Introduction into California of Cold-tolerant Biotypes of the Mealybug Predator, *Cryptolaemus montrouzieri*,[1] and Laboratory Procedures for Testing Natural Enemies for Cold-hardiness[2]

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ABSTRACT

A review is presented of the initial importation of the Australian mealybug lady beetle, *Cryptolaemus montrouzieri* Mulsant, in 1891–92 from mild climatic areas of eastern Australia, and the subsequent history of its periodic releases against mealybugs in California. Since its introduction, this predator, which can rarely survive California winters, has been maintained continuously in insectary culture and redistributed on a world-wide basis without further additions to its gene pool. New clones of the predator, collected in 1972 in 2 of the coldest areas of Australia, were propagated and colonized in California to try to broaden the genetic base for cold-hardiness of the species.

Special equipment was designed for laboratory testing of the cold tolerance of this predator. In tests with this equipment at −5.0°C for less than 12 h, the old (1891) race of *C. montrouzieri* was shown to be less cold-hardy than the newly imported biotypes. Evidence is supplied that a simple test of adult survival after 12-h exposure to −5.5± 0.5°C affords an easy procedure for measuring the cold-hardiness of this and other natural enemy species.

The Initial Introduction of *Cryptolaemus montrouzieri* Mulsant into California and the Program for its Periodic Release

In 1891–92, Albert Koebele sent several colonies of the predaceous lady beetle, *Cryptolaemus montrouzieri* Mulsant, from Australia to California for control of mealybugs attacking citrus (Koebele 1893). Neither the exact numbers received for propagation in California nor the exact origin of the several shipments are known, but available records indicate that the collections were probably from the environs of Sydney, N. S. W., and Brisbane, Queensland. Both of these localities have considerably milder winter climates than California. After being colonized in California citrus orchards heavily attacked by the citrus mealybug, *Planococcus citri* (Risso), these lady beetles sometimes showed remarkable ability to destroy many kinds of mealybugs (Hoyt 1912), but generally died out each winter except in some of the very warmest locations (Smith and Armitage 1920). Later, with the discovery that citrus mealybugs could be propagated on etiolated potato sprouts (Branigan 1916), the lady beetles were easily cultured over the winter season in insectaries to provide large numbers for early spring release in the orchards. This procedure was so successful that it was the main method of control for all kinds of mealybugs in California for many years (Smith and Armitage 1931). At one time, 26 "Cryps" insectaries were operated in the state, and propagation stocks were supplied from these to many insectaries built in foreign countries (Fisher 1963). Although the practice of releasing insectary-reared *C. montrouzieri* declined when better parasites and chemicals became available for mealybug control, 3 commercial insectaries are still raising this predator in southern California. Insofar as can be determined, except for exportations of this predator from Australia to Hawaii and Ceylon in 1894–95, all stocks of *C. montrouzieri* subsequently distributed to almost all of the major agricultural countries of the world have emanated from the easily accessible but highly inbred California insectary stocks.

Since 1891, no one seems to have expressed any interest in trying to enhance the performance of this predator by adding to its gene pool. This is not surprising since with the exception of a few experiments designed to produce better host adaptation (Turnock and Muldrew 1971), there has been little other than speculation on the value of supplementation of natural-enemy gene pools. This has been so despite general recognition that with most parasite and predator importations there are usually serious reductions in the heterozygosity of the introduced species (Remington 1968). Furthermore, there has also been only meager research conducted on cold-hardiness in natural enemies although climatic extremes are generally accepted as being among the most crucial factors affecting establishment. Among the most pertinent studies on cold susceptibility in natural enemies are those of Bodenheimer (1928), who reported on the effects of cold on *C. montrouzieri* in Palestine, and DeBach et al. (1955) on scale-parasite responses to cold in California. Macphee (1964) examined winter survival factors of natural enemies in rigorous climates, and Wilkes (1942) sought cold-tolerant races by laboratory selection. These studies, however, have thrown but little light upon the possible genetic basis for cold tolerance and its prevalence as a racial trait among natural enemies.

Objectives

The primary purpose of this research was to improve the practical biological control of mealybugs through supplementation of the cold-tolerance gene

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pool of *C. montrouzieri*. An offshoot or secondary goal was the development of laboratory techniques for testing and predicting the cold-tolerance of natural enemies.

