

# Best Mosquito Management - Santa Cruz County

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<http://www.agdept.com/AgriculturalCommissioner/MosquitoAbatementVectorControl.aspx>

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The Santa Cruz County Mosquito and Vector Control Department (SCCMVC), County Service Area 53 has provided mosquito control services to Santa Cruz County since 1994. Our formation came about because of prodigious public and local government support following years of complaints about biting mosquitoes. SCCMVC was formed through the Government Code, though responsibilities are similar to those outlined within the California Health and Safety Code (Section 2000 *et seq.*). The language contained in these codes underscore the landowner's responsibility to manage their property, including wetlands, to prevent mosquito breeding.

A **vector** is any insect, rodent, or other arthropod or animal that can threaten health by transmitting disease agents or causing discomfort.

Our public health program employs sustainable **Integrated Mosquito Management (IMM)** methods and emphasizes the prevention of mosquito production by reducing breeding sources and the control of aquatic stages to interrupt the mosquito cycle. This includes the use of least-toxic biorational materials selected on the basis of maximum safety to the public, applicator and environment, and otherwise follows general guidelines of the California Department of Public Health (CDPH), the Mosquito and Vector Control Association of California (MVCAC), the American Mosquito Control Association (AMCA) and the University of California (UC).

The sources of mosquitoes will always be with us, because of rainfall and myriad natural, residential and agricultural standing water sources. With a warming climate and vulnerable population, it is important that this SCCMVC be increasingly diligent in providing reduction of mosquitoes, protection from mosquito-borne diseases and relief from annoyance and biting nuisance. We continue to strive for the support from property owners and residents, regulators and government officials that is necessary for us to be successful in this endeavor.

## Seasonality and Variability

Wetlands have the potential to produce large outbreaks of mosquitoes at certain times of the year. SCCMVC, through education and pro-active aerial, boat and ground-based treatments of larval stages, can reduce significantly but not eliminate the nuisance. The SCCMVC makes frequent outreach appeals to the public to reduce standing water and artificial container breeding on their property, but the **proximity** of residents to large bodies of standing water increases their chance of exposure to mosquitoes and mosquito-borne diseases.

Restoration, enhancement and disturbance of wetlands for wildlife can produce mosquitoes as an unintended byproduct. Also, mosquitoes are often the pioneering species in newly flooded or disturbed sites. They can spike to high levels and disperse before a diverse aquatic regime and stasis through predation and competition is established. At least fifteen mosquito species can exploit sites as diverse as tree holes, neglected septic systems and swimming pools, small containers, stormwater systems, freshwater sloughs and brackish marshes in the mild climate of our County.

Our Vector Control Specialists follow a route list of known breeding sources on a routine basis. The water sources are dip sampled to determine breeding using a standard twelve-ounce dipper cup. Mosquito management decisions are made by first establishing a **threshold** level of breeding for each aquatic site based on species type, number per dip, distance to residents, larval age, the presence of predators, presence of sensitive species and other factors.

Even when protected species are not present, marshes have great ecological and educational value as suburban wetlands, a rarely encountered environment whose biological diversity must be protected. Yet it is because of this proximity to human habitation and activity that management of mosquitoes is occasionally required to protect human health and the annoyance associated with the nuisance species. Selective reduction of mosquito populations in this protected environment is a marsh management element that increases the benefit values to the surrounding community and has benefits to avian life, particularly nestlings and species susceptible to West Nile virus. West Nile Virus has the potential to cause human, wildlife and equine morbidity/mortality in this area.

### **Materials and Techniques**

There have been remarkable advances in mosquito control materials and methodology, incorporating IMM approaches anchored around the use of public education, source reduction and biocontrols. When appropriate, larvicides are employed, preferably the selective and non-persistent mosquito growth regulator methoprene (synthesized juvenile hormone) organic microbials (*Bacillus thuringiensis israelensis* and *B. sphaericus*) or organic fermented soil bacterium (*Spinosad*). These are “least toxic” materials that provide maximum safety for the applicator, non-target organisms, residents and their pets and property, with minimum impact on the food web of the water source. Dosage rates must be both sufficiently high to kill targeted species (and delay resistance) and sufficiently low to minimize non-target effects. Also, it is important that materials be rotated periodically to forestall resistance.

To achieve satisfactory, sustainable and consistent results with these materials, it is essential that they be alternated and used in conjunction with a comprehensive monitoring and source reduction program, and with other elements incorporating Best Management Practices. Knowledge of the biology of the target species and habitat, the timing of the application and other environmental factors related to operational success is crucial to obtaining cost-effective results of a reduction in the mosquito population. SCCMVC staff is experienced in non-intrusive monitoring and treatments of mosquito larvae and adults, and in determining threshold levels for different species that would initiate treatments.

Treatments are conducted on foot using backpack sprayers for liquids or backpack blowers for granular materials, from an inflatable boat, airboat, or by contracted drone treatments. Pesticides are applied when winds are less than 10 mph to avoid drift. Compromises are made in sensitive sites, such as a less intrusive management protocol arranged with the Fish and Wildlife Service for the federal refuge (see Wildlife Refuges section or Draft Monitoring and Treatment Plan). Temporary trails may be cut to less accessible areas, invasive plants reduced, and poison oak kept at bay with spot treatments of herbicide, where appropriate. If brushing a trail is necessary, care is taken to reduce the impact to green limbs or saplings.

The SCCMVC is involved in the development review process in a pro-active role as part of our source reduction program. Due to the potential disruption and public health threat that infestations of mosquitoes could cause residents of this area, their existence is a **significant biological hazard** that requires mitigation and should be included on initial study checklists.

Intense efforts are made to monitor mosquito breeding through trapping of adult mosquitoes and inspecting breeding sites by sampling water for larvae and treating when necessary to reduce emergence. Alternative management strategies and biological control methods are first considered. Some sources require checking and treating several times a year. Pre-treatment and post-treatment larval counts are taken to measure effectiveness and detect resistance. The contents of adult mosquito monitoring traps are also identified and counted to determine abundance and distribution of species. They are set once a week during the mosquito season and help technicians pinpoint potential aquatic habitats of each species based on the adults caught and can be controlled accordingly with larvicides.

Quality of life would seriously be impacted without mosquito reduction measures. With present or even increased levels of service, surviving mosquitoes in some years could still be numerous enough to result in complaints because breeding sites are frequently near human activity and mosquitoes can disperse long distances. Priority is placed upon requests for service from residents, ahead of our other routine operations.

The SCCMVC would only consider adulticiding measures (fogging) in a West Nile Virus disease outbreak, as it is more selective and effective to control larvae before they emerge and disperse as adults. Although fogging is perceived by some citizens as offering the best relief, it is unacceptable to others and is not a pro-active strategy. It would be considered by the SCCMVC as a last alternative and after approval by the West Nile Virus Technical Advisory Committee and the County Board of Supervisors, in cases of public health emergency.

