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LABORATORY AND FIELD EVALUATIONS OF NOVALURON, A NEW INSECT GROWTH REGULATOR (IGR), AGAINST *CULEX* MOSQUITOES[1]

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ABSTRACT. Limited laboratory and field studies have indicated that the insect growth regulator (IGR) novaluron exhibits good activity against larvae of pest species in the orders Coleoptera, Hemiptera (suborder Heteroptera), and Lepidoptera by both ingestion and contact. We completed laboratory and field studies to evaluate activity and efficacy of novaluron against *Culex* mosquitoes. In laboratory studies, novaluron was highly active against *Cx. quinquefasciatus,* as indicated by low levels of inhibition of emergency (IE), at the 50% level, IE_{50} (0.159 ppb for 2nd-stage larvae and 0.118 ppb for 4th-stage larvae) and IE_{90}, at the 90% level, (0.604 ppb for 2nd-stage larvae and 0.595 ppb for 4th-stage larvae). In outdoor microcosm and mesocosm studies against natural populations, novaluron yielded excellent control of immature *Culex* mosquitoes for up to 14 days at 1.25, 2.5, and 5 ppb in microcosms, and for up to 7 days at the dosages of 1, 5, and 10 mg/m^2 in mesocosms. Based on qualitative observations, novaluron seemed to have a favorable margin of safety for nontarget aquatic invertebrates cohabiting with mosquito larvae. Further large-scale field studies are warranted to evaluate initial efficacy and longevity of novaluron against various mosquito species, as well as its safety for nontarget biota.

KEY WORDS Novaluron, insect growth regulator (IGR), mosquito control

INTRODUCTION

Mosquito control by larviciding is facing serious challenges, as there has been very few new materials introduced or registered for larval control during the past 15 years. Today, *Bacillus thuringiensis* var. *israelensis* (*Bti*), *B. sphaericus* (*Bsph*), methoprene, oils, and few others are the only materials registered for mosquito control in California and most of the USA. Among these materials, *Bti* is the only one to offer minimum risk of resistance emergence (Vasquez-Garcia 1983, Becker and Ludwig 1993). Numerous cases of resistance have been reported in mosquitoes to *Bsph* (Wirth et al. 2000, Zahiri et al. 2002). In some areas in the USA and abroad, resistance to the insect growth regulator (IGR) methoprene has been detected (Amin and White 1984, Dame et al. 1998, Cornel et al. 2000). Other IGRs, e.g., diflubenzuron (Dimilin) used under restricted conditions for mosquito control also has potential for resistance development (Amin and White 1984, Walker and Wood 1986, Montada et al. 1989). Pyriproxyfen, another promising mosquito larvicide (Estrada and Mulla 1986, Mulla et al. 1986, Mulligan and Schaeffer 1990), not yet labeled for field use in the USA, did not result in increased tolerance in laboratory studies (Schaeffer and Mulligan 1991).

Due to the availability of few larvicides for mosquito control, screening and development of new promising bioactive agents against mosquito larvae are urgently needed. Novaluron, a new IGR, has shown high levels of activity against many pests with broad application potential for the control of agricultural, forestry, and urban pests.

This new acylurea compound acts as a chitin-synthesis inhibitor. Limited laboratory and field research has indicated good IGR activity against agricultural and forestry pests. It was found that novaluron is active against larvae in the orders Coleoptera, Hemiptera (suborder Heteroptera), and Lepidoptera by both ingestion and contact (Malinowski and Pawinska 1992, Malinowski 1995, Ishaaya et al. 1996, 2001).

To date, no information is available about the mosquitocidal activity of novaluron as a new chitin-synthesis inhibitor. There is an interest in developing this compound in global vector-control programs. Therefore, studies were initiated to gather pertinent information on novaluron in terms of its activity and efficacy against various species of mosquitoes in the laboratory and in field habitats. The current studies were initiated to determine the spectrum of larvicidal activity of novaluron against mosquitoes under laboratory and field conditions.

MATERIALS AND METHODS

Test materials

Technical powder of novaluron (99.4%, BN: 9860726), also known as Rimon®, MCW-275, GR 572, and Rimon EC10 (BN: 911304) with 10% (w/v) of active ingredients were supplied by WHOPES/WHO, Geneva, Switzerland, and received on January 30, 2002, for laboratory and field evaluations against *Culex* mosquitoes. Both materials were manufactured by Makhteshim Chemical Works, Ltd., Beer-Sheva, Israel.

[1] This investigation has been carried out as part of the WHO Pesticide Evaluation Scheme (WHOPES). However, it is not an endorsement of the product by WHO.

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Laboratory evaluation

Laboratory evaluation was conducted against *Culex quinquefasciatus* Say by using standard bioassay protocols (Mulla et al. 1985, 1986, 1989; Estrada and Mulla 1986). In brief, 1% stock solutions and serial dilutions of the technical-grade material were prepared in acetone (Spectrum Chemical Mfg. Corp., Gardena, CA, CAS 67-64-1). The required amount of the appropriate dilution was added to 240-ml disposable waxed-paper cups, each containing 200 ml of distilled water and 20 mosquito larvae. Two larval stages (2nd and 4th stages) were tested concurrently in 2 separate experiments. In each test, 5 concentrations within the activity range were used with 3 replicates for each concentration. In each test, 3 cups were left untreated as the control. An appropriate amount of rabbit pellets (0.1 g, once) was added to each cup as larval food after the larvae were introduced in the water in the cups. Water lost due to evaporation was replenished every other day. Mortality at larval, pupal (including intermediate forms between larvae and pupae), and adult stages (attached to pupal exuviae) was assessed every other day until all individuals died or emerged as adults. Adult emergence was evaluated by counting and removing pupal skins from the cups. All tests were run under a standard photoperiod (16L:8D) and room temperature (30–33°C). Overall inhibition of emergence and stage-related mortality at various concentrations were calculated (Estrada and Mulla 1986, Mulla et al. 1989). Percent mortality values obtained at each concentration were subjected to probit regression analysis with POLO-PC (LeOra Software 1987) to determine IE_{50} (inhibition of emergence) and IE_{90} values and their 95% confidence intervals.