The Search for a Cold-hardy Race of C. montrouzieri

While I was on a parasite collecting trip to Australia during January–May 1972, an opportunity arose to examine the native geographical distribution records of *C. montrouzieri* in museum collections. Aside from numerous records from very warm climatic zones, the records disclosed single collections each from the area around Canberra, A. C. T., and between the cities of Armidale and Stanthorpe in the high New England range country of N. S. W. These 2 areas rank among the coldest general areas in Australia, and are reported to experience winter cold extremes surpassing those of any of the citrus districts of California.

With the assistance of members of the Australian Federal Department of Entomology, a search of these 2 localities led to the discovery of 26 *C. montrouzieri* feeding on *Pseudococcus albizzia* (Maskell) on *Acacia dealbata* Link at a Canberra site and 34 individuals feeding on the same host at 2 nearby sites between Armidale and Stanthorpe. These predators were all received alive at the quarantine facility of the Division of Biological Control, University of California, Riverside, during late February and early March 1972 where they were maintained on *Planococcus citri* as 2 separate stocks hereafter designated as the Canberra and the Armidale races.

With only their origin from cold climates as assurance that the newly imported stocks were more tolerant to cold extremes than those cultured in California since 1891, the first priority was to increase the 2 stocks and colonize substantial numbers of each at a few selected release sites for long-term observation. To this purpose, about 1000 individuals of each race were released at 2 sites in the warmer coastal citrus areas on *P. citri* and in the colder San Joaquin valley district on the newly established Comstock mealybug, *Pseudococcus comstocki* (Kuw.). Afterward, stocks for further propagation and release of the 2 races were given to 2 commercial insectaries interested in the general distribution of this possibly cold-hardy biotype. Separate stocks of both races were maintained at Riverside to study their ability to withstand cold temperature extremes.

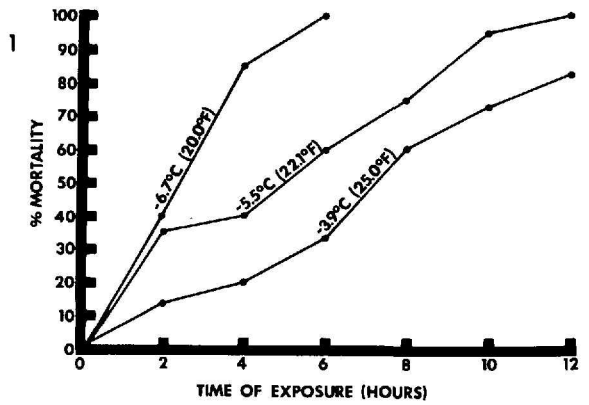
Development of Laboratory Methods for Assessing Cold Susceptibility in C. montrouzieri

Recognizing that the final proof of any improvement in climatic adaptation by the newly imported races of this lady beetle must await the evidence of permanent field establishment in areas where the species had heretofore failed to persist, there still remained the interesting possibility that a short-term laboratory test might be devised which could serve as a means of predicting if a predator might successfully overwinter in climates corresponding to those of the California citrus-growing districts. Since it appeared that we had here a predator species with races capable of surviving in areas of Australia too cold for citrus, and another borderline race which could not survive in most of the citrus areas of California, this was a unique opportunity to try to establish a predictive guideline for natural enemy cold-hardiness below which species newly imported to California citrus areas would be unlikely to survive. This line of reasoning was stimulated by some of my earlier unpublished research relative to the borderline cold tolerance of the adults of *Metaphycus helvolus* (Comp.), an encyrtid parasite of lecaniine scale insects, which cannot quite survive the most debilitating frosts of the California citrus areas (Flanders 1949). The results of these earlier trials, involving exposure of the adults of this parasite to subfreezing temperatures for periods of 24 h, had produced a 95.2% kill at −6.7°C (25°F); and no kill at −1.1°C (30°F). These temperatures critical to the parasites' survival were not unlike those critical for production of oranges, and the kills obtained seemed to conform in a remarkably similar way with the recognized winter destruction of this parasite in the coldest citrus districts. These results had not only suggested that winter survival of a natural enemy might rest primarily upon the cold tolerance of the adult stage, but had also suggested the possibility that cold destruction of the adult parasites, as with certain plants, might be related in large measures to their susceptibility to short-term exposures at critically low temperatures.