### **Important Mosquitoes of Santa Cruz County**

The County is home to over a dozen mosquito species, but of particular concern is *Culex tarsalis*, the encephalitis mosquito. It breeds through summer and fall and has the potential of transmitting West Nile Virus, St. Louis Encephalitis (SLE) and Western Equine Encephalitis (WEE) to humans and horses. They can be found among emergent vegetation such as smartweed and flooded grasses, algal mats and inundated blackberry thickets. The increase in warm-season human activity at dusk in areas surrounding the sloughs requires that we lower the threshold at which treatments are initiated to reduce mosquito breeding to acceptable levels. Criteria used to determine the threshold are larval density, species significance (nuisance or disease), flight range, proximity to people, dispersal patterns and other environmental and meteorological factors.

The other major *Culex* species that is of concern to us is the Northern House Mosquito, *Culex pipiens*. In North America, this mosquito is widely distributed in urban, suburban, and rural areas. *Culex pipiens* can transmit pathogens, such as West Nile virus (WNV) and Saint Louis encephalitis virus (SLE) to humans, birds, and horses. These mosquitoes readily enter homes and can bite throughout the night. Their larval breeding habitats are most often polluted with organic matter like decaying vegetation or sewage. A septic system with cracks or openings in the lid can lead to an infestation of *Culex pipiens*.

Treatment decisions are based on threshold levels of larvae determined for sites by evaluating species risk, proximity to residents, stage of development and abundance, ecological value, presence, number and type of predators and other aquatic life and other environmental factors. This number is dynamic, based on qualitative as well as quantitative observation and may change spatially and temporally. When threshold levels are exceeded, larviciding ensues using material appropriate for larval instars present: the microbial formulations described below, the insect growth regulator Methoprene, a duplex combination of the two, or Natular spinosad products. Pre-flood treatments are employed in some seasonal sources where historical breeding has existed and access is a problem post-flooding.

At present the program makes maximum effort to use *Bacillus thuringiensis israelensis* larviciding products for their efficacy and selectivity. A similar microbial larvicide, *Bacillus sphaericus* is used increasingly in highly organic sources such as sewage, dairy and apple processing ponds because the live bacteria recycles in the mosquito larvae it controls, thereby providing residual control. This is also OMRI accepted for use around organic crops.

The larvicide methoprene is used with water or granular carriers (for penetration of vegetation), or in pellet or briquet form for residual slow release and pre-flood. This insect juvenile growth hormone mimic prevents emergence of live adult mosquitoes and so requires post-treatment collection and emergence of pupae to determine efficacy. These products and the bacilli are applied to larger aquatic areas by contracted drone, boat/airboat or on foot with a backpack sprayer or motorized backpack blower.

Natular is a more recent product that has been registered in California for controlling mosquito larvae. Its active ingredient Spinosad is the product of a naturally occurring soil bacterium *Saccharopolyspora spinosa*, which acts as a neurotoxin and binds to nicotinic acetylcholine receptor (nAChR) and GABA receptors of its target species. This is the first larvicide to be evaluated as a Reduced Risk Product by the EPA. It possesses a unique mode of action not shared by any other insecticide and is shown to be minimally disruptive to most nontarget species tested thus far at its proposed field use rates. This allows the technicians to reduce the risk of building up pesticide resistance in mosquito populations by offering another mode of action to control larvae that can be rotated with other pesticides.

Mature marshes with diverse species seldom contain many mosquitoes, as predators, parasites and competing invertebrates keep them in check. Open water areas with good circulation seldom breed because of wave action and access by predators. Permanent aquatic sources usually contain natural mosquito predators and do not require treatment, unless vegetation like pennywort, water milfoil, or floating primrose is so dense that it prevents natural predation.

Mosquitoes are often the pioneer species, which accounts for their peak density in disturbed aquatic environments. Frequently, the aquatic habitats targeted for larviciding are temporary or semi-permanent. Temporary sites such as marshes and flooded agricultural areas or woodland depressions produce prolific numbers of floodwater mosquitoes. These sites are generally very low in species diversity and mosquito predators, due to the time needed for most species to locate and colonize them.

The Willow Mosquito, *Aedes washinoi*, is a day and dusk-biting species whose aquatic larvae appear in temporary pools in late winter, emerging to seek blood meals in the spring. This mosquito breeds in woodland depressions that fill with water, especially those areas dominated by willow trees and berry vines. Eggs are laid on the mud and organic material along the edges of the receding water in these areas. Adults are generally present in the early spring and are very aggressive.

While floodwater mosquitoes start development during the first week post-inundation, it may take two to three weeks for the first macro invertebrate predators to become established. Mosquito larvicides and growth hormones work effectively in the first week or so against larval mosquito stages. If ponds are treated late in the mosquito cycle for pupal mosquitoes by using less selective surface films, non-target aquatic invertebrates may also be killed. Although these are capable of eventually recovering from localized population declines via recruitment and re-colonization from proximal areas, surface films are usually used only in stagnant conditions where mosquitoes are the dominant organisms.

Another *Aedes* of concern is *Aedes sierrensis*, or the Western Treehole Mosquito. Like other *Aedes* species, this mosquito lays its eggs on a substrate instead of open water, in this case nutrient-rich holes and cavities formed in trees. The eggs can survive being dry and dormant for years before filling back up with water and hatching. Once wet, the eggs will hatch, and a very dense larval habitat will form. Adults emerge in spring after the equinox as they need a 12-hour day to trigger emergence from pupae to flying adult. Biting females will often swarm any large or small mammal they encounter, including humans and dogs.

Pets including dogs and cats are most at risk when it comes to *Aedes sierrensis* as they are the primary vector of dog heartworm (*Dirofilaria immitis*). Heartworms go through several life stages before they become adults, infecting the pulmonary artery of the host animal. The worms require the mosquito as an intermediate host to complete their lifecycles. The rate of development in the mosquito is temperature-dependent, requiring about two weeks of temperature at or above 27 °C (80 °F). Below a threshold temperature of 14 °C (57 °F), development cannot occur, and the cycle is halted. As a result, transmission is limited to warm weather, and duration of the transmission season varies geographically. The period between the initial infection when the dog is bitten by a

mosquito and the maturation of the worms into adults living in the pulmonary arteries takes six to seven months in dogs and is known as the "prepatent period".

Due to the prevalence of this mosquito in the forested parts of this county, we recommend canine owners put their dogs on a preventative heartworm medication, especially if living in an area with a lot of trees that sees extended periods of elevated temperatures and periods of rain, like the Santa Cruz Mountains. Treeholes can be filled in with sand or another granular material, but it is very unlikely that every single treehole in an area can be found and filled in.

Mosquito management and source reduction decisions are enhanced by knowledge of the distribution and life stage of protected species. The most important aim is to reduce mosquitoes below the nuisance threshold without harm to the environment, with emphasis on targeting vector species. The SCCMVC is signatory to CDPH compliance agreements (H&S Code section 116180) including the reporting of any adverse pesticide-related effects. We give high consideration to the direction and research provided by vector ecologists and IPM specialists within the UC system, and consultation with wetland managers and health authorities.

