	*Aphis fabae* Scopoli	E, L, P, A
Prunus sp. (Plum)	*Hyalopterus pruni* (Geoffrey)	A
Quercus rubra L.	*Myzocallus occultus* Richards	L, P, A
Rosa sp. (Ornamental)	*Macrosiphum rosae* (L.).	L, A
Salix sp. (Exotic)	*Tuberolachnus salignus* (Gmelin)	A
Spirea douglasi Hook.	Pollen & Nectar	L, P, A
Tilia americana L.	*Eucalypterus tiliae* (L.)	E, L, P, A

Prey species. On 17 host-plants, a total of 17 species of aphids were identified as prey for *H. axyridis* (Table 3.6). Adult and immatures co-occurred in all but four cases, including alfalfa and peppermint, confirming the habits of *H. axyridis* as a polyphagous, arboreal predator (Hodek, 1973). The apparent ability of *H. axyridis* to complete development on the black bean aphid (*Aphis fabae* Scopoli) is interesting in that many species cannot develop on, and usually will not eat, this aphid.

Parasitism. A braconid, *Dinocampus coccinellae* (Schrank), was the only parasitoid reared from *H. axyridis* collected in Oregon. Rates of parasitism were low, less than 1%. Only two *D. coccinellae* were observed from over 2000 wild adult and larval beetles held in mass cultures in the laboratory. The wasps were reared from two field collected adults collected in Benton Co. No parasites were reared from beetles collected as immatures.

The dominance of the guild of arboreal, aphidophagous coccinellids in western Oregon by *H. axyridis* is remarkable in both rate of geographic spread and the magnitude of the resulting population. Low rates of parasitism, bivoltinism, and polyphagy may all have contributed to the dominance of the arboreal habitat by *H. axyridis*. The range of the species in the Pacific Northwest is still increasing to the south, and relative abundances of species of arboreal coccinellids are likely still in flux. Because of the fortuitous spread of this species into Oregon, biological control of arboreal aphids, e.g. filbert aphid and pests of urban shade trees such as tulip poplar, linden, and European birch, is likely to be improved.

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Chapter 4

Temperature-dependent egg development and survival in some arboreal and semi-arboreal coccinellids in western Oregon

Abstract

Temperature-dependent rates of egg hatch and survival were assessed at seven constant temperatures from 10-34°C for of nine species of arboreal and semi-arboreal, aphidophagous coccinellids occurring in western Oregon. Survival of eggs differed among species within temperatures, and among temperatures within species. Species differed in both real and physiological time to egg hatch at constant temperatures. Lower-threshold temperatures ranged from 6.7°C for *Calvia 14-guttata* to 12.6° for *Olla v-nigrum*. Heat-units above these respective thresholds for eclosion differed among species and ranged from 30.5DD° for *O. v-nigrum* to 53.2DD° for *C. 14-guttata*. Based on a novel ranking procedure that incorporates both hatch time and survival, species were classified on a scale of cold to warm temperature adapted. Of the two introduced species, *Coccinella septempunctata* L. was more adapted to very warm temperatures than were other species in its guild. Conversely, *Harmonia axyridis* Pallas was moderately adapted to cooler temperatures, relative to other species.

Introduction

At least 180 species of homopterous Coccinellidae have been imported to North America for classical biological control programs since the late 1880's, and over 91% have failed to establish (Gordon,1985). Successfully introduced species have often dominated the aphidophagous coccinellid guild. For example, *Coccinella septempunctata* L. was first recorded as established in New Jersey in 1973, and comprised over 85% of individuals amongst the 16 aphidophagous coccinellid species in a marsh habitat, *C. septempunctata* (Angelet et al., 1979). *Hippodamia variegata* (Goeze) is another Old World coccinellid that was intentionally released for biological control of aphids (Gordon, 1985). Similarly, this species dominated the coccinellid guild on weedy, disturbed sites in New England, accounting for 37% of individuals, at the time of its first United States record.

Numerical domination of coccinellid guilds by exotic species has also been documented in the Pacific Northwest. *Coccinella undecimpunctata* L was released in Washington in 1965 and by 1970 had become widespread in British Columbia (Frazer & Gilbert, 1976) A study of aphidophagous coccinellids in herbaceous crops in southwestern British Columbia suggested that the increase in numbers of *C. undecimpunctata* there was partly due to this species developmental pre-adaptation to the cool, maritime climate of the area (Frazer & McGregor, 1992).

In western Oregon, 13 species of aphidophagous coccinellids are commonly found in trees and shrubs, but only four - *Harmonia axyridis* Pallas, *Adalia bipunctata* (L.), *C. septempunctata*, and *Cycloneda polita* Casey - are common (see Chapter 3). *Harmonia axyridis* and *C. septempunctata* are Old World natives recently introduced into the Pacific Northwest for biological control of homopterous pests (Coulson, 1992; Gordon and Vandenberg, 1991). During six-months of collecting from arboreal habitats in Benton Co., Oregon, these four species accounted for over 96%, and *H. axyridis* alone comprised over 70%, of all individuals. One possible reason for the dramatic domination of arboreal coccinellids by *H. axyridis* is that intrinsic, autecological attributes of this species confer a competitive advantage.

Temperature-dependent development and survival, higher fecundity, and larval voracity are properties that may combine to pre-adapt coccinellids to local climates (Frazer & McGregor, 1992, Stewart et al., 1991b). Because coccinellid species are highly polyphagous and often co-occur (see Chapter 3), immatures and eggs face hazards to which they must adapt. For example, coccinellid eggs are especially at risk of cannibalism and intraguild predation from adults and larvae (Agarwala & Dixon, 1993). Large coccinellids are both less likely to suffer intraguild predation than smaller ones (J. C. Miller, unpublished), and require more food resources to reach their large size. Thus, in a potentially hostile community, advantages to rapid eclosion of coccinellid eggs include reduced risk of cannibalism (Brown, 1972; Kaddou, 1960) and predation (Agarwala & Dixon, 1992), enhanced survival through ingestion of unhatched coccinellid eggs (Brown, 1972), and synchrony with appropriate-sized aphid prey (Frazer, 1988).

This paper examines differences in temperature-dependent rates of survival and development for eggs of nine species of arboreal and semi-arboreal, aphidophagous coccinellids commonly found in western Oregon. If thermal adaptation of *H. axyridis* has been a factor in its spread through Oregon, we would expect to observe differences from native guild members in its egg survival and / or speed of hatching. The goals of this study were to : 1) document size differences among co-occurring aphidophagous coccinellids, 2) measure temperature-dependent survival of eggs among these species, and 3) test for differences in developmental threshold temperatures and heat-unit requirements for egg hatch in each species.

Materials and Methods

Adult *H. axyridis, A. bipunctata, C. polita, C. septempunctata, Hippodamia sinuata* Mulsant, *Coccinella californica* Mannerheim, *Mulsantina picta* (Randall), and *Calvia quatuordecimguttata* L. were collected in the vicinity of Corvallis, Benton Co., Oregon during the summers of 1992 and 1994. Adult *Olla v-nigrum* (Mulsant) were collected near Medford, Jackson Co., Oregon, in July of 1994.

Adult beetles were immediately weighed and maintained in the laboratory at 22 ± 2°C and a 16 L:8 D photoperiod. All beetles were held in 1 oz. plastic cups with cardboard tops and fed the pea aphid, *Acyrthosiphon pisum* (Harris), which were reared on fava bean (*Vicia fabae* L.) in a greenhouse. Five females of each species contributed eggs to this study with the exception of *H. axyridis* for which 13 females were used. Adult beetles were inspected every 12 h for oviposition and were supplied with fresh aphids. Numbers of eggs in each clutch were recorded, and the whole clutch was assigned as a cohort to a temperature treatment. Seven constant temperature treatments were used: 10, 14, 18, 22, 26, 30, and 34°C; 16 L:8 D; 50 - 70% RH. Eggs were inspected every 12 h for eclosion and mortality. When eclosion was asynchronous within a clutch, first instars were documented and removed, and the remaining unhatched eggs immediately returned to the temperature treatment. Eggs that had turned brown and shriveled were counted as dead and removed from the experiment.

Differences in egg survival among species and temperature-treatments were analyzed via G-test for independence (Sokal & Rohlf, 1981). Differences in time to eclosion were analyzed by one-way ANOVA within each temperature treatment (Statistical Graphics, 1986), and homogeneous groups of mean hatch times were differentiated with Duncan's new multiple range test (Zar, 1974). Assuming rapid hatch is advantageous, a novel ranking method was devised to compare relative adaptedness of the eggs of these nine species at different temperatures. At each temperature, species were ranked one through nine based on the mean time required to hatch (one = slowest, nine = fastest), and these ranks were then downwardly adjusted to account for temperature-dependent mortality by multiplying each species rank by that species probability of hatching (= % survival) at that temperature. Thus, the most 'adapted' species at a temperature could have a maximum adaptedness index of nine if its eggs hatched soonest, and it suffered no mortality.