Field microcosm studies

Because technical material showed promising IGR activity in laboratory bioassays, a formulation of novaluron (Rimon EC10) was tested for its efficacy against natural populations of *Culex* mosquitoes breeding in outdoor microcosms. In total, 16 fiberglass tubs, each with 1-m^2 surface area and containing 240 liters of reservoir water were used. Water depth was 30.5 cm and water level was kept constant by float valves on the water lines feeding each tub. These tubs were located in an open, sunlit area at the Aquatic and Vector Control Research Facility in the Agricultural Experimental Station of the University of California (UC) at Riverside. In order to have sustainable mosquito production, the tubs were enriched before flooding with rabbit pellets (Brookhurst Hill, Riverside, CA) at the rate of 0.02%, equaling 50 g/tub.

Because environmental factors such as ultraviolet light and organic pollution, which would degrade novaluron, approximately 2.5×, 5×, and 10× the observed laboratory IE_{90}, equaling 1.25, 2.5, and 5 ppb, respectively, were used as dosages in this field test.

Novaluron Rimon EC10 was diluted with distilled water to 0.1% active ingredient (AI), and appropriate amounts of this stock suspension (0.3, 0.6, and 1.2 ml) were added to each of the assigned tubs, yielding the 1.25, 2.5, and 5 ppb AI, respectively. Three treatments and untreated controls were assigned randomly with 4 replicates each. Treatments were made 7 days after flooding when late stages (3rd- and 4th-stage larvae) were present in large numbers.

Larvae (early and late stages), pupae, and exuviae (pupal skins) were sampled by the dipping technique before and 4, 9, 14, 21, and 27 days after treatment to assess the initial and persistent efficacy. In each tub, 4 dips were taken, 1 from each of the 4 corners. During sampling, 1 dip was taken from each corner of all replicates and the remaining 3 dips were taken sequentially from the remaining corners. This sampling procedure minimized sampling error because diving larvae and pupae after physical disturbance resurfaced before the next sampling round. Average densities of larvae, pupae, and exuviae in various treatments and controls were calculated and compared by one-factor ANOVA with a repeated-measures design (Abacus Concepts, Inc. 1987). Other invertebrates were observed qualitatively during sampling to get some idea about the margin of safety of novaluron for nontargets.

Species composition in larval populations was determined by identification of 4th-stage larvae collected from untreated control tubs on each sampling day. During the test period, water-quality parameters were also determined in untreated control tubs. Water temperature was determined by submerging a minimum-maximum thermometer in water of a tub that was in the center of the microcosm arrangement. The dissolved oxygen, corrected electrical conductivity, and salinity were measured by YSI Model 85 Handheld Oxygen, Conductivity, Salinity and Temperature System (YSI Inc., Yellow Springs, OH).

Field mesocosm studies

Because technical material showed promising IGR activity in laboratory bioassays and the formulated novaluron (Rimon EC10) was effective against natural populations of *Culex* mosquitoes breeding in outdoor microcosms, this EC formulation was further evaluated in mesocosms. In total, 12 bare ground dirt ponds, each with 27-m^2 surface area and containing approximately 8,100 liters of reservoir water were used. Water depth was 30.5 cm and water levels were kept constant by float valves on the water lines feeding each pond. These ponds were also located in an open, sunlit area at the Aquatic and Vector Control Research Facility in the Agricultural Experimental Station of UC Riverside. In order to have sustainable mosquito pro-

duction, the ponds were enriched before flooding with rabbit pellets at the same rate of 0.02% as in tub test, equaling approximately 2,000 g/pond.

These ponds in the past have been used for research on the biology of a tadpole shrimp (Notostraca: Triopsidae), which decimates mosquito larvae and pupae (Tietze and Mulla 1991). Their eggs occur in large numbers in the dry bottom soil, and hatch on flooding and feed on immature mosquitoes. In order to have sustainable immature mosquito populations for the planned tests, Karate EC1 containing 12% lambdacyhalothrin (ICI Americas, Inc., Richmond, CA) was initially applied at 250 mg AI/hectare on day 3 after flooding and applied additionally at 90 mg AI/hectare on day 4 after flooding, to control the newly hatched tadpole shrimp. Based on our previous studies, this dosage eliminated tadpole shrimp without negative impact on immature mosquitoes (Mulla et al. 1992). For application of lambdacyhalothrin, Karate EC1 was diluted 1,000 times to 0.012% AI using tap water. The appropriate aliquots of this diluted Karate EC1 (0.012%), 5.56 ml for 250 mg AI/hectare (initial treatment) and 2 ml for 90 mg AI/hectare (additional treatment), were transferred to a 150-ml squeeze plastic bottle and further diluted using tap water to the final volume of 120 ml and applied to the water surface of these ponds.

In the present pond test, in addition to the factors in tubs such as organic pollution and ultraviolet irradiation, edaphic factors were involved. Therefore, the dosages of 1, 5, and 10 mg/m^2, equaling 3.3, 16.5, and 33.3 ppb, respectively, were evaluated.

Novaluron EC10 (10% AI) was diluted with tap water to 1% AI, appropriate amounts of this stock suspension (2.7, 13.4, and 26.7 ml) were transferred to a 150-ml squeeze plastic bottle and further diluted using tap water to the final volume of 120 ml and applied to the water surface of the ponds, which yielded 3.3, 16.5, and 33.3 ppb AI, respectively. Three treatments and untreated control were assigned randomly with 3 replicates each. Treatments were made 8 days after flooding when late stages (3rd and 4th stages) were present in large numbers.