To test these possibilities, initial trials were conducted for gross examination of the short-term effects of sub-freezing temperatures on the old (1891) line of *C. montrouzieri*. The results showed that, as with the previous parasite tests, adults of the predator were destroyed by very short-term exposures to sub-freezing temperatures in refrigerated cabinets. The results also were in general accord with the field evidence on their overwintering survival in only the warmer citrus districts. All adults and larvae were killed in less than 1 h at −12.2°C. The LT_{50} of adults at −6.7°C averaged from 7 curves was 2.7 h (range: 1.3–4.3 h). The average LT_{50} of 4 curves at −3.9°C rose sharply to 8.4 h (range: 7.5–11.0 h), and at −1.1°C there was almost no mortality of the predator in 12 h. Representative exposure-mortality curves[3] for accumulative 2 h-exposure intervals over a period of 12 h at −6.7°C and −3.9°C are shown in Fig. 1.

These preliminary trials with the 1891 race of *C. montrouzieri* provided some information pertinent to further cold-testing procedures with this insect. It was established that the adults of this coccinellid were particularly susceptible to CO_2 or ether anaesthesia so it was necessary to handle them manually or by aspirator without inactivation. Also, following cold stupefaction of adults in the tests, 2–3 h recovery periods at room temperature were required be-

[3] Points for these 2 curves are averages of 2–4 replicates of 10 insects each.



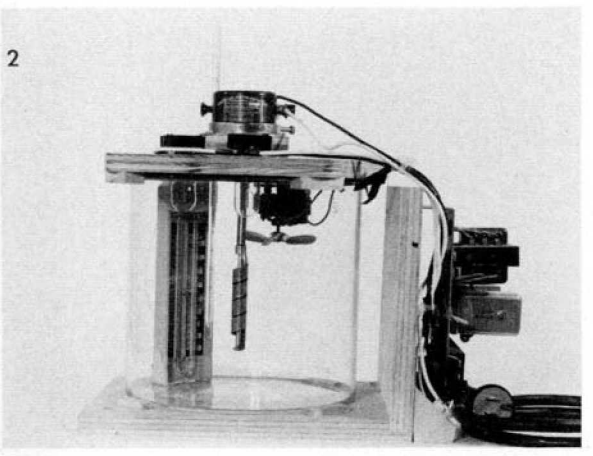


FIG. 1.—Comparative mortality curves for adults of the old (1891) race of *C. montrouzieri* when exposed for up to 12 h to temperatures of −6.7°C, −5.5°C, and −3.9°C.

FIG. 2.—Battery-jar temperature control unit for use in a deep-freeze cabinet to obtain stable sub-freezing temperatures.

fore movement indicated their survival. Relative humidity apparently was of little importance in the tests, possibly because of the complete inactivation of the adults, and with tests restricted to 12 h no check mortality occurred.

Standardization of refrigerated temperature cabinets at the sub-freezing temperatures required turned out to be more difficult than anticipated even though they were placed inside a 5°C cold room. With available cabinets accuracy was limited to about ±1.5°C. More precise temperature control equipment was designed to reduce this variation to ±0.5°C. This equipment (Fig. 2) consisted of individual battery-jar temperature control units with wooden tops on the inside of which were mounted circulating fans and thermoregulators activating 5 W heating lights. The heated jar units were operated within a deep-freeze cabinet which was kept in a 5°C cold room. The holding vials were 8-ml plastic cylinders, screened at either end. With as few as 10 individuals/vial for removal at 2-h intervals in a 12-h period, (i.e., 60 insects), reasonably accurate LT_{50} points could be calculated from eye-fitted time-mortality curves. Differences in LT_{50}'s were calculated from curves derived only from simultaneous paired comparisons in the same cabinet.

Results of Comparisons of LT_{50} Values of Old and New Races

On the basis of data from preliminary trials with the 1891 race of *C. montrouzieri*, temperatures of −5.0°C were selected as most appropriate for showing differences in cold tolerance between races at their LT_{50} points.