Linear regressions of developmental rates (1/days) as the dependent variable on temperature were used to establish lower developmental thresholds (mean x-intercept) and total degree-day requirements for eclosion (1/ slope estimate) (Stinner et al., 1974). Because development rate becomes non-linear relative to temperatures near thermal thresholds, best linear models for each species were chosen by excluding extreme temperature-treatments to form reduced models and comparing these with the full model (all temperature treatments) via extra-sum-of-squares F-tests (Ramsey & Schafer, unpublished manuscript). Confidence intervals for degree-day (DD°) and threshold (D_{th}) estimates were constructed based on standard error estimates for linear model parameters (slope and y-intercept) from regression output (Statgraphics v 5.0). For D_{th} (the x-intercept), confidence intervals were calculated as the difference between the two most extreme slope and y-intercept values from their respective 95% confidence intervals.

Results

Beetle Size. Weights of field collected adult beetles differed significantly among species (one-way ANOVA, $F = 582.5$, $DF = 14, 2912$, $P < 0.001$) (Fig 4.1). *Coccinella septempunctata* and *H. axyridis* were the largest and second largest species, weighing (mg. ± SEM) 39.6 mg. ± 1.7 and 33.8 mg. ± 0.6 respectively, and are the only two guild members not native to North America. These species average three to five times larger than the smallest species, *M. picta* (7.9 mg. ± 0.5) and *C. polita* (10.0 mg. ± 0.5). The largest native species were *C. californica* (22.3 mg. ± 1.3)

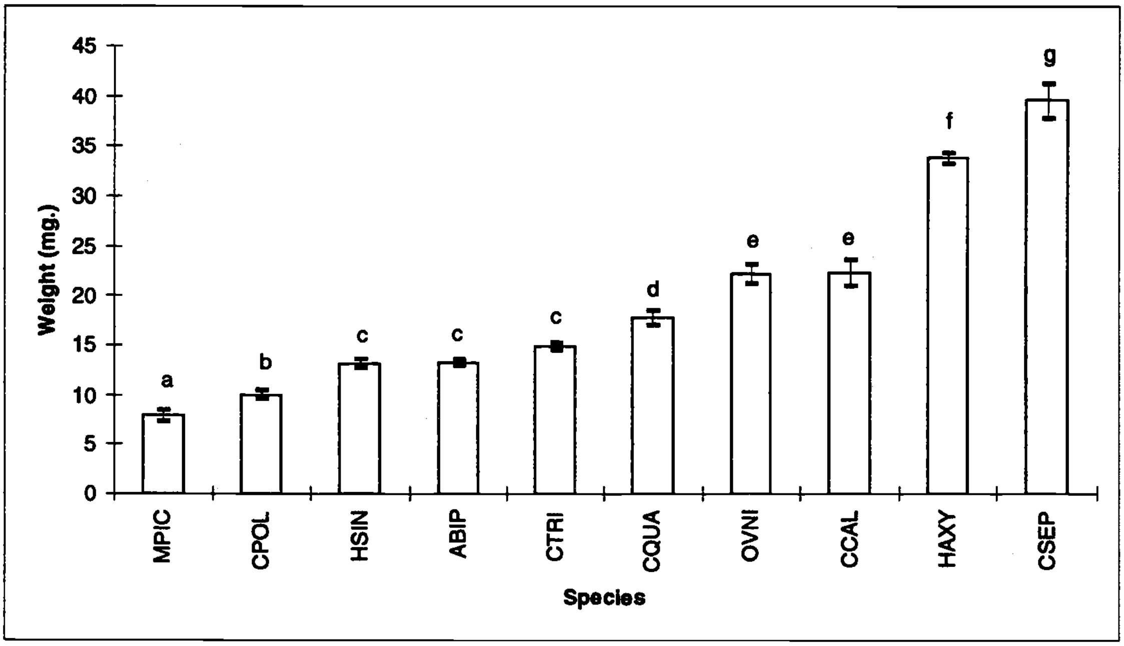

Figure 4.1. Field weights (mean ± SEM) of ten aphidophagous coccinellid species

and *O. v-nigrum* (22.1 mg. ± 0.9). Among the intermediate-sized species, *C. quatuordecimguttata* was larger (17.8 mg. ± 0.8) than *C. trifasciata* (14.9 mg. ± 0.3), *A. bipunctata* (13.2 mg. ± 0.3), and *H. sinuata* (13.1 mg. ± 0.4).

Temperature-dependent egg survival. Within each of seven temperatures (10 - 34°C), survival among these nine species differed significantly (G 101.8 - 454.3, DF = 1, $P < 0.001$) (Table 4.1). Similarly, within each species survival differed among temperature treatments (G = 88.8 - 677.8, DF = 1, $P < 0.001$). Thus, for each temperature, eggs of some species survived better than others, and for each species certain extreme temperatures were more lethal than others. At 34°C, *C. californica, C. septempunctata, H. sinuata,* and *O. v-nigrum,* had eclosed (%) at 41.8, 88.0, 69.4, and 67.3, respectively. The tolerance of the eggs of these four species to high temperature is also evident at 26 and 30° where they showed consistently higher survival than any other species, while suffering average to very high mortality at 14°C. Similarly, species that showed the highest survival at 14°C tended to suffer the most mortality at high temperatures. For example, *A. bipunctata* and *C. polita* hatched at levels of over 70% at 14°, but suffered over 50% mortality at 30°C and complete egg death at 34°. At 14°C hatching success was greatest for *A. bipunctata* (73.3%), *C. quatuordecimguttata* (97.3%), *C. polita* (88.2%), and *H. axyridis* (73.5%).

Table 4.1. Temperature-dependent mortality (%) for eggs of nine species of aphidophagous, arboreal coccinellids

Species	10°C	14°C	18°C	22°C	26°C	30°C	34°C
Ca. 14-guttata	10.0	2.7	0.0	7.0	31.1	30.2	100
Cy. polita	100	11.8	18.6	18.7	24.1	52.3	100
Ha. axyridis	100	26.5	19.3	39.1	39.9	46.9	100
Ad. bipunctata	100	26.7	45.6	25.8	33.0	51.2	100
Co. californica	100	29.5	5.6	36.5	8.2	19.4	58.2
Co. septempunctata	100	30.5	4.4	10.0	19.7	11.3	12.0
Mu. picta	100	35.0	17.4	69.4	68.4	100	100
Ol. v-nigrum	100	65.4	8.9	11.0	1.9	3.5	32.7
Hi. sinuata	100	97.1	15.7	24.2	5.3	18.6	30.6

Table 4.2. Average hatching time (days) for eggs of nine species of aphidophagous, arboreal coccinellids

Species	10°C	14°C	18°C	22°C	26°C	30°C	34°C
Ca. 14-guttata	12.9 a	7.3 a	5.9 b	3.8 c	2.5 b	2.5 d	N / A
Mu. picta	N / A	12.8 b	7.1 e	4.0 d	3.0 cd	N / A	N / A
Ad. bipunctata	N / A	12.9 b	5.4 a	3.6 b	3.0 cd	2.3 c	N / A
Ha. axyridis	N / A	13.6 b	6.6 cd	3.8 c	2.9 c	2.5 d	N / A
Ol. v-nigrum	N / A	14.9 c	6.5 c	3.2 a	2.3 a	2.1 ab	2.2 b
Cy. polita	N / A	15.7 cd	6.0 b	4.1 de	3.0 cd	2.5 d	N / A
Co. septempunctata	N / A	17.4 ef	5.9 b	3.8 c	2.6 b	2.0 a	2.0 a
Co. californica	N / A	18.0 f	6.9 de	4.4 f	3.0 cd	2.1 bc	2.5 d
Hi. sinuata	N / A	22.0 g	8.2 f	4.8 h	3.1 d	2.5 d	2.2 b

1> Species within a temperature treatment followed by the same letter are not significantly different (Duncan's multiple range test, $\alpha = 0.05$)

Egg death at 10°C was complete for all species except *C. quatuordecimguttata* which achieved 90% eclosion.

Temperature-dependent egg development. There was significant variation among species in the real time (days) and physiological time (degree-days) required for successful eclosion. Among species, time to eclosion differed significantly at each temperature treatment (one-way ANOVA, F = 2,600.8 - 34,995.7, P < 0.001). At 14°C *C. quatuordecimguttata* hatched an average of nearly two weeks faster (7.3 d) than the slowest species, *H. sinuata* (22.0 d) (Table 4.2). Also, time to hatch was generally low for *A. bipunctata* (12.9 d), *H. axyridis* (13.6 d), and *M. picta* (12.8 d). As temperature increased, the significant differences in development time between species decreased in number and magnitude. For example, at 22°C O. v-nigrum had the fastest eclosion (3.2 d), while the slowest species, *H. sinuata*, hatched an average of 1.6 d later. At 30°C, *C. septempunctata* was the fastest hatching species (2.0 d) but averaged only a 12-hour advantage over the slowest species, *C. quatuordecimguttata*.

The relative adaptedness index (RAI) which incorporated both time to hatch and mortality showed that thermal adaptedness within the guild varied with species and temperature (Table 4.3). For example, eggs of *C. quatuordecimguttata* were the most adapted of the nine species at 14°C (RAI = 8.76), followed by *M. picta* (5.20) and *A. bipunctata* (5.13). Eggs of *O. v-nigrum, C. septempunctata, C. californica*, and *H. sinuata* ranked lowest (RAI < 2.1). At 18°, *C. septempunctata* and *O. v-nigrum* began increasing in rank, and at warmer temperatures (30 & 34°C) ranked as the most adapted. *H. axyridis* shows adaptation to cooler temperatures, and decreases steadily from 4.41 at 14° to a low of 2.1 at 30°. *Hippodamia sinuata* ranked lowest (RAI < 1.0) at the four lowest temperatures. Because of mortality no species achieved the perfect rank of nine. Only one species hatched successfully at 10°, and that treatment was excluded from the rank procedure.