As in the tub test, larvae (early and late stages), pupae, and exuviae were sampled by the dipping technique before and 3, 7, 13, and 20 days after treatment to assess efficacy. In each pond, 1 dip was taken from each of 4 corners. Average densities of larvae, pupae, and exuviae in various treatments and control were calculated and compared by one-factor ANOVA with a repeated measures design (Abacus Concept, Inc. 1987). Other invertebrates were observed qualitatively during sampling to assess safety margin of novaluron for nontargets.

Species composition in larval populations was determined by identification of 4th-stage larvae collected from untreated control ponds on each sampling day. During the test period, the water-quality parameters were also determined in untreated control ponds. Water temperature was determined by submerging a minimum-maximum thermometer in water. The dissolved oxygen, corrected electrical conductivity, and salinity were measured by a YSI Model 85 Handheld Oxygen, Conductivity, Salinity and Temperature System.

RESULTS AND DISCUSSION

Laboratory studies

The tests lasted 16 days when 2nd-stage larvae were treated or 10 days when 4th-stage larvae were treated. The cumulative mortality of the controls (no novaluron) was 4.2 and 3.4% when using 2nd- and 4th-stage larvae, respectively. In treatments using the range of 0.01–1.00 ppb, the cumulative mortality steadily increased over time and across concentrations. Most mortality occurred within 10 and 6 days after treatment for 2nd- and 4th-stage larvae, respectively (Fig. 1). For stage-specific mortality, somewhat higher adult mortality was noted at lower concentrations. In contrast, more larval and pupal mortality occurred at the higher concentrations, leaving few larvae and pupae to make it to the adult stage (Fig. 2). Overall, more larval mortality at each concentration was noted when 2nd-stage larvae were treated as compared with the treatment of the 4th-stage larvae (Fig. 2). When the 2nd-stage larvae were treated at 1.00 ppb (the highest concentration), most mortality occurred in the larval stage with little in the pupal stage. With high and complete mortality of larvae and pupae, there was no emergence of adults (Fig. 2). Adult mortality, although low, was noted in all other concentrations.

The probit analysis of dosage–response data indicated that the IE_{50} was 0.159 ppb (95% CI: 0.127–0.191 ppb) and IE_{90} was 0.604 ppb (95% CI: 0.423–0.785 ppb) for 2nd-stage larvae, IE_{50} was 0.118 ppb (0.094–0.142 ppb) and IE_{90} was 0.595 ppb (95% CI: 0.417–0.774 ppb) for 4th-stage larvae.

In laboratory studies, most mortality occurred within 10 and 6 days after treatment was made to 2nd- and 4th-stage larvae, respectively, indicating novaluron acts as an IGR against immature mosquitoes. Mortality occurred in all stages, i.e., in larvae, pupae, and adults. Increased larval mortality was achieved at higher treatment concentrations, which is different from the action of methoprene, a juvenile hormone analog (JHA), where most mortality occurred in the pupal stage regardless of the test dosage (Mulla et al. 1989). Based on these laboratory data, novaluron was highly active against the test species, even more active than diflubenzuron (IE_{90} 2.0 ppb) and methoprene (IE_{90} 50 ppb), and similar to pyriproxyfen (IE_{90} 0.3 ppb) against the same species (Mulla 1995). It seems that the