The SCCMVC is committed to improving the quality of life and economic productivity of area residents and the habitability of the surrounding community. A wide variety of aquatic habitats, ranging from residential receptacles to larger agricultural and marshland areas, may be treated with larvicides. Fauna inhabiting the latter sites may include amphibians, fish, other vertebrates and invertebrates, particularly insects and crustaceans. The use, description, and safety of our selected larviciding materials are detailed in the attached appendix and in our environmental review documents online at

<http://www.agdept.com/AgriculturalCommissioner/MosquitoAbatementVectorControl.aspx>

## **Invasive Aedes**

Of most concern to Mosquito and Vector Control agencies across the state is the proliferation and spread of the invasive *Aedes aegypti* and *Aedes albopictus* mosquito species. Confirmed breeding populations of *Ae. aegypti* in California were first reported in the summer of 2013, when they were found in the Central Valley counties of Fresno and Madera and the coastal county of San Mateo. Since then, *Aedes aegypti* and *albopictus* have become established in the Central Valley and in Southern California and are spreading to more areas of the State as the climate warms up.

These species are competent vectors of Dengue, Chikungunya, Yellow Fever, and Zika virus and present a very challenging shift in operations for California mosquito control districts. In 2023, multiple Southern California counties reported local transmission of Dengue for the first time in over 50 years. While almost every case of Dengue reported in California is travel-related with a resident returning from an area endemic with *Aedes aegypti* or *albopictus*, 2023 was the first time in the modern era that multiple residents tested positive for Dengue with no recent history of travel, indicating someone had traveled to the area with an active Dengue infection that was then spread by the bite of a locally established *Aedes aegypti* population.

*Aedes aegypti* is especially problematic with its cryptic container breeding habitats. They can breed in habitats as small as a bottle cap of water and will readily find any little bit of water in a container and lay their eggs on the sides. *Aedes* mosquitoes, unlike the other genera of mosquitoes we have, lay their eggs on container walls or in moist soils of ephemeral ponds as they dry in the spring and summer. The eggs can survive multiple years of drought between hatching, and in the case of the container breeding *Aedes aegypti*, can be unknowingly transported to a new locale and establish a new population.

Another factor that sets them apart from the native mosquito species we have in the County is the time of day they are active. The native mosquito species are mostly crepuscular (active at dawn and dusk) while the invasive *Aedes* species are day-biters that often swarm people. Across the

state, the number one indicator of an invasive *Aedes* outbreak is service request calls by the public. They do not fly far from where they have emerged as adults and trapping them is much more difficult than the native mosquito species. **We ask the public to report any day-biting, small, black and white mosquitoes to our department.**

In October 2022, we had our first detection of *Aedes aegypti* in the County in a small Watsonville neighborhood. A homeowner and fellow County employee reported an uptick in biting mosquitoes, specifically in the daytime. After catching a couple adult mosquitoes in a trap we set out and confirming them as *aegypti*, our team began contacting all the homeowners in the neighborhood to warn them of the dangers and risks of establishing an invasive *Aedes* species in the county. We went door-to-door asking for permission to survey the home's yards and inspect any containers holding water for larvae or eggs. Countless hours by the entire team through extensive trapping and yard inspections at the end of 2022 and throughout 2023 has yielded a positive result as no invasive *Aedes* were detected in 2023.

For more information and to read our full Invasive *Aedes* Response Plan click here (hyperlink to our plan once we have it finalized, probably spring).

### **Other Biological Control**

Although established in California in many permanent natural sources, mosquitofish are non-native and it is **not** the policy of the SCCMVC to stock these opportunistic feeders in natural sources. The SCCMVC warns against the re-release of *Gambusia affinis* before stocking these useful fish for the public in ornamental ponds, swimming pools and troughs. They can compete with other native fishes and are known to be fin biters, nipping at the fins of other fish and reducing their survival.

Bats have proven their usefulness against arthropod pests when they can be lured to stay in properly built bat-houses. However, besides preferring larger (sometimes beneficial) flying prey to maximize protein intake vs. energy expenditures, bats feed at dusk. The CDPH warns that a small percentage of bats are rabid, and that bats often prefer attics to a bat-house. Studies have been done examining the stomach contents of bats in the United States and they can very greatly from small flying insects like mosquitoes to larger insects like moths and beetles. In general, while some bats will feed on adult flying mosquitoes, they should not be considered as the sole means of controlling them, especially considering the risk of rabies, histoplasmosis, or bat parasites associated with their roosting.

Violet-green swallows are likewise often recognized as a supplement to mosquito management when they can be established, but are likewise not preferential in feeding or nest sites, and their mud homes, mites and excrement can, unfortunately, be a nuisance around structures.

Mosquitoes spend most of their lives as larvae and pupae in water before emerging as flying adults and thus promoting natural aquatic predators is the best way to manage mosquito populations, especially in large natural bodies of water like the Sloughs. If an aquatic predator like a dragonfly or damselfly naiad eats a mosquito larvae, it will never have the chance to become a flying and blood seeking female adult mosquito that can annoy people or spread disease.

### **Environmental Protection**

Our area has myriad mosquito breeding sites near and within populated areas. Without ongoing and effective vector control, substantial mosquito activity would significantly and adversely

affect the human environment. The SCCMVC's mosquito control program, including chemical materials, is essential to maintain the vectors in the environment at a tolerable level. The SCCMVC's program will never alleviate all mosquitoes. Rather, it is a resource maintenance program aimed at striking a balance to allow comfortable and healthful human existence within the natural environment, while protecting and maintaining the environment.

History has shown us that the control and abatement of vectors are necessary for our human environment to continue to be habitable. Malaria was largely responsible for the tragic and precipitous decline of native Californians in the 19<sup>th</sup> century, and with yellow fever, encephalitis and dengue continued to sicken thousands of Americans into the 20<sup>th</sup> century. Mosquito-borne diseases are currently a leading cause of human mortality worldwide. Modern recognition of the value of wetlands should not overshadow their potential for pestilence.

The Endangered Species Act has language that limits the regulations from placing undue burden upon essential local operations, including mosquito management, and places the burden of proof of harm on the regulators. Indirect effects causing harm must be close and actual, without remote causal links (effect on effect on effect). Ecologists recognize that the value to the food web of mosquitoes is not substantially missed when they are reduced in wetlands close to human activity, as that niche is adequately filled by other invertebrate scavengers.

In addition to the environmental protection measures and procedures inherent in the SCCMVC's IMM program as discussed above, there are other practices unique to the SCCMVC's chemical control program that protect the environment:

There are numerous federal and state laws and regulations that strictly control and regulate the storage, transport, handling, use and disposal of the pesticides to protect against surface and groundwater contamination and other impacts to the environment and public health. (E.g., Federal Insecticide, Fungicide and Rodenticide Act; Cal. Food & Agric. Code divisions 6 & 7; Cal. Code of Regs., title 3, division 6.) The SCCMVC applies aquatic larvicides under an NPDES permit required in waters of the U.S. and reports use to the Central Coast Regional Water Quality Control Board and has a Best Management Plan on file with that agency. The SCCMVC and its staff consistently comply with these laws and regulations.

The SCCMVC uses only pesticides registered by the U.S. Environmental Protection Agency and California Department of Pesticide Regulation. The SCCMVC then strictly complies with the pesticide label restrictions and requirements concerning the storage, transport, handling, use and disposal of the pesticides.

Consistent with the SCCMVC's integrated mosquito management principles, when using pesticides, the SCCMVC selects the least hazardous material that will meet its goals and the SCCMVC avoids using restricted materials-type pesticides.

The SCCMVC is an active member of the Mosquito and Vector Control Association of California (MVCAC), a statewide association representing the interests of vector control districts throughout the state. The Association, and its member districts, participates in the U.S. Environmental Protection Agency's Pesticide Environmental Stewardship Program, a program to encourage less pesticide use and greater environmental stewardship by vector control districts.