The first series of tests at −5.0°C was run to see if there were distinctive differences between the 2 new Australian races. In 8 paired comparisons between the Canberra and the Armidale races, the average LT_{50} for the Canberra race was 7.85 h, and 6.65 h for the Armidale race. The difference between the 2 newly imported races, while favoring the Canberra race, was not statistically significant.

The 2nd series of tests were run to see if the combined new races differed from the old. In 5 paired comparisons of curves of the 1891 race with 5 of the Canberra and 5 of the Armidale races, the average LT_{50} of the 1891 race was 6.41 h compared to 7.41 h for the combined new races. This difference in LT_{50} values favoring cold tolerance of the new races was statistically significant at the 5% probability level. From these data it was concluded that the new races originating from cold climates in Australia also represented distinctive biotypes with respect to endurance of cold in refrigerated cabinet tests.

Although the new races could be shown to be cold-hardy biotypes, the test procedure for demonstrating this was not simple. However, an interesting feature of some of the comparisons suggested another possibly less involved test procedure. It was noted in certain paired curves that after all the individuals of the 1891 race had succumbed, a fairly large number of individuals of the new races survived. In 8 such paired curves wherein all of the old race died, there still remained an average of 31.3% of the new biotypes alive. Populations of the new biotypes apparently had a much broader base of variability with curves skewed at the higher exposures toward greater cold tolerance, whereas the old race was much more homogeneous. These data suggested that a single measurement of survival of natural enemies after 12 h continuous exposure to a slightly lower cold regime might provide a much simpler cold-hardiness test procedure. Accordingly the temperatures in the test jar units were set for −5.5°C, and a 12-h total exposure curve for 2-h intervals established at this temperature with the 1891 line of *C. montrouzieri*. This curve, as depicted also in Fig. 1, showed this temperature should produce a nearly ideal LT_{100} for comparisons after a continuous 12-h test period. Using this continuous 12-h exposure method in a series of 6 paired comparisons at −5.5°C with a total of 120 insects

Table 1.—Comparative percent of survival of 3 races of *Cryptolaemus montrouzieri* and 2 hymenopterous parasites at temperatures of −5.5°C for 12 h.

Species tested	No insects tested	Avg % survival
C. montrouzieri Muls. old (1891) race	210	0
C. montrouzieri Muls. Armidale race	60	51.7
C. montrouzieri Muls. Canberra race	70	48.6
Metaphycus helvolvus (Comp.)	272	58.6
Coccophagus lycimnia (Walk.)	460	97.2

of each of the 1891, the Canberra, and the Armidale biotypes, it was shown that after all of the 1891 individuals had succumbed, an average of 62.2% of the Canberra and 52.9% of the Armidale biotypes survived. These survival results surprisingly were higher than those obtained in previous trials. A possible explanation is that because of a shortage of mealybug food, cold-test survivors were returned to the stock cultures for reproduction. This inadvertent selection for cold-hardiness could have contributed to the increased survival in subsequent comparisons. If so, it may indicate that cold-hardiness in this species may have a rather simple genetic basis.

Discussion and Illustration of the Cold-hardiness Test Procedure with Other Natural Enemies

At this juncture, it appeared that we might have an acceptable laboratory method for judging cold-hardiness among different races of *C. montrouzieri;* but we did not have positive assurance that this was synonomous with winter cold tolerance of that species outdoors, or that the procedure could be employed to categorize cold-hardiness in other kinds of natural enemies. Therefore, additional evidence was sought to indicate whether or not this proposed short-term assessment of laboratory cold-hardiness might have predictive value with other species of natural enemies. For this purpose, paired comparisons using 12-h exposures to −5.5°C were made between the 3 races of *C. montrouzieri* and 2 parasites whose relative overwintering capabilities were well known. The 2 parasites were an encyrtid soft-scale parasite, *Metaphycus helvolus* Compere, and an aphelinid soft-scale parasite, *Coccophagus lycimnia* Walker. Of these, *M. helvolus* fails to overwinter in the very coldest citrus areas of California, whereas *C. lycimnia* survives these readily. The average percentage of survival as seen from simultaneous comparisons (Table 1) support the conclusion that this simple refrigerated-unit test of the cold-hardiness of certain natural enemy species may afford a reasonably good overall measure of their overwintering capabilities in climates like those of the California citrus-growing areas.

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