Interspecific differences in developmental rates were differentiated according to best linear models (Table 4.4). For each species studied, rates were linear over the majority of temperature treatments. The rate of development was linearly related to temperatures between 10 and 30°C for *A. bipunctata* and *C. quatuordecimguttata* only. Other species expressed linear developmental rates from 14 to 26°C, except

Table. 4.3. Relative adaptedness index (RAI)1> at constant temperatures of eggs of nine species of aphidophagous, arboreal coccinellids.

Species	14°	18°	22°	26°	30°	34°	(°C)
Ca. 14-guttata	8.76	7.50	5.58	5.51	2.44	0.00	
Mu. picta	5.20	1.65	1.22	1.11	0.00	0.00	
Ad. bipunctata	5.13	4.90	5.94	2.35	2.93	0.00	
Ha. axyridis	4.41	3.23	3.65	3.61	2.10	0.00	
Cy. polita	3.53	4.88	2.44	2.66	1.67	0.00	
Co. septempunctata	2.08	7.17	5.40	4.82	7.98	8.82	
Ol. v-nigrum	1.73	4.55	8.01	8.83	7.24	5.05	
Co. californica	1.41	2.83	1.27	3.21	6.05	2.51	
Hi. sinuata	0.03	0.84	0.76	0.95	2.85	5.21	

1> Rank of species mean time to hatch @ given temperature (1 = slow, 9 = fast) multiplied by probability of successful eclosion.

C. septempunctata, H. sinuata, and *O. v-nigrum*, for which rate of development at 30°C was included. From these equations, lower-developmental threshold temperatures (D_{th}) and heat-units over that threshold required for eclosion (DD°) were determined (Table 4.5). Values of D_{th} differed between species, and ranged from a low of 6.7°C for *C. quatuordecimguttata* and 10.1°C for *A. bipunctata*, to a high of 11.9 - 12.6°C for *C. septempunctata, H. sinuata*, and *O. v-nigrum*. Intermediate thresholds (10.8 - 11.7°) were observed for *C. californica, C. polita*, and *H. axyridis*, among which they did not differ. Heat-unit requirements for egg hatch differed significantly among species and ranged from a low of 30.5 DD° for *O. v-nigrum* and 36.5 DD° for *C. septempunctata*, to a high of 53.2 DD° for *C. quatuordecimguttata* and 47.3 DD° for *M. picta*.

Table 4.4. Temperature-dependent development parameters for eggs of nine species of aphidophagous, arboreal coccinellids

Species	Equation	F	P	R^2	N	Best Model
Ad. bipunctata	y = -0.227656 + 0.022432 (°C)	8,622	<0.0001	92.0	753	10 - 30°C
Ca. 14-guttata	y = -0.126157 + 0.018789 (°C)	2,838	<0.0001	88.7	364	10 - 30°C
Co. californica	y = -0.264516 + 0.022703 (°C)	11,029	<0.0001	98.0	221	14 - 26°C
Co. septempunctata	y = -0.326168 + 0.027425 (°C)	34,996	<0.0001	98.5	530	14 - 30°C
Cy. polita	y = -0.247133 + 0.022547 (°C)	8,671	<0.0001	98.5	314	14 - 26°C
Ha. axyridis	y = -0.257973 + 0.023467 (°C)	33,884	<0.0001	97.2	969	14 - 26°C
Hi. sinuata	y = -0.280135 + 0.022614 (°C)	12,740	<0.0001	97.1	383	14 - 30°C
Mu. picta	y = -0.227593 + 0.021145 (°C)	2,198	<0.0001	96.5	80	14 - 26°C
Ol. v-nigrum	y = -0.413605 + 0.032771 (°C)	2,600	<0.0001	93.2	192	14 - 30°C

Table 4.5. Estimates of developmental threshold values and degree-days to eclosion for nine species of aphidophagous coccinellids

Species	D_{th} (°C)	95% Conf. Interval	DD°	95% Conf. Interval
Ca. 14-guttata	6.7 a[1>]	6.2 - 7.2	53.2 f	52.2 - 54.2
Ad. bipunctata	10.1 b	9.8 - 10.5	44.6 d	44.1 - 45.1
Mu. picta	10.8 bc	10.2 - 11.4	47.3 e	46.3 - 48.3
Cy. polita	11.0 c	10.6 - 11.3	44.4 d	43.9 - 44.8
Ha. axyridis	11.0 c	10.8 - 11.2	42.6 c	42.4 - 42.8
Co. californica	11.7 cd	11.4 - 12.0	44.0 d	43.6 - 44.5
Co. 7-punctata	11.9 de	11.7 - 12.1	36.5 b	36.3 - 36.6
Hi. sinuata	12.4 e	12.1 - 12.7	44.2 e	43.8 - 44.6
Ol. v-nigrum	12.6 e	12.0 - 13.6	30.5 a	29.9 - 31.1

[1>] Species within a column followed by the same letter are not significantly different (95% confidence intervals, $\alpha = 0.05$)

Discussion

Species with reduced D_{th} values are predicted to begin embryogenesis at lower ambient temperatures than others and may, depending on the heat-unit requirement to complete development, hatch sooner. Based on the estimates of D_{th} and DD°, predicted times to hatch for the nine species were calculated (Table 4.6). The benefit of low threshold temperatures is realized most at low temperatures. For example, *C. septempunctata* has a significantly higher threshold than *H. axyridis*, and at 15° should hatch significantly slower. However, because of the relatively small heat-unit it requires, *C. septempunctata* should be at a competitive advantage at temperatures of 18° or greater. The effect of disparate heat-unit requirements when threshold are equivalent is exemplified by D_{th} group "c" (see Table 4.5), which includes, *M. picta, C. polita, H. axyridis,* and *C. californica*. Although threshold values of these three species do not differ, their heat-unit requirement does, and the lowest is that of *H. axyridis* which is predicted to hatch sooner at four temperatures (Table 4.6).

Table 4.6. Predicted hatch times of nine species of aphidophagous coccinellids at constant temperatures

Species	15°	20°	25°	30°C
Ca. 14-guttata	6.4	4.0	2.9	2.3
Ad. bipunctata	8.7	4.3	2.9	2.1
Mu. picta	11.3	5.1	3.3	2.5
Cy. polita	11.1	4.9	3.2	2.3
Ha. axyridis	10.7	4.7	3.0	2.2
Co. californica	13.3	5.3	3.3	2.4
Co. septempunctata	11.8	4.5	2.8	2.0
Hi. sinuata	17.0	5.8	3.5	2.5
Ol. v-nigrum	12.7	4.1	2.5	1.8

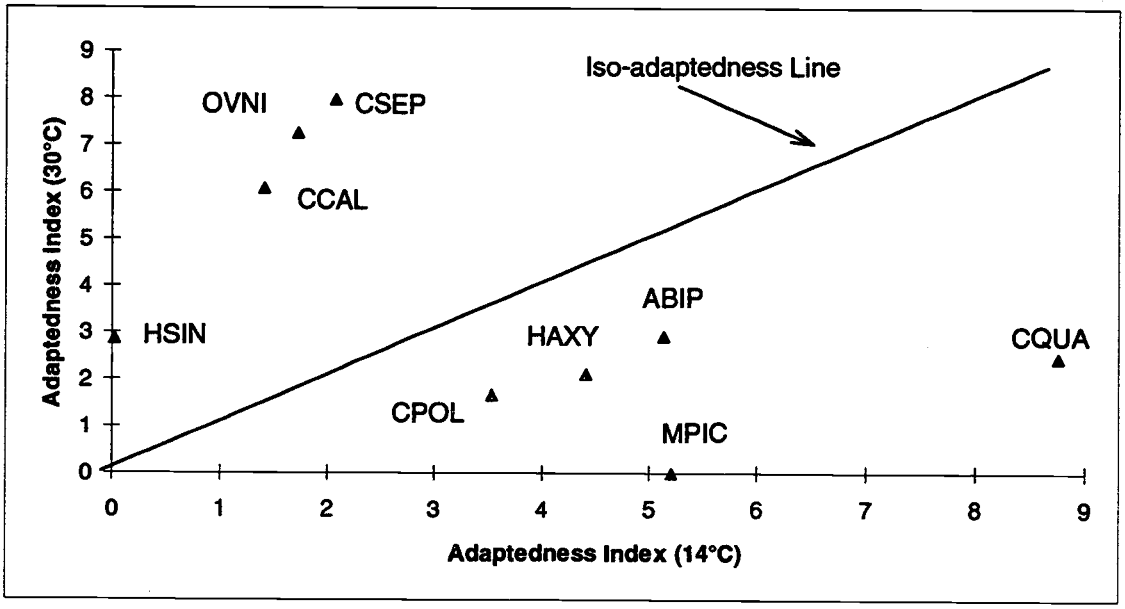

Figure 4.2 Relative adaptedness index (RAI) for eggs of nine species of coccinellids at two extreme temperatures.