Pesticides are applied only by CDPH-certified and trained vector control technicians. The frequent training includes continuing education on appropriate practices to avoid environmental impacts and assure compliance with regulatory requirements.

The SCCMVC regularly calibrates its pesticide application equipment to ensure that it distributes the proper quantities of material. It stores the material safely and under scrutiny of the Agricultural Commissioner and Environmental Health departments. Pesticide use is well documented and overseen by the Agricultural Commissioner. Application sites are measured and

digitized into geographic information systems maps and related to trap monitoring and field records in the database, which is shared on a UC server with MVCAC and CDPH.

## **Wildlife Refuges**

The SCCMVC maintains good communication and cooperation with environmental regulatory agencies. Consultation is provided to these agencies for their wetlands restoration projects to ensure compatibility with mosquito management goals.

In our current Zones of Benefit, there are about 15 acres of wetland within the 300-acre Ellicott Slough Refuge/Reserve jointly managed by U.S. Fish and Wildlife Service (USFWS) and California Fish and Game (CDFG) that offers protection for the endangered Santa Cruz long-toed salamander and threatened red-legged frog and tiger salamander, and another 130 acres managed by CDFG as ecological reserve in the Watsonville Slough. The SCCMVC also reports pesticide applications to the State Park system on its coastal freshwater marshes at Sunset State Beach, Twin Lakes and Natural Bridges.

In 2000 the SCCMVC met with USFWS representatives, and it was determined to defer using the larvicide methoprene in the Santa Cruz Long-toed Salamander ponds on the Ellicott Slough State Ecological Reserve and at the Calabassas unit of the Ellicott Slough National Wildlife Refuge (ESNWR) pending a USFWS Section 7 (inter-agency consultation) for the Refuge, which will result in a permit or Comprehensive Conservation Plan that will determine mosquito management alternatives. The SCCMVC submitted Pesticide Use Proposals (PUP's) to the USFWS for larviciding materials to be used in the ESNWR. The PUP's outline application rates, target mosquito species, methods of application and the listed sensitive species and helps to ensure compatible use through the SCCMVC conforming to Best Management Practices (BMP) in the Refuge area.

A protocol agreed upon with the USFWS for treatment of mosquito larvae in ponds and ditches of the Ellicott area amphibian refuge reduces the possibility of non-target impacts. Applications are made under the following conditions:

- Applications are made from shorelines of ponds. The SCCMVC staff does not enter the water. If the source is too large to treat from the edges, SCCMVC contracts with a drone company to treat the source in the most minimally invasive way possible, which approval is requested each time before application.
- The San Francisco Bay Area National Wildlife Refuge office is notified so that a USFWS biologist can be present when larvicides are applied.
- All staff sampling mosquito larvae with a dipper are informed of the appropriate techniques for avoiding the capture of amphibian larvae or dislocating eggs and egg masses and for their release if they are inadvertently caught. Staff conducting monitoring activities do not enter the water. Dippers are cleaned and sanitized before sampling to reduce the risk of introducing foreign species into the water or spreading Chytrid fungus.

Note: In addition, the SCCMVC informs USFWS of surveillance activities at Ellicott and emails a treatment map following applications. Similarly, amphibian protection measures are taken on a voluntary basis on other properties where amphibian recovery efforts are being conducted. Other areas within the South County are being considered for acquisition within the federal refuge and State reserve systems.



Previously Submitted to the USFWS:

1. Appendix: The use, description, and safety of methoprene and microbial materials
2. Treatment Criteria table used as basis for building treatment threshold determination.
3. "Long-term effects of the mosquito control agents Bti and methoprene on non-target macro-invertebrates in wetlands in Wright County, MN (1997-98)", Lake Superior Research Institute.
4. USGS News Release: X-ray Studies Shed Light on Frog Deformities.
5. "Methoprene concentrations in freshwater microcosms treated with sustained-release Altosid formulations", Ross, Judy, Jacobsen and Howell, 1994.
6. "Nontarget effects of mosquito larvicides used on national wildlife refuges", Lawler, Jensen and Dritz, 1997.
7. "Laboratory and field evaluation of the efficacy of four insecticides for *Aedes vigilax* and toxicity to the non-target shrimp *Leander tenuicornis*." Brown, Thomas, Mason, Greenwood and Kay, 1999.
8. "Insect developmental inhibitors. 3. Effects on nontarget aquatic organisms." Miura and Takahashi, 1973.
9. "Field evaluation of the effects of slow-release wettable powder formulation of Altosid on nontarget organisms." Creekmur, Russell and Hazelrigg.
10. "Potential effects of Altosid briquet treatments on *Eubbranchipus bundyi*." Batzer and Sjogren.
11. "Environmental degradation of the insect growth regulator methoprene. II. Metabolism by aquatic microorganisms." Schooley, Bergot, Dunham and Siddall.
12. "Effects of methoprene on nontarget organisms when applied as a mosquito larvicide." Hester, Rathburn and Boike.
13. Report for the Ministry of Health (New Zealand): Environmental and health impacts of *Bacillus thuringiensis israelensis*. Glare and O'Callaghan, 1998.
14. Report for the Ministry of Health (New Zealand): Environmental and health impacts of the insect juvenile hormone analogue, s-methoprene. Glare and O'Callaghan, 1999.

## APPENDIX

### **More about Methoprene**

**INTRODUCTION.** s-Methoprene does not produce non-discriminatory, rapid toxic effects that are associated with nervous system toxins. s-Methoprene is a true analogue and synthetic mimic of a naturally occurring insect hormone called Juvenile Hormone (JH). JH is found during aquatic life stages of the mosquito and in other insects but is most prevalent during the early instars. As mosquito larva matures, the level of JH steadily declines until the 4<sup>th</sup> instar molt, when levels are very low. This is a sensitive period when all the physical features of the adult begin to develop.

s-Methoprene in the aquatic habitat can be absorbed on contact and the insect's hormone system becomes unbalanced. When this happens during the sensitive period, the unbalance interferes with 4<sup>th</sup> instar larval development.

One effect is to prevent adults from emerging. Since pupae do not eat, they eventually deplete body stores of essential nutrients and then starve to death. For these and perhaps other reasons, s-Methoprene is considered an insect growth regulator (IGR). An advantage that methoprene has over other larvicides is that the mosquito immatures remain alive as prey for aquatic predators.

There have been widely distributed reports regarding the effect methoprene may have on certain amphibians. Reports of frog abnormalities have been widely circulated, but these reports have not stood up to scientific scrutiny. Often it is in a lab setting where amphibians were treated at levels 100s of times stronger than what is used in the field based on the label rates.

**FORMULATIONS AND DOSAGES.** Currently, seven s-methoprene formulations are sold under the trade name of Altosid. These include Altosid Liquid Larvicide (A.L.L.) and Altosid Liquid Larvicide Concentrate, Altosid Briquets, Altosid XR Briquets, Altosid SBG (single-brood granules), Altosid XR-G and Altosid Pellets. Altosid labels contain the signal word "CAUTION" and all products are Category 4.