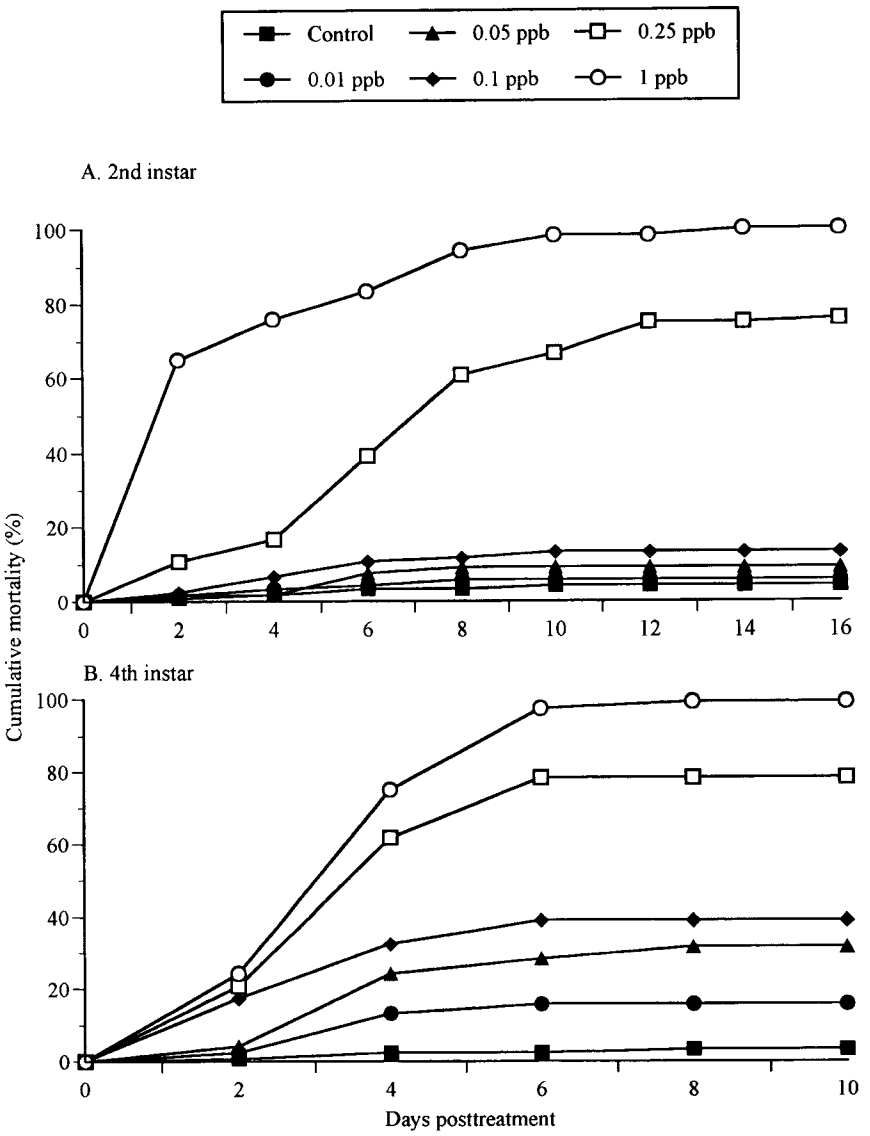

Fig. 1. Cumulative mortality of larvae, pupae, and adults of *Culex quinquefasciatus* when 2nd (A) or 4th (B) instars were treated with novaluron technical powder solution in the laboratory.

susceptibility levels of 2nd- and 4th-stage larvae of *Cx. quinquefasciatus* to novaluron were equivalent.

Field microcosm studies

Mosquito control. The average number of early-stage larvae per dip provided a less important indication of control efficacy than late-stage larvae and pupae, as some newly hatched larvae had not been exposed for a long enough time to the control material. Nevertheless, the population trend of early-stage larvae did decline after the treatments, especially at the higher dosages. The initial population densities in all assigned treatments and control tubs before treatment were essentially the same. On day 4 after treatment, all treated tubs at the 3 different dosages had significantly lower counts than the control (Fig. 3A). A dosage-related difference was noted on this sampling day as the highest dosage, 5 ppb, further lowered the average counts of early-stage larvae, while no difference was indicated between the two lower dosages, 1.25 and 2.5 ppb (Fig. 3A). On day 9 after treatment, the lowest dosage, 1.25 ppb, lost its efficacy, while the two higher dosages, 2.5 and 5 ppb, still exhibited comparable activity against early-stage larvae (Fig. 3A). From day 14, 21–27, no noticeable control activity was indicated, as the average counts of early-stage larvae in treatments were either equal to or higher than those in the controls (Fig. 3A).

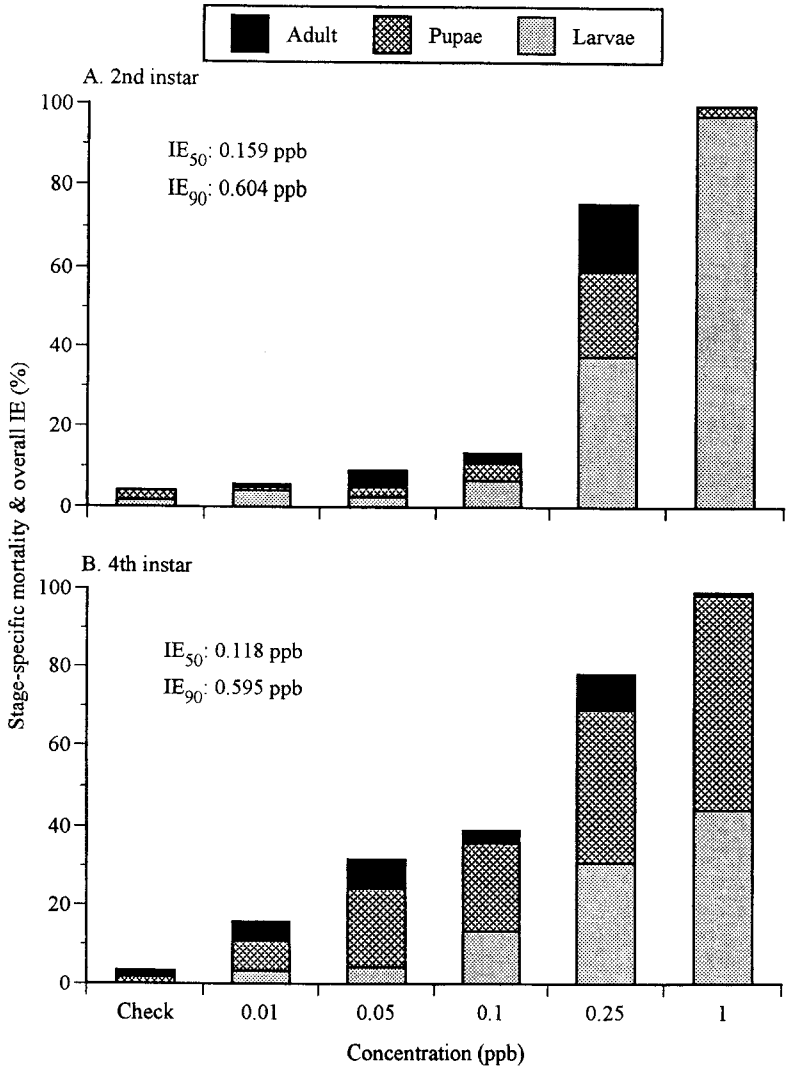

Fig. 2. Stage-specific mortality and overall inhibition of emergence of *Culex quinquefasciatus* when 2nd (A) or 4th (B) instars were treated with novaluron technical powder solution in the laboratory.

Compared with the population trends of early-stage larvae, later stage larvae provided a better indication of control, which clearly showed that novaluron significantly reduced populations at the lowest dosage for up to 9 days and at the two higher dosages for up to 14 days (Fig. 3B). The 3 dosages were equally effective on day 4 after treatment. Some dosage-dependent differences were noted on day 9 after treatment when the lowest dosage was less effective than the 2 higher dosages (Fig. 3B). On day 14, the lowest dosage was no longer effective, but efficacy was still fairly good for the two higher dosages. On days 21 and 27 after treatment, however, no reduction was shown; the population densities of late-stage larvae in the lowest and the middle dosages even surged above the level in untreated controls (Fig. 3B).