Predicted times for egg hatch also show that at 15°C, *C. quatuordecimguttata* and *A. bipunctata* are the only species tested that should have an advantage in development time over *H. axyridis*. For example, although *H. axyridis* is predicted to develop an average of two days sooner than *O. v-nigrum* at 15°, the effect of that advantage is multiplied by the ca. 40% better survival of *H. axyridis* in that thermal regime (Table 4.1). A comparison of the two extreme temperatures for which RAI was calculated demonstrates this more clearly (Fig. 4.2)

When relative adaptedness indices of the eggs of these nine species are contrasted at the coolest and warmest temperatures, a pattern is evident. The line in the center of Fig. 4.2 represents the set of values that RAI could take for a species that is equitably adapted at both warm and cool temperatures. Such a theoretical 'thermal-generalist' was not found among the nine species studied. Instead, the species that were able to hatch at 34°C are all above this line indicating they are more tolerant of warm than cool temperatures, and this is not surprising. However, the utility of the RAI concept may lie in its ability to quantify thermal tolerance among species in a guild as the distances from the iso-adaptedness line. For example, *C. septempunctata* and *C. quatuordecimguttata* show a dramatic tradeoff in tolerance between extreme temperatures, while *H. axyridis* and *A. bipunctata* do not.

Interestingly, the two most recently introduced species show dissimilar response to temperature, although they are roughly comparable in size Enhanced survival of *C. septempunctata* at warm temperatures is contrasted by the moderate pre-adaptation to cooler temperatures shown by *H. axyridis*. Although temperature-dependent development profiles for *H. axyridis* in this study support this attribute conferring a moderate advantage in cool climates, this remains to be proved in the field. The role of such enhanced temperature-dependent development in one dominant species in determining guild structure and function should be investigated for aphidophagous predators.

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Chapter 5

A comparison of temperature-dependent development among three seasonal generations of *Harmonia axyridis* Pallas

Abstract

Temperature-dependent development of *Harmonia axyridis* Pallas, a coccinellid newly introduced for biological control of Homoptera, was compared among three seasonal generations. The lower developmental threshold for immature development averaged 11.2°C and did not differ among the three seasonal populations. However, populations did differ in real time and total heat-units required to complete development, ranging from 252.0 to 290.2 DD°. The lower threshold for egg, larval, and pupal development differed between generations. The lower threshold temperature and heat-unit requirement for total development are significantly higher in Oregon than is reported for a strain of this species introduced into France. The relevance of differences in developmental rate traits in aphidophagous coccinellids is discussed.

Introduction

In the practice of classical biological control, individuals of unknown genetic make-up are selected from a source population, artificially reared to increase numbers of individuals and remove parasites, and then released (DeBach, 1964). The haphazard selection of individuals during exploration may result in laboratory populations with different genotypic / phenotypic profiles than source population because random variation may be amplified during mass rearing (Hoy, 1979; Mackauer, 1976). Traits conferring climatic adaptation are known to vary within populations of natural enemies (Hoy, 1979). One component of climatic adaptation, developmental rate, has been demonstrated to vary within populations of aphidophagous Coccinellidae. For example, variation in temperature-dependent development rates has been demonstrated within a population of the lady beetle *Coccinella trifasciata* LeConte

(Miller, in press). Also, Rodriguez-Saona (1994) demonstrated that this characteristic varied within a local population of *Hippodamia convergens* Guerin-Meneville, and that this trait responded to directional selection in the laboratory.

In contrast, interpopulation differences may be negligible. Miller (1992) studied temperature-dependent development among populations of *H. convergens* from Arizona and Oregon and compared his findings with published reports for New York and Texas populations. Populations did not differ in either lower threshold temperatures or total heat-unit requirement for development. Thus, although temperature-dependent development has been shown to vary within populations of aphidophagous Coccinellidae, constancy in these traits among populations has also been demonstrated. Whether the environments of release areas act as a selective force on developmental rate attributes of introduced natural enemies has not been investigated for a coccinellid, and the recent introduction of one such species to the United States provided an opportunity to do so.

An aphidophagous lady beetle, *Harmonia axyridis* Pallas, was released in the United States as part of a classical biological control program focusing on various arboreal, homopterous pests (Coulson,1992; Chapin and Brou, 1991). Native to western and central Asia, beetles collected from Japan, Korea, China and Russia were the source of cultures subsequently released during 1978-82 (Coulson, 1992), and the species has been established in Washington and Oregon since 1991. Releases made in Washington of Japanese source-material are the probable source of *H. axyridis* populations that have expanded south into Oregon (see Chapter 1).

Because *H. axyridis* is presently undergoing a range expansion following its introduction to the Pacific Northwest, the goal of this study was to assess whether temperature-dependent development rates of this species in western Oregon differed: 1) from previously reported results on development from egg to adult and 2) among generations during expansion of its range. A study of temperature-dependent development of this species in France has been published (Schanderl et al., 1985) and this provides the basis from which to test whether temperature-dependent development traits differ in *H. axyridis* across geographical populations. If generations display different developmental threshold and/or degree-day requirements for development, we may conclude that the species is undergoing selection for these traits. Four outcomes to selection on temperature-dependent development traits are possible. Lower thresholds and / or total heat-units required for development may 1) increase through time, 2) decrease through time, 3) oscillate about some average value, or 4) remain fixed.

Materials and Methods

Three separate temperature-dependent development studies were undertaken. Adult female *H. axyridis* were collected during fall (November), 1993, spring (April), 1994, and fall (September) 1994, and their F_1 progeny were used in seperate studies of developmental rates. Adults were reared individually in the laboratory at 22° ± 2°C, and fed pea aphids, *Acyrthosiphon pisum* (Harris), cultured in a greenhouse on fava bean, *Vicia fabae* L. Eggs from seven to eleven field-collected females were placed individually into clear, paper-topped, 28-ml plastic cups within 12 h of oviposition, and systematically assigned to a temperature treatment to assure equal representation of females. Development through egg, larval, and pupal stages was observed at seven constant temperatures: 10, 14, 18, 22, 26, 30, and 34°C, at 16:8 (L:D), and 50-70% RH. All individuals were monitored every 12 h for eclosion, molts, and mortality. Because of egg desiccation at 34°C, newly hatched first instars (n = 50; 56 in fall 1994 trial) were moved to that temperature from 30°C to observe larval-adult development. Only individuals completing pupation were used to assess larval developmental times. Larvae were kept supplied with excess pea aphids. Each individual was weighed 24 h after pupation.

Statistical analyses were conducted to assess effects of temperature on mortality, developmental times, and pupal weights. All tests for significance were conducted at $\alpha = 0.05$. Temperature-dependence of mortality was assessed via a G-test for independence (Sokal & Rohlf 1981). Equality of development times among seasonal populations was tested by one-way ANOVA at each temperature. Analysis of developmental rates of eggs, larvae, and pupae, was via simple linear regression models (Statistical Graphics 1986). For development rates, the inverse of duration of each life stage (1/days to molt) was used as the dependent variable and constant temperatures as independent variable. These models estimate total degree-day requirements as the inverse of the slope parameter, and lower developmental-threshold as the x-intercept (Stinner et al. 1974). Differences in developmental heat-unit requirement (DD°) and developmental threshold (D_{th}) were differentiated by confidence intervals of these parameters. Best models were chosen by testing linear models with all temperature-treatments included against models with extreme temperature treatments removed, using extra-sum-of-squares F-tests (Ramsey & Schafer, unpublished).

Results and Discussion

Mortality. Total mortality from first instar to adult differed significantly among the three seasonal generations at 34° (G = 52.2, DF = 1, P < 0.001), 30° (G = 8.27, DF = 1, P = 0.004) and 14°C (G = 80.66, DF = 1, P < 0.001), but did not differ at 26° (G = 0.48, DF = 1, P = 0.498), 22° (G = 2.94, DF = 1, P = 0.086), or 18° (G =0.88, DF = 1, P = 0.348). Average mortality at 26, 22, and 18°C was 7.2, 8.5, and 14.5%, respectively (Fig. 5.1). At 34°C, mortality was relatively high for all three generations,

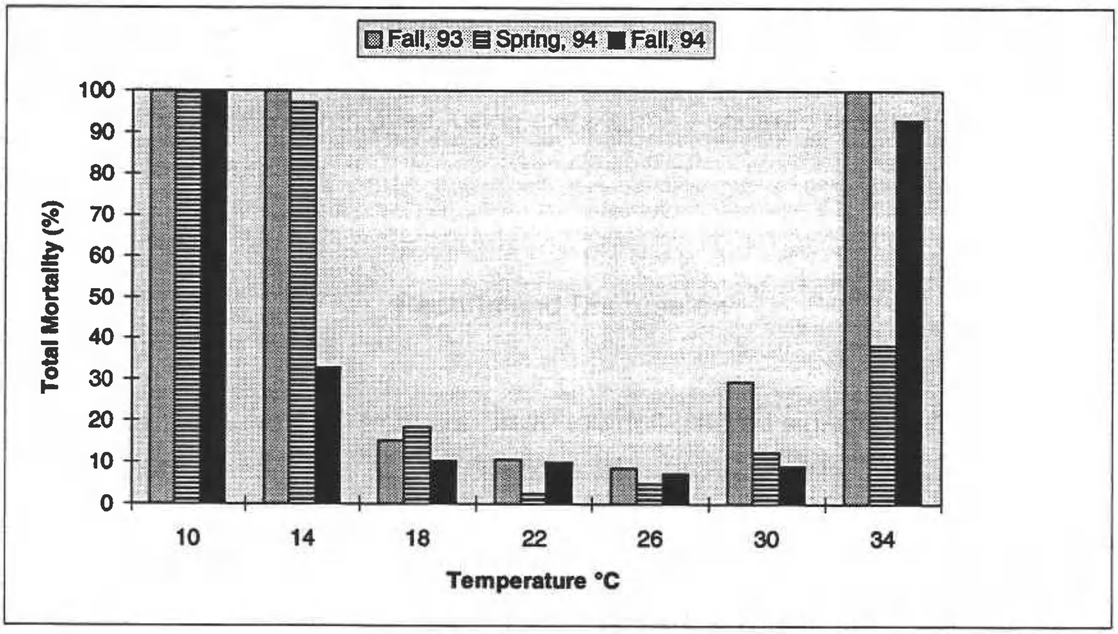

Figure 5.1. Mortality for three seasonal generations of *Harmonia axyridis* at constant temperatures.

and was 100% for fall, 1993 beetles. Mortality at 30° ranged from 29.4% for the fall, 1994 generation to 9.1% for fall, 1993 progeny. At 14°C, mortality was very high for both fall, 1993 (100%) and spring, 1994 (97.4%) generations, but considerably lower (32.2%) for the fall, 1994 beetles.