**ALTOSID LIQUID LARVICIDE (A.L.L.) & A.L.L. CONCENTRATE.** These two flowable formulations have identical components except for the difference in the concentration of active ingredients. A.L.L. contains 5% (wt./wt.) s-Methoprene while A.L.L. Concentrate contains 20% (wt./wt.) s-Methoprene. The balance consists of inert ingredients that encapsulate the s-Methoprene, causing its slow release and retarding its ultraviolet light degradation.

**DOSAGES.** Use rates are 3 to 4 ounces of A.L.L. 5% and  $\frac{3}{4}$  to 1 ounce of A.L.L. Concentrate (both equivalent to 0.01008 to 0.01344 lb. AI) per acre, mixed in water as a carrier and dispensed by spraying with conventional ground and aerial equipment. Because the specific gravity of Altosid Liquid is about that of water, it tends to stay near the target surface. No rate adjustment is necessary for varying water depths when treating species that breathe air at the surface.

**TARGET SPECIES.** Liquid formulations are designed to control fresh and saline floodwater mosquitoes with synchronous development patterns. Cold, cloudy weather and cool water slow the release and degradation of the active ingredient as well as the development of the mosquito larvae. Accordingly, formulation activity automatically tracks developing broods.

**ALTOSID BRIQUETS.** The Altosid Briquet was the first solid methoprene product marketed for mosquito control beginning in 1978. It is made of plaster (calcium sulfate), 3.85 % (wt./wt.) r-methoprene, 3.85% s-methoprene (.000458 lb. AI/briquet) and charcoal (to retard ultra violet light degradation). Altosid Briquets release methoprene for about 30 days under normal weather conditions.

**DOSAGES.** Application should be made at the beginning of the mosquito season, and under normal weather conditions, repeat treatments should be carried out at 30-day intervals. The recommended application rate is 1 Briquet per 100 sq. ft. in non-flowing or low-flowing water up to 2 feet deep.

**TARGET SPECIES.** Flood water *Aedes* and permanent water *Anopheles*, *Culex*, and *Culiseta* larvae are usual targets. Typical treatment sites include storm drains, catch basins, roadside ditches, ornamental ponds and fountains, cesspools and septic tanks, waste treatment and

settlement ponds, flooded crypts, transformer vaults, abandoned swimming pools, construction and other man-made depressions.

**ALTOSID XR BRIQUETS.** It is made of hard dental plaster (calcium sulfate), 1.8% (wt./wt.) s-methoprene (.00145 lb. AI/briquet) and charcoal (to reduce ultraviolet light degradation). Despite containing only 3 times the AI as the “30-day briquet”, the comparatively harder plaster and larger size of the XR Briquet change the erosion rate allowing sustained s-methoprene release up to 150 days in normal weather.

**DOSAGES.** XR Briquets should be applied 1 to 2 per 200 sq. ft. in no-flow or low-flow water conditions, depending on the species.

**TARGET SPECIES.** Targets are the same as for the smaller briquets. Appropriate treatment sites for XR Briquets include storm drains, catch basins, roadside ditches, ornamental ponds and fountains, cesspools and septic tanks, waste treatment settlement ponds, flooded crypts, transformer vaults, abandoned swimming pools, construction and other man-made depressions, cattail swamps and marshes, water hyacinth beds, pastures, meadows, rice fields, freshwater swamps and marshes, woodland pools, flood plains and dredge spoil sites.

**ALTOSID PELLETS.** Altosid Pellets were approved for use in April 1990. They contain 4% (wt./wt.) s-methoprene (0.04 lb. AI/lb.), dental plaster (calcium sulfate), and charcoal. Like the Briquets discussed above, Pellets are designed to slowly release s-methoprene as they erode. Under normal weather conditions, control can be achieved for up to 30 days.

**DOSAGES.** Label application rates range from 2.5 lbs. to 10.0 lbs. per acre (0.1 to 0.4 lb. AI/acre), depending on the target species and/or habitat.

**TARGET SPECIES.** The species are the same as listed for the briquet formulations. Listed target sites include pastures, meadows, rice fields, freshwater swamps and marshes, salt and tidal marshes, woodland pools, flood plains, tires and other artificial water holding containers, dredge spoil sites, waste treatment ponds, ditches, and other man-made depressions, ornamental pond and fountains, flooded crypts, transformer vaults, abandoned swimming pools, construction and other man-made depressions, tree holes, storm drains, catch basins, and waste water treatment settling ponds.

**ALTOSID SBG** at 1.5% (wt./wt.) s-methoprene it has a five to seven day residual for use with mosquitoes having synchronous development patterns (single-brood). It has a small particle size but high density and a broad target site list.

**DOSAGES.** Label application rates range from 5 lbs. to 20.0 lbs. per acre, depending on the target species and/or habitat.

**ALTOSID XR-G** was approved for use in 1997. This product contains 1.5% (wt./wt.) s-methoprene. Granules are designed to slowly release s-methoprene as they erode. Under normal weather conditions, control can be achieved for up to 21 days.

**DOSAGES.** Label application rates range from 5 lbs. to 20.0 lbs. per acre, depending on the target species and/or habitat.

**TARGET SPECIES AND APPLICATION SITES.** The species are the same as listed for the briquet formulations. Listed target sites include snow pools, meadows, rice fields, freshwater swamps and marshes, salt and tidal marshes, woodland pools, tires and other artificial water holding containers, dredge spoil sites, waste treatment ponds, ditches, and other natural and man-made depressions.

## **More About Bacillus Products**

Mosquito control makes use of two stomach toxins whose active ingredients are manufactured by bacteria. These control agents are often designated as Bacterial Larvicides. Their mode of action requires that they be ingested to be effective, which can make them more difficult to use than the contact toxins and surface-active agents. Bacteria are single-celled parasitic or saprophytic microorganisms that exhibit both plant and animal properties and range from harmless and beneficial to intensely virulent and lethal.

A beneficial form, *Bacillus thuringiensis* (Bt), is the most widely used (especially in agriculture) microbial pesticide in the world. It was originally isolated from natural Lepidopteran (butterflies and moths) die-offs in Germany and Japan. Various Bt products have been available since the 1950's, and in 1976, Dr. Joel Margalit and Mr. Leonard Goldberg isolated from a stagnant riverbed pool in Israel, a subspecies of *B. thuringiensis* that had excellent mosquito larvicide activities. It was named B.t. variety israelensis (B.t.i.) and later designated *Bacillus thuringiensis* Serotype H-14. Either of these two designations may be found on the labels of many bacterial mosquito larvicide formulations used today. Another species of bacteria, *B. sphaericus*, also exhibits mosquito larvicide properties.

**INTRODUCTION.** Like a tiny chemical factory capable of only one production run, each **B.t.i.** organism may produce, if the environmental conditions around it are favorable, five different microscopic protein pro-toxins packaged inside one larger protein container or crystal. The crystal is commonly referred to as delta (d-) endotoxin. If the d-endotoxin is ingested, these five proteins are released in the alkaline environment of an insect larvae's gut. The five proteins are converted into five different toxins if specific enzymes also are present in the gut. Once converted, these toxins work alone or in combination to destroy the gut wall. This leads to paralysis and death of the larvae.