We consider the population trends of pupae and collection of pupal exuviae to provide accurate assessment of the impact of IGR treatments on immature mosquitoes. Reduction in pupae and exuviae is reflected in reducing or preventing adult emergence, which is the primary goal of larvicidal treatments. On days 4 and 9 after treatment, all treatments were equally effective in inhibiting pupation, while high numbers of pupae were collected from untreated control tubs (Fig. 3C). On day 14 after treatment, the number of pupae was still high in control tubs. Statistically, the same numbers of pupae were noted in the tubs treated by the lowest dosage and the control tubs. The two higher dosages were still effective in preventing pupation on day 14 (Fig. 3C). On days 21 and 27 after treatment, low numbers of pupae were present in all

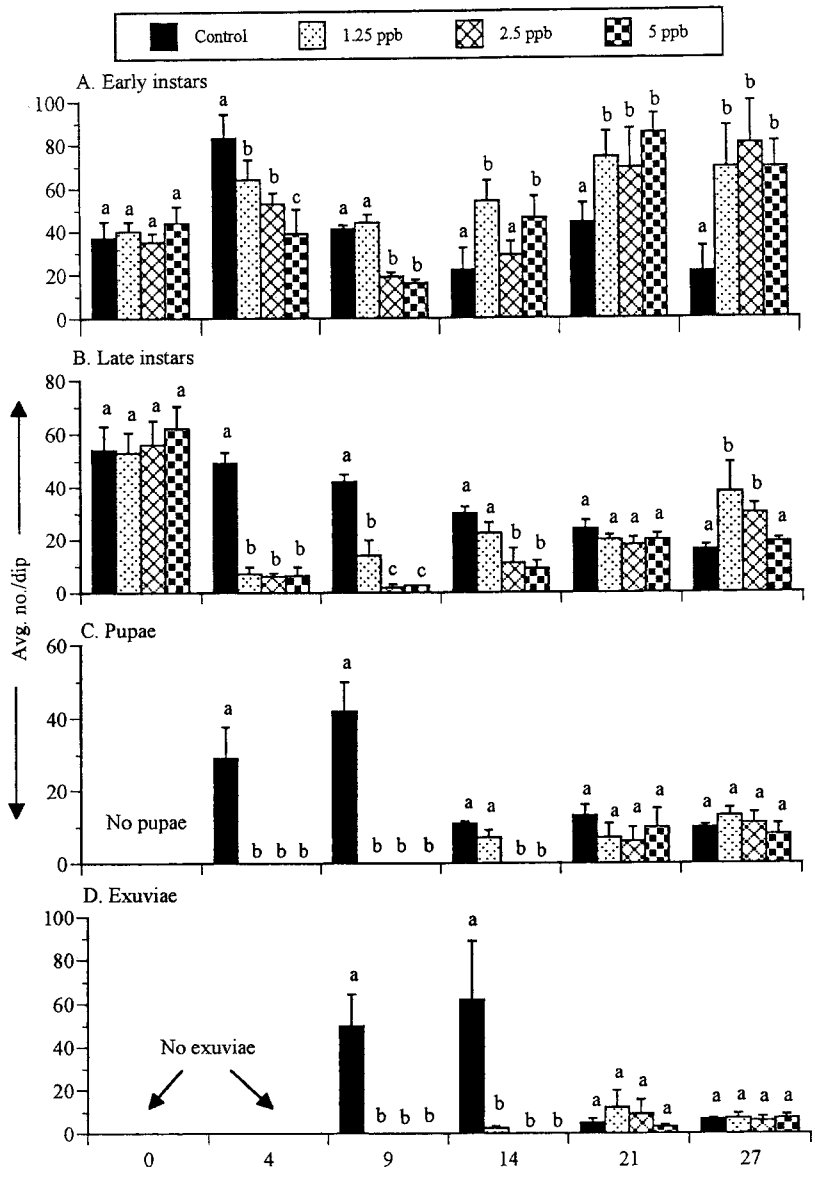

Fig. 3. Population trends of early instar, late instar, pupae, and exuviae of mosquitoes (primarily *Culex* spp.) in microcosms (Riverside, CA, USA; May 6–June 10, 2002) treated with novaluron EC10 at various dosages. In each age group, columns of the same day posttreatment marked with different letters are significantly different from each other at the 0.05 level, as analyzed by one-factor ANOVA with a repeated-measures design.

treatments and controls, no significant differences were noted among various treatment regimens and controls (Fig. 3C).

No adult emergence was noted on day 0 (before treatment) and day 4 after treatment, as no exuviae were present in dip samples because of the short time of development. On days 9 and 14 after treatment, large numbers of adults emerged from the control tubs, while emergence was almost completely inhibited in treated tubs, except that a very few exuviae were noted on day 14 in the tubs treated with the lowest dosage (Fig. 3D). On days 21 and 27, as in pupal counts, low numbers of exuviae were collected in all tubs and there were no significant differences among various treatment regimens and the controls (Fig. 3D).