Mortality at those temperatures where differences existed is difficult to interpret as being correlated with other temperature-dependent development traits (see below). The spring 1994 generation had high mortality at the cool extreme (14°C) and low mortality at the warm extreme (34°C). This is odd because these were progeny of females that had overwintered and that had, presumably, been selected for cold hardiness. The opposite is true of the fall 1994 generation, for which the parental generation had experienced summer-long selection for heat tolerance and yet suffered very high mortality at warm temperatures.

Development times and rates. Average time to complete development ranged from 14.0 days at 30° for the spring 1994 generation to 45.9 days at 18°C for the fall, 1994 generation (Fig. 5.2). Development time from egg-to-adult differed among the three generations at 18° (F = 9.34, DF = 2,129, $P < 0.001$), 22° (F = 95.21, DF = 2,145, $P < 0.001$), 26° (F = 59.95, DF = 2,137, $P < 0.001$) and 30°C (F = 11.83, DF = 2,114, $P < 0.001$). Average egg-to-adult development time was lower for the spring 1993 generation than for the others at 30, 26, and 22°C. At 18°C the fall 1993 generation generations was significantly faster than the other two. Statistical

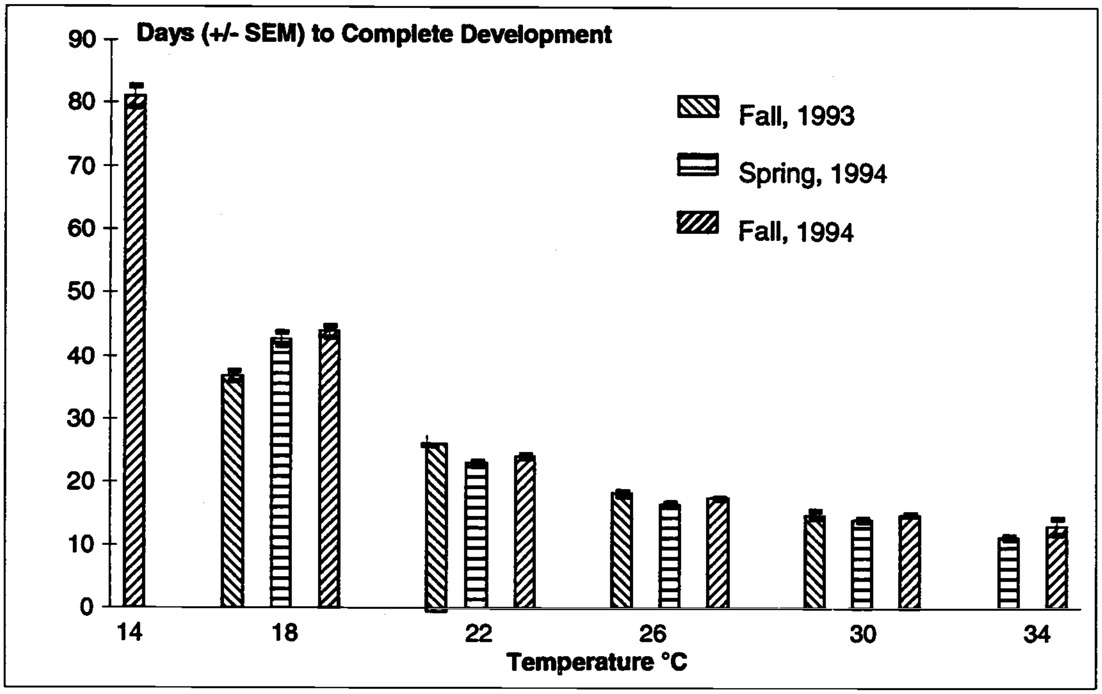

Figure 5.2. Days for egg-to-adult development for three seasonal populations of *Harmonia axyridis*.

comparison of development times at extreme temperatures (14, 30, and 34°C) was not conducted because of mortality-induced, low sample sizes.

Days required to complete development at each temperature were lowest for the spring 1994 population at each temperature except 18°, for which the fall 1993 developed significantly faster than did the other two populations. Total development time did not differ between the fall 1993 and fall 1994 populations at 30°, but was significantly faster for the latter at 22 and 26°C.

From these development times, temperature-dependent development rates and D_{th} values for each life stage of each population were calculated (Table 5.1). Overall, average D_{th} values among life stages ranged from 8.7° for eggs of the fall 1993 beetles to 11.4°C for pupal development of the fall 1994 population. Thresholds of life-stages did not differ within seasonal populations except for the fall 1993 population, where egg-hatch threshold was ca. 2°C lower than respective larval and pupal thresholds. Constancy among lower thresholds for development among life stages is unusual for aphidophagous coccinellids. Thresholds are reported to increase by about 1°C for each successive stadium in many species (Miller and Paustian, 1992; see also Chapter 4).

As was true of total development, the lower threshold for completion of four larval stages did not differ among the three populations. However, heat-units required for completion of larval stages did differ among all three populations and was lowest for the spring 1994 population (143.0 DD°), highest for the fall 1993 population (172.9 DD°), and intermediate for the fall 1994 population (143.0 DD°). Threshold for egg hatch was lowest for the fall 1993 population (8.7°C) and differed from that of the fall 1994 population (11.0°C), while the spring 1994 threshold (10.6°C) did not differ from either. The thermal requirement for the completion of pupation differed in both D_{th} and DD° values. The spring 1994 population required 74.9 DD° above a D_{th} of 10.0°C, and this did not differ from the 70.7 DD° over 10.8°C for the fall 1993 beetles. However, progeny of the fall 1994 females had a significantly elevated threshold for pupation (11.4°C) than the spring 1994 generation, as well as a lower heat-unit requirement (65.7 DD°) than either of the other two.

When the three generations are taken as a composite, they develop significantly more slowly from egg to adult than did the population of *H. axyridis* studied in France, especially at cooler temperatures. Although the source of the French population and the selection procedure used during introduction is unknown,

Table 5.1 Lower developmental thresholds and heat-unit requirements of life-stages of three seasonal populations of *Harmonia axyridis* from Corvallis, Benton County, Oregon.

		Fall 1993		Spring 1994		Fall 1994	
Life Stage		Estimate	C.I.	Estimate	C.I.	Estimate	C.I.
Eggs	DD°[1]	48.6 b	46.9 - 50.4	45.1 a	43.5 - 46.8	45.5 a	45.3 - 45.8
	D_{th}[1]	8.7 a	7.6 - 9.9	10.6 ab	9.3 - 11.9	10.5 b	10.4 - 10.7
Larva	DD°	172.9 c	168.7 - 177.5	143.0 a	139.3 - 146.8	161.7 b	159.7 - 163.7
	D_{th}	10.7 a	9.8 - 11.6	11.3 a	10.4 - 12.3	10.8 a	10.4 - 11.3
Pupae	DD°	70.7 b	69.2 - 72.3	74.9 b	73.1 - 76.9	65.7 a	64.9 - 66.6
	D_{th}	10.8 ab	10.1 - 11.6	10.0 a	9.1 - 10.9	11.4 b	11.0 - 11.8
Total	DD°	290.2 c	284.6 - 296.0	252.0 a	248.5 - 255.6	274.9 b	272.4 - 277.4
	D_{th}	10.6 a	9.9 - 11.2	11.4 a	10.8 - 11.9	10.9 a	10.6 - 11.2

[1] Numbers in a row followed by the same letter are not significantly different (95% confidence interval)