B.t.i. is grown commercially in large fermentation vats using sophisticated techniques to control environmental variables such as temperature, moisture, oxygen, pH and nutrients. The process is similar to the production of beer, except that B.t.i. bacteria are grown on high protein substrates such as fishmeal or soy flour and the spore and delta endotoxin are the end products. At the end of the fermentation process, B.t.i. bacteria exhaust the nutrients in the fermentation machine, producing spores before they lyse and break apart. Coincidental with sporulation, the delta endotoxin is produced. The spores and delta endotoxins are then concentrated via centrifugation and microfiltration of the slurry. It can then be dried for processing and packaging as a solid formulation(s) or further processed as a liquid formulation(s). Since some fermentation medium (e.g. fish meal) is always present in liquid formulations, they generally smell somewhat like the medium.

**FORMULATIONS AND DOSAGES.** There are five basic B.t.i. formulations available for use: liquids, powders, granules, pellets, and briquets. Liquids, produced directly from a concentrated fermentation slurry, tend to have uniformly small (2-10 micron) particle sizes, which are suitable for ingestion by mosquito larvae. Powders, in contrast to liquids, may not always have a uniformly small particle size. Clumping, resulting in larger sizes and heavier weights, can cause particles to settle out of the feeding zone of some target mosquito larvae, preventing their ingestion as a food item. Powders must be tank mixed before application to an inert carrier or to the larval habitat, and it may be necessary to mix them thoroughly to achieve a uniformly small consistency. B.t.i. granules, pellets, and briquets are formulated from B.t.i. primary powders and an inert carrier. B.t.i. labels contain the signal word "CAUTION" and B.t.i. is Category 4.

Since fourth instar mosquito larvae quit feeding prior to becoming pupae, it is necessary to apply B.t.i. prior to this point in their development. Although the details are poorly understood, evidence suggests that larvae also undergo a period of reduced feeding or inactivity prior to molting from 1<sup>ST</sup> to 2<sup>ND</sup>, 2<sup>ND</sup> to 3<sup>RD</sup>, and 3<sup>RD</sup> to 4<sup>TH</sup> instars. If we apply B.t.i. at these points in their development, the toxic crystals may settle out before the larvae resume feeding, and with

synchronous broods of mosquitoes, complete control failures may result. With asynchronous broods, efficacy may be reduced. Kills are usually observed within 24 hours of toxin ingestion.

The amount of toxins contained within B.t.i. products are reported indirectly as the result of at least two different bioassays and are difficult to equate to one another. Prepared volumes of toxins are applied to living mosquito larvae and the resulting mortality is produced through formulae numerical measures known as International Toxic Units (ITU's) and *Aedes aegypti* International Toxic Units (AA-ITU's). These measures are only roughly related to observed efficacy in the field and are therefore inappropriate to consolidate and report on like other toxicants.

**BTI LIQUIDS.** Currently, three commercial brands of B.t.i. liquids are available: Aquabac XT, Teknar HP-D, and Vectobac 12AS.

**DOSAGES AND FORMULATIONS.** Labels for all three products recommend using 4 to 16 liquid oz/acre in unpolluted, low organic water with low populations of early instar larvae (collectively referred to below as clean water situations). The Aquabac XT and Vectobac 12 AS (but not Teknar HP-D) labels also recommend increasing the range from 16 to 32 liquid oz/acre when late 3<sup>rd</sup> or early 4<sup>th</sup> instar larvae predominate, larval populations are high, water is heavily polluted, and/or algae are abundant. The recommendation to increase dosages in these instances (collectively referred to below as organic water situations) also is seen in various combinations on the labels for all other B.t.i. formulations discussed below.

B.t.i. liquid may also be “Duplexed” with the Altosid Liquid Larvicide discussed above. Because B.t.i. is a stomach toxin and lethal dosages are somewhat proportional to a mosquito larvae's body size, earlier instars need to eat fewer toxic crystals to be adversely affected. Combining B.t.i. with methoprene (which is most effective when larvae are the oldest and largest) allows a public health agency to use less of each product than they normally would if they would use one or the other. Financially, most savings are realized for treatments of mosquitoes with long larval

**BTI CORNCOB GRANULES.** Granular formulations use a carrier that is dense enough to penetrate heavy vegetation. There are currently two popular corncob granule sizes used in commercial formulations. Aquabac 200G, Bactimos G, and Vectobac G are made with 5/8 grit crushed cob, while Aquabac 200 CG (Custom Granules) and Vectobac CG are made with 10/14 grit cob. Aquabac 200 CG is available by special request. The 5/8 grit is much larger and contains fewer granules per pound. The current labels of all B.t.i. granules recommend using 2.5 to 10 lb./acre in clean water and 10 to 20 lb./acre in highly organic water situations.

**BTI PELLETS.** Bactimos Pellets are the only extruded B.t.i. product on the market today. They are manufactured using a larval food as the B.t.i. carrier, and the manufacturer claims that this helps attract feeding larvae. The Pellets contain twice the amount of toxic units as Bactimos (corn-cob) Granules, and the label correspondingly recommends using only half as much by weight in both clean water and organic water situations.

**BTI BRIQUETS (donuts).** B.t.i. donuts are a sole source product manufactured by Summit Chemical Company under a Bactimos B.t.i. subregistration. They are a mixture of B.t.i., additives, and cork. They are designed to float and slowly release B.t.i. particles for up to 30 days. They apparently are attractive to raccoons and possibly other wildlife because of their odor, and may sometimes be disturbed or carried off. Donuts may be staked in place to prevent wind from moving them from a site's littoral zone into open water. The use rate is one donut per 100 square feet in clean water and up to four donuts per 100 square feet in dirty water. Many districts have not found these to be practical in most larval sites due to their expense and the possibility of them being moved by wind or animals. Homeowners, however, may find practical uses for these in ornamental ponds or other very small habitats.

**TARGET SPECIES.** B.t.i. adversely affects larval stages of insect species in the Order Diptera, Suborder Nematocera, Families Culicidae (Mosquitoes) and Simuliidae (Black Flies). B.t.i. has

been shown to be effective for numerous mosquito species, including members of the mosquito genera *Aedes*, *Anopheles*, *Culex*, and *Culiseta*, commonly targeted in California.

Products containing B.t.i. are ideally suited for use in integrated pest management programs because the active ingredient does not interrupt the activities of most beneficial insects and predators. Since B.t.i. has a highly specific mode of action, it is an insecticide of minimal environmental concern. B.t.i. controls all larval instars provided they have not quit feeding and can be used in almost any aquatic habitat with no restrictions. It may be applied to irrigation water and any other water sites except treated finished drinking water. B.t.i. is fast acting and its efficacy can be evaluated almost immediately. It usually kills larvae within 1 hour after ingestion, and since each instar must eat for the larvae to grow that means B.t.i. usually kills mosquito larvae within 24 hours of application. It leaves no residues, and it is quickly biodegraded. Resistance is unlikely to develop simultaneously to the five different toxins derived from the B.t.i. delta-endotoxin since they have five different modes of action. This suggests that this mosquito larvicide will continue to be effective for many years.