As for species composition during the test period, from day 0 through day 9, *Cx. stigmatosoma* Dyar and *Cx. quinquefasciatus* were the predominant species; these 2 species declined from day 14 and remained at low levels to day 27, when the test was concluded (Fig. 5A). *Culex tarsalis* Coquillett, on the other hand, were present at low levels initially but increased from day 14 after treatment and

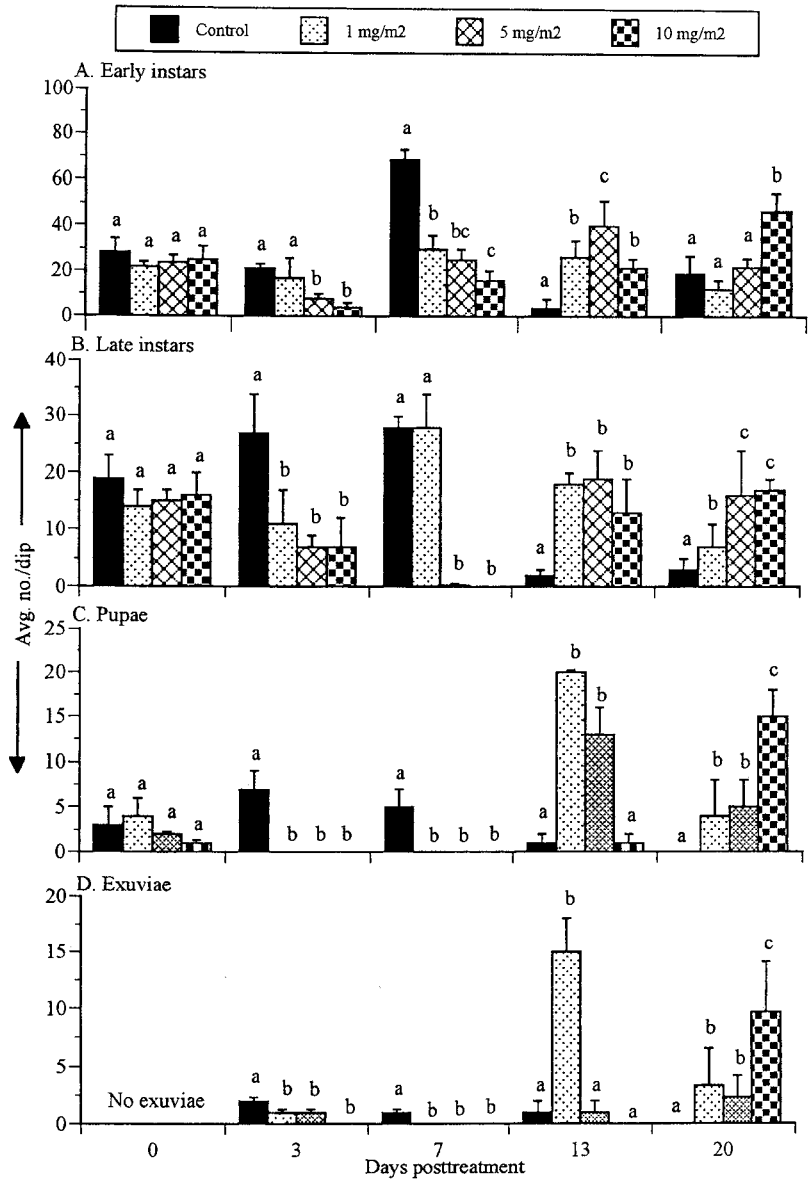

Fig. 4. Population trends of early instar, late instar, pupae, and exuviae of mosquitoes (primarily *Culex* spp.) in mesocosms (Riverside, CA, USA; June 24–July 23, 2002) treated with novaluron EC10 at various dosages. In each age group, columns of the same day posttreatment marked with different letters are significantly different from each other at the 0.05 level, as analyzed by one-factor ANOVA with a repeated-measures design.

remained high until the test was terminated. *Culiseta* species, including *Cs. inornata* Williston and *Cs. incidens* Thompson (winter mosquitoes) comprised a low proportion of the mosquito population throughout the test period (Fig. 5A).

Impact on macroinvertebrates. We made cursory and qualitative observations on the presence of macroinvertebrates. Based on qualitative observations, there were no noticeable differences in macroinvertebrate fauna between untreated control and treatments. Chironomid midges (egg masses and egg strings, larvae, and exuviae), ephydrid flies (larvae, pupae, and exuviae), and mayflies (nymphs and pupae) were among the most commonly encountered groups. Others such as copepods, ostracods, cladocerans, and aquatic beetles (larvae and adults) were also noted in samples.

Water quality. During the test period, minimum and maximum water temperatures ranged from 15.6 to 19.4°C and 28.9 to 34.4°C, respectively. Dis-