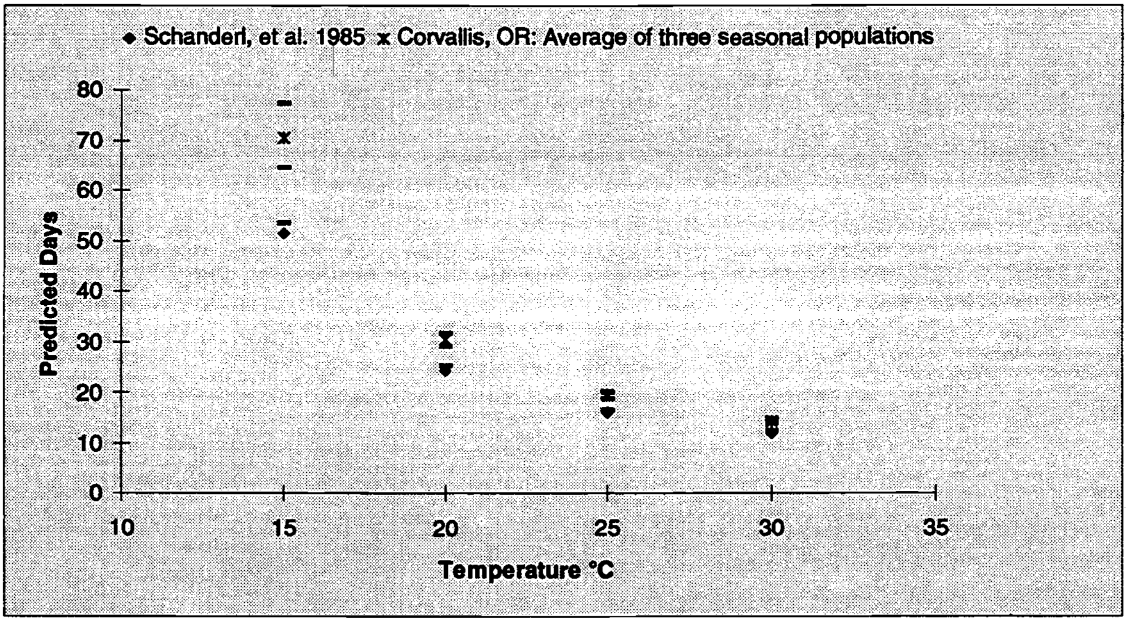

Figure 5.3. Comparison of predicted time to complete development between two geographically disparate populations: *Harmonia axyridis* in France vs. Corvallis, Oregon

the relevance of this disparity to biological control is clear. Variation in intrinsic traits such as temperature-dependent development in natural enemies should be considered before introductions.

All three seasonal generations of *H. axyridis* in western Oregon differed in total heat-units required for complete development but not in lower thresholds (Table 5.1). Using the combined estimate of D_{th}, the lowest heat-unit requirement for egg-to-adult development was that of the spring generation, which required 22.9 DD° less than the fall 1994 generation and 38.2 DD° less than the fall 1993 generation. Thus we reject three hypotheses of change in D_{th} values. Lower thresholds of the three seasonal populations of *H. axyridis* did not 1) increase, 2) decrease, or 3) oscillate over time. The hypothesis of no change in this trait could not be rejected. Conversely, changing values for total thermal requirement among seasonal generations force us to reject the hypotheses of 1) no change, 2) increase, and 3) decrease, but are consistent with an oscillation in this trait over time. Thus, in a western Oregon population *of H. axyridis* there is presently variation in temperature-dependent development traits, and evidence presented here suggests that natural selection may be changing the norm of that variation as this species expands its range.

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Chapter 6

Temperature-dependent development in a polymorphic ladybeetle, *Calvia quatuordecimguttata* (L.) (Coleoptera: Coccinellidae)

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Chapter 6

Temperature-dependent development in a polymorphic ladybeetle, *Calvia quatuordecimguttata* (L.) (Coleoptera: Coccinellidae)

Abstract

The influence of temperature on the development rate and expression of three elytral color patterns in the lady beetle *Calvia quatuordecimguttata* (L.) was assessed at 6 constant temperatures between 10 and 34°C. The expression of the three elytral color patterns was discrete and independent of temperature treatment. No differences were observed among the color morphs in the real or physiological time for egg-to-adult development, or pupal weight. However, temperature did influence survival and growth rates for the overall population. No eggs hatched at 34°C. Larval mortality was high at 10° (44%) and moderate (5-25%) between 14° and 30°C. Development time from oviposition to adult was 14.6 ± 0.9 days at 30°C and 95.6 ± 9.0 days at 10°C. The total heat-unit requirement for egg to adult development was 274.1 degree-days above a mean developmental-threshold of 8.2°C. Pupal weight differed among temperature treatments and was greatest at 10 to 22°C, and lowest at 26 and 30°C.

Introduction

Temperature-dependent development in temperate-zone aphidophagous coccinellids has been relatively well studied. Numerous studies have shown that temperature experienced by immature stages directly affects mortality, development-rates, and size (e.g. Miller 1992; Orr & Obrycki 1990; Obrycki & Tauber 1981) Developmental thresholds and heat-unit requirements may differ within and between species. For example, different developmental-thresholds and heat-unit requirements have been observed in isofemale lines of *Hippodamia convergens* Guerin (Rodiguez-Saona 1994) and *Coccinella trifasciata* LeConte (Miller, In Press). Also, development rates for larvae have been reported to vary with prey. Michels & Behle (1991) showed that *Hippodamia sinuata* Mulsant expressed different threshold and heat-unit requirements on different diets. Thus, developmental rates may differ among species, female source, and prey item. However, no studies have investigated differences in temperature-dependent development among color variants of a polymorphic species.

The polymorphic ladybeetle *Calvia quatuordecimguttata* (L.) is endemic to western and central Asia (Lusis 1971), North America and Europe (Gordon 1985). An uncommon and primarily arboreal predator of psyllids and aphids in Western Oregon (Belnavis 1989; Messing 1982), *C. quatuordecimguttata* is often associated with *Adalia bipunctata* (L.), *Harmonia axyridis* Pallas, *Olla v-nigrum* (Mulsant), and *Coccinella septempunctata* L. (see Chapter 1). Three color variants of adults occur sympatrically in North America: black elytra with 14 white spots (WB); black elytra with two or four red spots (RB); and orange elytra with twelve black spots (BO). Until 1976 the BO color morph was considered a distinct species, *Calvia duodecimmaculata* Korschefsky (Belicek 1976). We have observed all three morphs in the progeny of a single field-collected female.

The biology of *C. quatuordecimguttata* is poorly known, including the relationship between temperature, larval development, and adult coloration. The species provides an opportunity to investigate various aspects of the adaptive ecology of polymorphisms. It is possible that a particular genotype that confers a specific coloration pattern also confers other specific autecological attributes. For example, in the pepper moth (*Biston betularia* L.) melanic (*f. carbonaria*) individuals are thought to differ physiologically from the non-melanic (*f. typica*) morph in their ability to tolerate pollutants (Lees & Creed 1975). Similarly, coccinellid larvae that express a particular adult coloration may also express different temperature-dependent development rates, lower developmental-threshold temperatures, or size. Also, elytral coloration in some polymorphic coccinellids is dependent on rearing temperature. This has been shown for *Epilachna chysomelina* (F.) (Timofeeff- Ressovsky 1941) and *H. axyridis* (Sakai et al. 1974), but has not been investigated for species of *Calvia*.

This study was designed to test two hypotheses. The first hypothesis was that coloration of adult *C. quatuordecimguttata* is dependent upon rearing temperature experienced during larval/pupal development. This hypothesis could be rejected if similar proportions of each color morph occurred among the F_1 adults reared from larvae/pupae exposed to different constant temperature treatments. The second hypothesis we tested was that each morph of *C. quatuordecimguttata* would express a unique developmental threshold and degree-day requirement. We could reject this hypothesis if lower developmental-threshold temperatures, and total heat-unit requirements, were similar among color-morphs.

Materials and Methods

Adults representing each of the three color variants of *C. quatuordecimguttata* were collected from broadleaf trees between March and July, 1992, in the vicinity of Corvallis, Benton Co., Oregon. The beetles were reared individually in the laboratory at 22° ± 2°C, and fed pea aphids, *Acyrthosiphon pisum* (Harris), cultured in a greenhouse on fava bean, *Vicia fabae* L. All eggs from females of each color morph were placed individually into clear, paper-topped, 28-ml plastic cups within 12 h of oviposition, and systematically assigned to a temperature treatment. Development through egg, larval, and pupal stages was observed at six constant temperatures: 10, 14, 22, 26, 30, and 34°C, at 16:8 (L:D), and 50-70% RH. All individuals were monitored every 12 h for eclosion, molts, and mortality. Because of egg desiccation at 34°C, newly hatched first instars (n = 20) were moved to that temperature from 30°C to complete development. Only individuals completing pupation were used to assess developmental times. Treatment sizes, initially 22-74, were reduced over time because of mortality. Larvae were kept supplied with excess pea aphids. Each individual was weighed 24 h after pupation. The color morph of the F_1 adult was then recorded to assess temperature effects on morph frequency, as well as differences in developmental rates and pupal weights for each morph.

Statistical analyses were conducted to assess effects of temperature on morph frequency, mortality, developmental times, and pupal weights. All tests for significance were conducted at $\alpha = 0.05$. Independence of F_1 elytral color-morph on larval temperature treatment was subject to χ^2 analysis (Ott 1988). Independence of color expression upon rearing temperature was tested among two treatment categories: warm (26 and 30°C) and cool (14 and ca. 15°C). The additional temperature treatment (ca. 15°C) was included in this assessment of temperature-dependent morph expression to provide a larger sample size, and is excluded from other analyses.