B.t.i. labels carry the CAUTION signal word, suggesting the material may be harmful if inhaled or absorbed through the skin. However, the 4-hr Inhalation LC 50 in rats is calculated to be greater than 2.1 mg/liter (actual) of air, the maximum attainable concentration. The acute Dermal LD 50 in rabbits is greater than 2,000 mg/kg body weight and is considered to be non-irritating to the eye or skin. Toxicology profiles also suggest that the inert ingredients (not the B.t.i.) in liquid formulations, may cause minor eye irritations in humans. The acute Oral LD 50 in rats is greater than 5,000 mg/kg body weight (similar to an individual drinking over 5 quarts) suggesting the material is practically non-toxic in single doses. Common table salt has an LD 50 of 4,000 mg/kg of body weight.

B.t.i. applied at label rates has virtually no adverse effects on applicators, livestock, or wildlife including beneficial insects, annelid worms, flatworms, crustaceans, mollusks, fish, amphibians, reptiles, birds or mammals. However, non-target activity on larvae of insect species normally associated with mosquito larvae in aquatic habitats has been observed. There have been reported impacts in larvae in the Order Diptera, Suborder Nematocera, Families Chironomidae (midges), Ceratopogonidae (biting midges) and Dixidae (dixid midges). These non-target insect species, taxonomically closely related to mosquitoes and black flies, apparently contain the necessary gut pH and enzymes to activate delta-endotoxins. However, the concentration of B.t.i. required to cause these effects is 10 to 1,000 times higher than normal use rates.

Further, studies report these impacts are short-lived, with the population of these species rebounding quickly. **See study "Long-term effects of the mosquito control agents Bti and methoprene on non-target macroinvertebrates in wetlands in Wright County, Minnesota (1997-1998)".**

Concerning the operational use of B.t.i., timing of application is extremely important. Optimal benefits are obtained when treating 2<sup>nd</sup> or 3<sup>rd</sup> instar larvae. Treatments at other development stages may provide less than desired results. Therefore, a disadvantage of using B.t.i. is the limited treatment window available.

**INTRODUCTION.** *Bacillus sphaericus* is a commonly occurring spore-forming bacterium found throughout the world in soil and aquatic environments. Some strains produce a protein endotoxin at the time of sporulation. It is grown in fermentation vats and formulated for end use with processes similar to that of B.t.i. A standard bioassay like that used for B.t.i. has been developed to determine preparation potencies. The bioassay utilizes *Culex quinquefasciatus* 3<sup>rd</sup>-4<sup>th</sup> instar larvae. The endotoxin destroys the insect's gut in a way similar to B.t.i. and has been shown to have activity against larvae of many mosquito genera such as *Culex*, *Culiseta*, and *Anopheles*. The toxin is only active against the feeding larval stages and must be partially digested before it becomes activated. At present, the molecular action of *B. sphaericus* is

unknown. Isolation and identification of the primary toxin responsible for larval activity has demonstrated that it is a protein with a molecular weight of 43 to 55 kD.

**VECTOLEX CG.** VectoLex-CG is the trade name for Abbott Laboratories' granular formulation of *B. sphaericus* (strain 2362). The product has a potency of 50 BSITU/mg (*Bacillus sphaericus* International Units/mg) and is formulated on a 10/14 mesh ground corn cob carrier. The VectoLex-CG label carries the "CAUTION" hazard classification.

**DOSAGES.** VectoLex-CG is intended for use in mosquito breeding sites that are polluted or highly organic in nature, such as dairy waste lagoons, sewage lagoons, septic ditches, tires, and storm sewer catch basins. VectoLex-CG is designed to be applied by ground (by hand or truck-mounted blower) or aerially at rates of 5-10 lb./acre. The best results are obtained when applications are made to larvae in the 1<sup>st</sup> to 3<sup>rd</sup> instars. Use of the highest rate is recommended for dense larval populations. Larval mortality may be observed as soon as a few hours after ingestion but typically takes as long as 2-3 days, depending upon dosage and ambient temperature. VectoLex-G should be stored in a cool, dry place, in an intact product package. Once the VectoLex-G package is opened, moisture can be absorbed by the product leading to loss of activity over time. Refrigeration is not necessary.

**TARGET SPECIES.** *B. sphaericus* adversely affects larval stages of insect species in the Order Diptera, Suborder Nematocera, Family Culicidae (mosquitoes). *Culex* species are the most sensitive to *Bacillus sphaericus*, followed by *Anopheles* and some *Aedes* species. In California, *Culex* spp. and *Anopheles* spp. may be effectively controlled. Several species of *Aedes* have shown little or no susceptibility, and salt marsh *Aedes* species are not susceptible. *Bacillus sphaericus*, in contrast to B.t.i., is virtually non-toxic to Black Flies (Simuliidae).

*B. sphaericus* has demonstrated the unique property of being able to control mosquito larvae in highly organic aquatic environments, including sewage waste lagoons, animal waste ponds, and septic ditches. After a single application at labeled rates, field evaluations have shown VectoLex-CG to persist for 2-4 weeks. Field evaluations with VectoLex-CG have shown that *Bacillus sphaericus* may undergo limited recycling in mosquitoes in certain organically rich environments.

**VectoLex-CG** has been extensively tested and has had no adverse effects on mammals or non-target organisms. *B. sphaericus* technical material was not infective or pathogenic when administered as a single oral, intravenous or intratracheal installation in rats. No mortalities or treatment-related evidence of toxicological effects were observed. The acute oral and dermal LD 50 values are greater than 5000 mg/kg and greater than 2000 mg/kg, respectively. The technical material is moderately irritating to the skin and eye. Oral exposure of *B. sphaericus* is practically nontoxic to mallard ducks. No mortalities or signs of toxicity occurred following a 9000 mg/kg oral treatment. Birds fed diets containing 20% w/w of the technical material experienced no apparent pathogenic or toxic effects during a 30-day treatment period. Mallards given an intraperitoneal injection of *B. sphaericus* demonstrated toxicologic effects including hypoactivity, tremors, ataxia and emaciation. The LD 50 value was greater than 1.5 mg/kg.

Acute freshwater fish toxicity tests were done on bluegill sunfish, rainbow trout and daphnids. The 96-hour LC 50 and NOEC value for bluegill sunfish and rainbow trout was greater than 15.5 mg/liter; the 48-hour EC 50 and NOEC value for daphnids was greater than 15.5 mg/liter. Acute aquatic saltwater fish toxicity tests were done on sheep head minnows, shrimp and oysters. The 96-hour LC 50 value for both sheep head minnows and shrimp was 71 mg/liter, while the NOEC (no observable effect concentration) value was 22 mg/liter for sheep head minnows and 50 mg/liter for shrimp. The 96-hour EC 50 value for oysters was 42 mg/liter with a NOEC of 15 mg/liter.

Invertebrate toxicity tests were done on mayfly larvae and honeybees. The LC 50 and NOEC value for mayfly larvae was 15.5 mg/liter. Honeybees exposed to 10E4-10E8 spores/ml for up to 28 days demonstrated no significant decrease in survival when compared to controls. Acute

toxicity of *B. sphaericus* to non-target plants was evaluated in green algae. The 120-hour EC 50 and NOEC values were greater than 212 mg/liter.

*Bacillus sphaericus* will not regenerate in salt water, rendering its use impractical for control of salt-water mosquitoes. Cycling is limited to permanent fresh water bodies, and if organics are very high, recycling may be minimal.