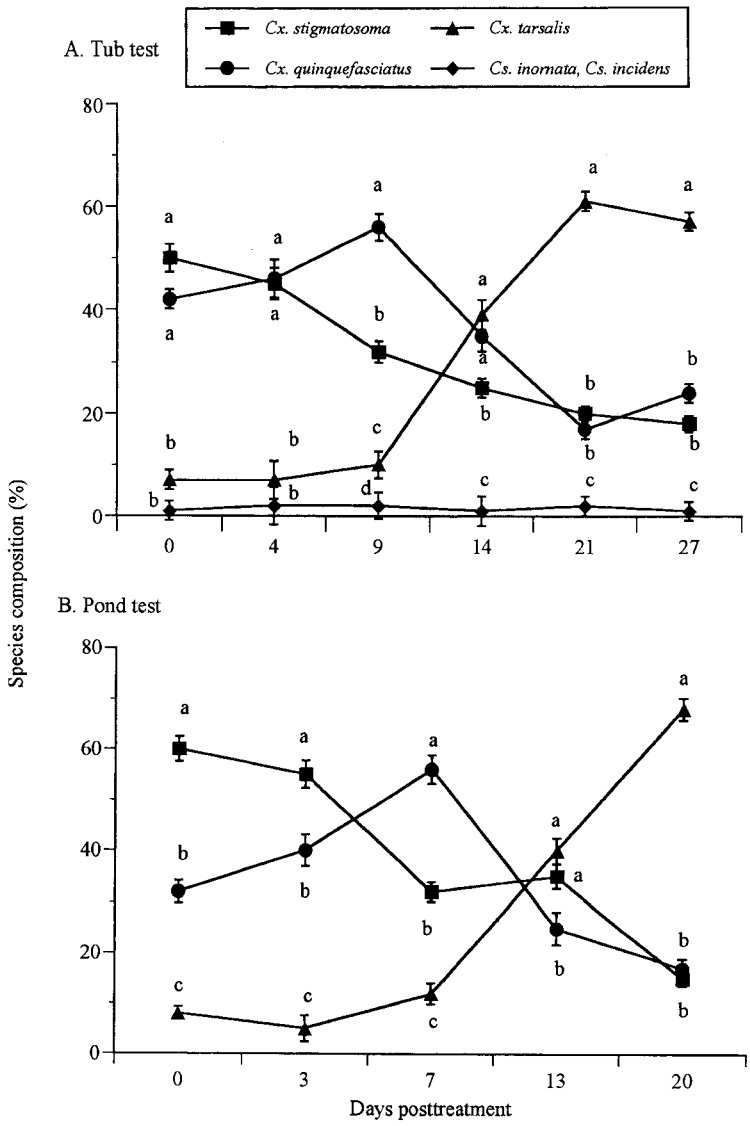

Fig. 5. Species composition in microcosm (A) and mesocosm (B) tests by identification of the 4th instar. Percentages on the same day posttreatment marked with different letters are significantly different from each other at the 0.05 level, as analyzed by chi-square test.

solved oxygen fluctuated in the range of 7.7–11.9 ppm. Corrected electrical conductivity measured 497–639 μS and salinity was 0.2–0.3 ppt (Fig. 6A).

Field mesocosm pond studies

Mosquito control. As in the tub test, the average number of early-stage larvae per dip provided a less sensitive indication of control efficacy. Nevertheless, the population trend of early-stage larvae did show some decline due to treatments, especially at the higher dosages. The initial population densities in all assigned treatments and control ponds before treatment were essentially the same. On day 3 after treatment, the treated ponds at 2 higher dosages (5 and 10 mg/m^2) had significantly lower counts than the control. No significant reduction in early-stage larvae was noted in the lowest dosage, 1 mg/m^2 (Fig. 4A). On day 7 after treatment, all 3 dosages, i.e., 1, 5, and 10 ppb, exhibited good activity against early-stage larvae, with the highest dosage being more effective than the 2 lower dosages (Fig. 4A). On days 13 and 20 after treatment, some differences in densities of early-stage larvae were noted among the 3 treatments (Fig. 4A). But mosquito populations in untreated controls declined significantly, speculatively as a result of predation by the few tadpole shrimp surviving the Karate treatments.

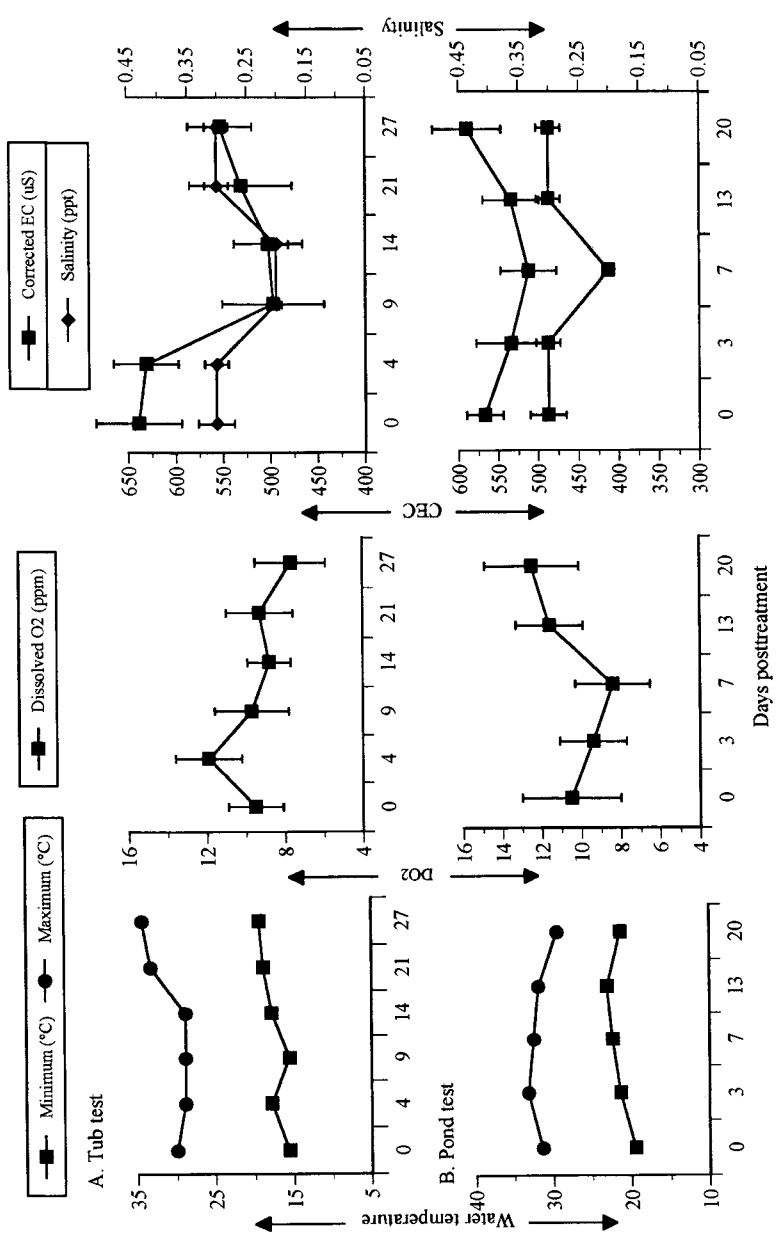

Fig. 6. Minimum and maximum water temperature (°C) and other water parameters in microcosm (A) and mesocosm (B) tests during the test period.