Temperature-dependence of mortality was assessed via a G-test for independence (Sokal & Rohlf 1981). Analysis of developmental rates and proportion of time spent as egg, larva, and pupa, included both simple and multiple linear regression models (Statistical Graphics 1986). For development rates, the inverse of duration of each life stage (1/days to molt) was used as the dependent variable, the constant temperatures as an independent variable, and F_1 elytral color-morph as indicator variables (see Table 3). These models estimate total degree-day requirements as the inverse of the slope parameter, and lower developmental-threshold as the x-intercept (Stinner et al. 1974). The multiple regression model which includes coloration variables tests for significant interactions between F_1 elytral color variant and development rate at each temperature. Between-morph differences in developmental heat-unit requirement (DD°) and developmental threshold (D_{th}) were differentiated by confidence intervals about these parameters, as well as by testing full and reduced regression models via extra sum-of-squares F-tests. Dependence of pupal weight upon color was tested via Duncan's new multiple range tests for each temperature treatment.

Results and Discussion

Field collection. A total of 99 adult beetles were collected from ornamental white birch (Betulaceae: *Betula pendula* L.) infested with *Euceraphis betulae* (Kalterbach), and tulip trees (Magnoliaceae: *Liriodendron tulipifera* L.), infested with the aphid *Illinoia liriodendri* (Monell). The ratio of the three morphs was 3.7(WB): 3.6(RB): 1.0(BO). Mean weights of field collected beetles did not differ among morphs, and were (mg. ± SD): 18.02 (± 4.18), 17.44 (± 4.13), and 17.99 (± 5.08), for WB, RB, and BO morphs, respectively.

Color expression of F_1 beetles. A total of 179 adult F_1 beetles were reared and subject to χ^2 analysis based temperature treatment and F_1 elytral coloration (Table 6.1). There was sufficient evidence to reject the null hypothesis of dependence of F_1 elytral-coloration upon temperature treatment ($\chi^2 = 1.09$; df = 2; P = 0.58). The proportion of beetles expressing each coloration was independent of temperature treatment experienced by immature stages. Additionally, there appeared to be no

Table 6.1. Chi-square analysis of temperature dependence of morph expression in *Calvia quatuordecimguttata*.

		F_1 Color Morph					
Temperature treatment	N	WB	RB	BO	χ^2	df	P
Hot (26° and 30°C)	84	33	33	18	1.09	3	0.58
Cool (14° and ca. 15°C)	95	38	43	14			

major alteration of color patterns within F_1 morphs correlated with temperature. Patterns in elytral coloration were discrete and fixed within the three morphs.

The absence of a temperature effect upon coloration in *C. quatuordecimguttata* suggests that each morph is genetically based. For the congener, *Calvia punctata* Mulsant, inheritance of a similar elytral color-trimorphism was shown to be consistent with three alleles operating at one locus in a sequence of complete dominance (Lusis 1971). Further studies on the inheritance of the color trimorphism in *C. quatuordecimguttata* are planned. Because these color morphs appeared to be based on particular genotypes, we investigated the possibility that each genotype also expressed different developmental attributes.

Temperature-dependent development and pupal weight of F_1 beetles by morph. Color morphs of *C. quatuordecimguttata* did not differ in developmental attributes. Based on linear-regression analyses of developmental rates between 10 and 26°C, developmental-threshold estimates (°C ± SE) for each morph were: 8.3° (± 0.8°) for WB beetles, 8.2° (± 0.7°) for RB beetles, and 8.6° (± 1.9°) for BO beetles (Table 6.2). Likewise, total heat-unit requirements (degree-days ± SE) did not differ among morphs, and were: 269.3 (± 7.0) for WB beetles, 277.2 (± 7.0) for RB beetles, and 265.1 (± 16.0) for BO beetles.

A multiple regression analysis of development rate on temperature, using F_1 color morph as independent variables (Table 6.3; Eq. [2]), was not better ($F = 2.03$; df = 1,119; $P = 3.09$; extra-sum-of-squares F-test) than the simple linear model (Eq. [1]) which included temperature as the only independent variable and ignored any correlation of adult color with developmental rate. Likewise, adding temperature and

Table 6.2. Confidence intervals (α = 0.05) for developmental thresholds and heat-unit requirements for three morphs of *Calvia quatuordecimguttata*

Morph	D_{th}	95% C. I.	DD°	95% C. I.
Black elytra w/ 14 white spots (WB)	8.3°	7.5 - 9.1°	269.3	262.4 - 276.6
Black elytra w/ 2 or 4 red spots (RB)	8.2°	7.5 - 9.0°	277.2	270.2 - 284.6
Orange elytra w/ 12 black spots (BO)	8.6°	6.7 - 10.6°	265.1	249.2 - 283.1

F_1 coloration interaction terms (Eq. [3]) did not improve the fit of the simple model (F = 1.12; df = 4,117; P = 1.12; extra-sum-of-squares F-test), and provided sufficient evidence to reject the null hypothesis of correlation of temperature-dependent development traits with elytral coloration.

Pupal weight did not differ among morphs within any temperature treatment (Table 6.4). One way ANOVA procedures for pupal weight by color morph were insignificant at 10° (F = 0.09, df = 8, P = .77), 14° (F = 0.08, df = 36, P = .45), 22° (F = 0.38, df = 36, P = .68), 26° (F = 0.86, df = 45, P = .43), and 30°C (F = 0.31, df = 37, P = .74). Thus, we were forced to reject the hypothesis of dependence pupal weight upon color morph. All morphs reached equivalent size after accounting for the effects of temperature treatment.

Overall temperature-dependent development and survival. For *C. quatuordecimguttata* as a whole (all three morphs combined), egg nonviability and mortality (G = 48.7; df = 1; P < 0.001), larval mortality (G = 28.0; df = 1; P < 0.001), and pupal mortality (G = 32.1; df = 1; P < 0.001) were significantly different by temperature treatments (Table 6.5). No eggs hatched at 34°C. No first instars transferred to 34° from 30° survived to the third instar. Egg mortality of was 10.0, 12.7, and 0% for 10, 14, and 18°C, respectively (Table 6.5). Eclosion of first instar *C. quatuordecimguttata* was consistently higher than reported for *A. bipunctata, C. septempunctata*, and five other species of coccinellids in British Columbia (Frazer &

Table 6.3. Comparison of linear regression degree-day models for different color variants of *Calvia quatuordecimguttata*

Eq. #	Model[1>]	F	df	P	r^2
[1]	$M({}^{1}/\text{days})=\beta_0+\beta_1(^\circ\text{C})$	3832.0	1,128	<.0001	0.97
[2]	$M({}^{1}/\text{days})=\beta_0+\beta_1(^\circ\text{C})+\beta_2F_{1RB}+\beta_3\,F_{1BO}$	1074.8	3,119	<.0001	0.96
[3]	$M({}^{1}/\text{days})=\beta_0+\beta_1(^\circ\text{C})+\beta_2F_{1BO}+\beta_3F_{1RB}+\beta_4F_{1RB}\,{}^*(^\circ\text{C})+\beta_5F_{1BO}\,{}^*(^\circ\text{C})$	638.6	5,117	<.0001	0.96

[1>] RB = Red-on-Black variant

BO = Black-on-Orange variant.

Table 6.4. Development time (days) for *Calvia quatuordecimguttata* at constant temperatures by F_1 color morph

		Temperature / F_1 Morph												
Life Stage		10°				14°				18°	22°			
		WB	RB	BO	All	WB	RB	BO	All	All	WB	RB	BO	All
n		7	2	0	9	12	21	4	37	22	12	16	2	30
Egg	Avg.	12.7	11.0	N/A	12.3	7.2	7.4	7.2	7.3	6.5	3.9	3.8	3.8	3.8
	SD	0.6	1.4	N/A	1.1	0.9	1.1	1.0	1.0	0	0.2	0.3	0.3	0.3
Larva	Avg.	54.5	50.0	N/A	53.5	27.9	26.6	28.4	27.2	19.8	13.2	12.9	12.0	13
	SD	7.9	6.4	N/A	7.5	2.6	1.8	2.5	2.2	0.9	1.0	1.1	N/A	1.0
Pupa	Avg.	29.3	29.0	N/A	29.2	13.0	12.8	12.8	12.8	N/A	5.1	5.2	5.0	5.2
	SD	1.9	0	N/A	1.6	1.2	1.1	1.2	1.1	N/A	0.7	0.4	0.5	0.6
Pupal Wt. (mg.)	Avg.	18.6	17.9	N/A	18.5	20.2	19.9	18.1	19.8	N/A	19.5	19.4	19.4	19.4
	SD	2.9	2.3	N/A	2.7	2.8	2.8	2.9	2.8	N/A	2.1	2.5	N/A	2.3
Total	Avg.	96.5	90.0	N/A	95.1	48.1	46.8	48.4	47.4	N/A	22.6	22.1	20.5	22.2
	SD	9.3	7.8	N/A	8.9	4.1	3.5	2.7	3.6	N/A	1.2	1.3	N/A	1.2

Table 6.4. continued

26°				30°			
WB	RB	BO	All	WB	RB	BO	All
24	17	6	47	10	15	10	35
2.5	2.6	2.4	2.5	2.4	2.5	2.5	2.5
0.2	0.2	0.4	0.2	0.4	0.2	0.3	0.3
8.7	8.7	9.3	8.8	8.8	8.4	9.2	8.8
0.6	0.5	1.2	0.7	1.2	0.6	0.8	1.0
3.5	3.7	3.6	3.6	3.2	3.5	3.5	3.4
0.3	0.4	0.4	0.3	0.6	0.5	0.4	0.5
18.2	17.9	16.6	17.9	16	16.6	17.1	16.6
2.7	2.6	3.0	2.7	1.4	1.3	3.3	2.2
14.7	14.9	15.3	14.9	14.3	14.4	15.2	14.6
0.6	0.7	1.5	0.8	0.6	0.7	1.1	0.9