Gradually increasing populations of dragonfly nymphs (predators of immature mosquitoes) in untreated control ponds (not in novaluron-treated ponds) were also responsible for mosquito population declines.

Compared with the population trends of early-stage larvae, later stage larvae provided a better indication of control, which clearly showed that novaluron significantly reduced populations at the lowest dosage for up to 3 days and at the 2 higher dosages for up to 7 days (Fig. 4B). The 3 dosages were equally effective on day 3 after treatment. Some dosage-dependent differences were noted on day 7 after treatment, when the lowest dosage was no longer effective, while the 2 higher dosages yielded equivalent control (Fig. 4B). On days 13 and 20, populations of late-stage larvae were present at comparable levels among the 3 treatments (Fig. 4B). Population reduction in untreated control was believed to be caused by tadpole shrimp surviving the Karate treatments and dragonfly nymphs, populations of which had increased substantially by days 13 and 20 after treatment in the control ponds.

Population trends of pupae and pupal exuviae are considered to be more sensitive parameters to assess the impact of IGR treatments on immature mosquitoes. Emergence prevention is the primary goal of IGR larvicidal treatments. On days 3 and 7 after treatment, all treatments were equally effective in preventing pupation, while higher numbers of pupae were sampled from untreated control ponds at the same time (Fig. 4C). On days 13 and 20 after treatment, as in early- and late-stage larvae, numbers of pupae declined in untreated control ponds as a result of the presence of tadpole shrimp and dragonfly nymphs. Pupal densities on day 13 in treatments by the 2 lower dosages had increased to higher levels than that in control, while in the highest dosage, pupal counts remained low. By day 20 after treatment, however, all treatments had lost their efficacy based on pupal counts (Fig. 4C).

Exuvial counts were attempted in order to evaluate the effect of this material on adult emergence. No adult emergence was noted on day 0 (before treatment), as no exuviae were present in dip samples because of the short time of development. On days 3 and 7 after treatment, some adults emerged from the control ponds, while emergence was almost completely suppressed or prevented in treated ponds (Fig. 4D). On day 13, exuviae counts in untreated ponds were low because of assumed predation on late-stage larvae and pupae by residual tadpole populations as well as dragonfly nymphs (Fig. 4D). Pupal exuviae were present in fairly high numbers in the ponds treated at the lowest dosage, while exuvial counts were still low in middle dosage, and no exuviae were sampled at the highest dosage. On day 20 after treatment, the situation in control was similar to that on day 13. Pupal exuviae counts in all treatments, however, had surged to high levels (Fig. 4D).

As to species composition during the test period, from day 0 through day 7, *Cx. stigmatosoma* and *Cx. quinquefasciatus* were the predominant species; these 2 species declined from day 13 and remained at low levels to day 20, when the test was concluded. *Culex tarsalis,* on the other hand, was present at low levels initially, but increased from day 7 after treatment and remained high until the test was terminated (Fig. 5B).

Impact on macroinvertebrates. We made cursory and qualitative observations on the presence of macroinvertebrates. Novaluron treatments at 1, 5, and 10 mg/m^2 (equaling 3.3, 16.5, and 33.3 ppb, respectively) in combination with Karate eliminated dragonfly nymphs and residual populations of tadpole shrimp after initial and additional treatments using Karate EC1 in treated ponds. Other invertebrates, such as chironomid midges and ephydrids, were less affected and they were equally abundant in treatments and control.

Water quality. During the test period, minimum and maximum water temperatures ranged from 19.5 to 23.1°C and 29.5 to 33.2°C, respectively. Dissolved oxygen fluctuated in the range of 8.4–12.5 ppm. Corrected electrical conductivity measured 512–589 μS and salinity was 0.2–0.3 ppt (Fig. 6B).

Novaluron has been proven to be an effective agent against immature mosquitoes breeding in outdoor microcosms, where degradation-conducive factors such as artificial organic pollution, microbial flora, and ultraviolet radiation were present. At the dosages of 2.5×, 5×, and 10× of IE_{90}, equaling 1.25, 2.5, and 5 ppb or 0.0025, 0.005, and 0.01 lb AI/ac, respectively, novaluron did exhibit excellent control of immature mosquitoes (primarily *Culex* spp.) for up to 14 days, as indicated by the average numbers of late-stage larvae, pupae, and pupal exuviae. Novaluron has also been proven to be an effective agent against immature mosquitoes in mesocosms supporting natural populations of *Culex* mosquitoes, where there were more degradation-conducive factors (edaphic factors and others as in the microcosm tubs) than in the microcosms. At the dosages 1, 5, and 10 mg/m^2 (equaling 3.3, 16.5, and 33.3 ppb, or 0.009, 0.045, and 0.09 lb AI/ac, respectively), novaluron provided excellent control of immature mosquitoes (*Culex* spp.) in mesocosms for up to 7 days in clear to moderately polluted water, as indicated by the observed average numbers of late-stage larvae, pupae, and pupal exuviae. The level of control by novaluron at various dosages was comparable with that of the most active IGR, pryiproxyfen (S-31183) and was better than that by methoprene (Mulla et al. 1986, 1989).

The dynamic changes in species composition during microcosm and mesocosm studies were attributable to habitat preference and difference in larval development rate of various species. *Culex stigmatosoma* and *Cx. quinquefasciatus* generally prefer polluted water and their larvae develop faster than those of *Cx. tarsalis.* As the organic materials

were microbially consumed, the water became clear and suitable for the development of *Cx. tarsalis*. *Culex tarsalis* more likely laid their eggs in less polluted water and the larval stage of this species lasted longer than those of *Cx. stigmatosoma* and *Cx. quinquefasciatus*.

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