Table 6.5. Percent mortality for each life-stage of *Calvia quatuordecimguttata* at constant temperatures

Life Stage	Temperature°C													
	10		14		18		22		26		30		34	
Egg	10.0%	(50)[1]	12.7%	(55)	0.0%	(22)	7.0%	(43)	31.1%	(74)	30.2%	(63)	100%	(51)
First instar	28.9%	(45)	16.7%	(48)	0.0%	(22)	17.3%	(40)	3.9%	(51)	11.4%	(44)	0.0%	(20)[2]
Second instar	6.2%	(32)	2.5%	(40)	4.5%	(21)	0.0%	(33)	2.0%	(49)	0.0%	(39)	35.0%	(13)
Third instar	0.0%	(30)	2.6%	(39)	0.0%	(21)	9.0%	(33)	2.1%	(48)	5.1%	(39)	100%	(0)
Fourth instar	16.7%	(30)	2.6%	(38)	0.0%	(21)	0.0%	(30)	0.0%	(47)	5.4%	(37)	N/A	(0)
Instar I-IV	44.4%	(45)	22.9%	(48)	4.5%	(22)	25.0%	(40)	7.8%	(51)	20.5%	(44)	100%	(20)
Pupa	64.0%	(25)	0.0%	(37)	N/A[3]		0.0%	(30)	0.0%	(47)	0.0%	(35)	N/A	(0)
Egg-Adult	82.0%	(9)	32.7%	(37)	N/A		30.2%	(30)	36.5%	(47)	44.4%	(35)	100%	(0)

1. Number beginning each life stage noted in parenthesis
2. Twenty first instars transferred from 30°C
3. Data not available

McGregor 1992) regardless of temperature. Egg death of *Hippodamia convergens* Guerin was 80% at 17°C (Miller 1992).

Larval mortality of coccinellids is often more severe at temperatures near developmental thresholds, as was the case for *C. quatuordecimguttata*. Larval mortality was relatively high (44%) at 10°C, and relatively low (7.8 - 25.0%) for temperature treatments between 14°C and 30°C. Similar levels of larval mortality were reported for *Eriopis connexa* Mulsant (Miller & Paustian 1992), which was categorized as a relatively cold-adapted species, while 61% of *H. parenthesis* larvae died at 14°C (Orr & Obrycki 1990). There was no pupal mortality in *C. quatuordecimguttata* between 14-30°. However, 64.0% of the pupae died at 10°C. Overall, mortality in the immature stages of *C. quatuordecimguttata* is low at cool temperatures relative to other aphidophagous coccinellids.

Weight of pupae differed among temperature treatments (one way ANOVA, $F = 7.18$, $df = 146$, $P < .0001$). Pupal weight was greatest at 10, 14, and 22°C, among which it did not differ, and least at 26 and 30°C (Duncan's new multiple range test). By comparison, mean adult weight in *H. parenthesis* was greatest at 18 and 22°C, and lowest at 10, 26, and 30°C (Orr & Obrycki 1990). Adult *H. axyridis* were heaviest at 20-30°C, and least so at 15°C (Schanderl et al. 1984). Thus, while size of aphidophagous coccinellids are reported to differ with temperature treatment, *C. quatuordecimguttata* is unusual among coccinellids in achieving maximum size at low temperatures.

Development time from oviposition to adult ranged from 14.5 days at 30°C to 114 days at 10°C (Table 6.4). The developmental threshold (D_{th}) and degree-day (DD°) requirement for each life stage of *C. quatuordecimguttata* were determined (Table 6.6). Values for D_{th} are low relative to those of other coccinellids, but increase by ca. 1°C for each life stage, a trend reported for many species (e.g. Obrycki & Tauber 1981). To complete egg to adult development, *C. quatuordecimguttata* required 274 DD° above a D_{th} of 8.2°C. One possible advantage of such a low threshold for development is the predicted speed of development at low temperatures. At 14 and 18°C, *C. quatuordecimguttata* is expected to develop faster than its common associates *A. bipunctata* and *C. septempunctata* (Obrycki & Tauber 1981), and *H. axyridis* (Schanderl et al. 1985).

In our study concerning the developmental biology of *C. quatuordecimguttata* we rejected our two hypotheses: 1) that F_1 color pattern was dependent upon larval/pupal rearing temperature, and 2) that temperature-dependent development

Table 6.6. Developmental threshold (D_{th}) and degree-day (DD°) requirements (± SE) of life stages of *Calvia quatuordecimguttata*

Life Stage	D_{th}	DD°	Equation	r^2
Egg	7.1 ± 0.2	50.8 ± 0.9	$y = -0.14078+0.019685x$	0.89
Larva	8.3 ± 0.2	162.6 ± 2.9	$y = -0.05089+0.006151x$	0.94
Pupa	9.1 ± 0.2	62.4 ± 1.3	$y = -0.14502+0.016024x$	0.93
Egg-Adult	8.2 ± 0.2	274.1 ± 4.4	$y = -0.02989+0.003649x$	0.97

differed among, and was correlated with, F_1 elytral color morph. Temperature treatment was not associated with departures from expected outcomes of morph expression, and all morphs had equivalent mean development times and pupal weights. Overall, *C. quatuordecimguttata* exhibits numerous attributes of a cold-adapted, early spring species. For example, completion of development from egg to adult in *C. quatuordecimguttata* at 10°C is unusual among the temperate-zone aphidophagous coccinellids. Also. relatively high survival at low temperatures, particularly 10 and 14°C, is exceptional. The low developmental threshold, low mortality, and substantial weight gain at low temperatures all conform with field observations of that *C. quatuordecimguttata* is as an early spring, high-latitude/altitude species (Semyanov 1980; Belicek 1976). Future studies of the function of color polymorphism in this species should investigate possible thermal or aposematic advantages conferred by coloration upon adults.

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Chapter 7

Summary

The intentional release to the state of Washington of *Harmonia axyridis,* an exotic coccinellid, has resulted in the fortuitous spread of this beetle to western Oregon. It was found to comprise over 70% of all coccinellids in an arboreal habitat in Benton County, Oregon, while accounting for over 82% of coccinellid biomass. In sampling of herbaceous, perennial crops *H. axyridis* was a minor component of the coccinellid fauna, comprising only 4% of coccinellids. In western Oregon, this species was associated with 16 species of homopteran prey on 16 host plants. Rates of parasitism were quite low, less than 1%, and the only parasite reared from over 2000 adults and larvae was the braconid, *Dinocampus coccinellae* (Schrank). Phenology of *H. axyridis,* determined by field sampling, indicates at least two generations per year in western Oregon. This species was observed forming large aggregations in late October of 1993 and 1994. The range of the species appeared to be extending south at the end of Summer, 1994.

The rapid establishment and expansion of the range of *H. axyridis,* as well as the high relative abundance of this species in Benton Co., leads to questions of mechanisms successful colonization by new species. One possible mechanism of preadaptation in poikilotherms- favorable temperature-dependent development and survival - was investigated for the eggs of nine species of western Oregon coccinellids. The nine species studied differed significantly in lower thresholds for development and heat unit requirements for egg hatch. In terms of rapidity and success of larval eclosion, *Olla v-nigrum* (Mulsant), *Coccinella californica* Mannerheim, *C. septempunctata* L., and *Hippodamia sinuata* Mulsant were most adapted to warm or very warm temperatures, while *Cycloneda polita* Casey, *H. axyridis, Adalia bipunctata* (L.), *Mulsantina picta* (Randall), and *Calvia quatuordecimguttata* L. were most adapted at cool to very cool temperatures. Thus, variation in this intrinsic trait may confer different competitive advantages in different thermal regimes. Of these nine species, egg adaptedness of *H. axyridis* was comparatively poor at all temperatures, especially so above 18°C.

Assessment of degree-day requirements in three seasonal generations of H. axyridis from western Oregon showed that the species lower developmental threshold temperature has not responded to a selective pressure in its new habitat, but that the total heat-unit requirement may have. For the three populations sampled (fall 1993, spring 1994, fall 1994), D_{th} did not differ and averaged 11.2°C. However, total heat-units above the 11.2° threshold required for development differed among all three populations, and was least for spring 1994 (252.0 DD), moderate for fall 1994 (274.9 DD), and greatest for fall 1993 (290.2 DD). Thus, generations with higher heat-unit requirements are predicted to take longer to complete development than those with a lesser requirement. One implication of this intra-population variation in degree-day profile to classical biological control may be the potential for selection of exotic natural enemies to more closely fit release area climate and guild constraints.

No variation in degree-day profiles among different forms of the polymorphic lady beetle, *C. quatuordecimguttata*, was demonstrated. In this species three elytral/notal color-forms are known, and it provides an opportunity to test hypotheses about the adaptiveness of coloration in different thermal environments. All three form (white/black; red/black; and black/orange) had equal mean development times, rates, and pupal weights in each temperature treatment. Additionally, unlike some Coccinellidae, expression of coloration in *C. quatuordecimguttata* was demonstrated to be independent of temperature treatment.

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