**VectoMax™** is a biological larvicide that combines B.t.i. and *Bacillus sphaericus* in one homogenous formulation. These two bacteria are combined in one molecule which ensures that larvae feed on both at the same time. VectoMax offers the faster, broad spectrum control of VectoBac® (B.t.i.), with the residual control of VectoLex® (*B. sphaericus*).

B.t.i. and *B. sphaericus* are naturally occurring bacteria that have insecticidal activity on mosquitoes. These bacteria contain protein crystals that, when ingested by mosquito larvae, will rupture the gut wall or the larvae. This results in rapid death of the larvae. Can be used in a wide range of sources and habitat. Urban and rural, agricultural sites, crop and non-crop, lakes, ponds, wetland and marshes, catch-basins and swimming pools, etc.

## More About Spinosad

**INTRODUCTION.** Spinosad is biologically derived from the fermentation of *Saccharopolyspora spinosa*, a naturally occurring soil organism. This Group 5, organic-rated larvicide overstimulates the nervous system of mosquito larvae. Spinosad contains a mix of two spinodoids, spinosyn A and spinosyn D in a 17:3 ratio respectively. It was discovered in 1982 in isolates from crushed sugarcane in an abandoned rum distillery in the Caribbean. Spinosad has been used around the world for the control of a variety of insect pests, including Lepidoptera, Diptera, Thysanoptera, Coleoptera, Orthoptera, and Hymenoptera. As a product of naturally occurring soil bacteria, it is considered a biorational pesticide that is highly specific in its activity with little effect to non-targets and thus many nations have approved it for use in organic agriculture. It is not mutagenic or considered carcinogenic.

**NATULAR.** Natular is the trade name of Clarke's proprietary formulation of Spinosad for use in controlling mosquito larvae. Natular larvicides became the first public health label for spinosad, and also the first aquatic use pattern with the active ingredient as well. In natural water systems, spinosad degrades rapidly in sunlight. A water column half-life of less than one day has been observed in artificial pond systems in outdoor conditions. In field conditions, spinosad breaks down rapidly into carbon dioxide and water by soil microbes.

**Naturlar G30** is the trade name of the 30-day granular formation of spinosad. It has an active ingredient concentration of 2.5% of spinosad. The granule is mostly comprised of a sand mixture made from quartz, aluminum oxide, calcium oxide, and titanium oxide. A dust mask is recommended when handling the product to avoid risk of inhalation. It is registered as a Group 5 insecticide by the EPA that carries the least toxic to human life signal word, "CAUTION." Do not apply to natural or artificial containers intended for consumption by people, animals, or livestock.

**DOSEAGE.** Natular G30 can be applied to a variety of sources including temporary standing water sites like woodland pools, retention ponds, roadside ditches, other freshwater sites like ponds, canals, and creek edges, dormant rice fields between harvests, freshwater swamps and marshes, and coastal areas such as mangroves, brackish swamps, and coastal impoundments. Apply Natular G30 at rates between 5-12 lbs per acre for targeted treatment sites with mild-moderate organic content such as the ones listed above. Use lower labeled rate when water is shallow, vegetation and/or pollution are minimal, and mosquito populations are low. Within the labeled rate range, higher rates may be used when water is deep, vegetation and/or pollution are



high, and mosquito populations are high in number. Natular G30 may be applied at rates up to 20 lb per acre in waters high in organic content, deep-water mosquito habitats or those with dense surface cover, and where monitoring indicates a lack of kill at typical rates. Sites include storm sewers, catch basins, sewage lagoons, cesspools, oxidation ponds, fruit and vegetable processing waste. Other sources include natural and artificial containers such as tree holes, leaf axils, flowerpots, urns, rain barrels, buckets, tires, and abandoned swimming pool. Natular G30 may be applied to standing water associated with agricultural crops as long as the waters are not intended for irrigation. Reapply after 30 days, if needed for extended control in continuously flooded habitat. Applications have best results when applied to 1<sup>st</sup> through early 4<sup>th</sup> stage larval instars and begins to work immediately through both contact and ingestion. Optimal control is reached within 24-72 hours.

**Natular 2EC** is the trade name for the liquid emulsifiable concentrate formulation of spinosad produced by Clarke. It has an active ingredient concentration of 20.6% spinosad, a mixture of spinosyn A and spinosyn D in a ratio of 17:3 respectively. The main inactive ingredient is natural oil of wintergreen at a concentration of approximately 75%. It is registered as a Group 5 Insecticide by the EPA and carries a “CAUTION” signal word. Can cause eye irritation. Avoid contact with clothes, skin, or eyes. Can cause adverse allergic reactions in those sensitive to wintergreen oil. Do not apply to natural or artificial containers intended for consumption by people, animals, or livestock.

**DOSEAGE.** Natular 2EC is a concentrated emulsifiable liquid that needs to be mixed with water before use. Agitate solution while mixing and continue agitating to avoid separation. Only mix as much 2EC as needed to treat each source as solution has limited shelf life once combined with water. Natular 2EC can be applied to a variety of sources including temporary standing water sites like woodland pools, retention ponds, roadside ditches, other freshwater sites like ponds, canals, and creek edges, dormant rice fields between harvests, freshwater swamps and marshes, and coastal areas such as mangroves, brackish swamps, and coastal impoundments. Storm water/drainage systems like catch basins and wastewater systems like sewage lagoons and cesspools as well as natural and artificial containers of many types may need higher concentrations of 2EC for complete control. Apply Natular 2EC solutions at a rate between 1.1-2.8 fl oz/acre. It may be applied at rates up to 6.4 fl oz/acre in rich organic water sources such as sewage ponds, animal waste lagoons, and waters with high concentrations of leaf litter or other organic debris.

**Natular T30** is the trade name for the 30-day tablet formulation of Spinosad made by Clarke. The active ingredient Spinosad (a mixture of spinosyn A and spinosyn D) is present in a concentration of 8.33%. It is registered with the EPA (Reg. No. 8329-85) as a Group 5 insecticide. It carries a “CAUTION” signal word on the label. It is harmful if swallowed. Can cause moderate eye irritation. Do not apply to natural or artificial containers intended for consumption by people, animals, or livestock.

**DOSEAGE.** Natular T30 tablets are designed to kill and control mosquito and midge larvae in small bodies of water for 30 days. Examples include catch basins, storm water drainage areas, woodland pools, ditches, retention ponds, ornamental ponds, abandoned swimming pools, artificial containers, sewage lagoons, wastewater impoundments, and fruit and vegetable processing ponds to name a few. Use 1 Natular T30 tablet per 100 sq. ft. at a water depth of 0-2 ft. Add another tablet for every 2 feet of depth per 100 sq. ft.

**Natular Extended Release Tablets (XRT)** are dust free, long lasting tablets that are rated for 180 days of control. It contains 6.25% of the active ingredient Spinosad (a mixture of spinosyn A and spinosyn D) and 93.75% other ingredients. It carries a signal word “CAUTION” and is classified as a Group 5 Insecticide. It is registered with the EPA (Reg. No.8329-84). Harmful if swallowed and can cause moderate eye irritation.

DOSEAGE. Natular XRT has been designed specifically with storm water catch basins in mind but can still be used in most natural and manmade depressions that hold water, such as sewers, woodland pools, roadside ditches, tire tracks, potholes, fishponds, ornamental ponds, fountains, abandoned swimming pools, streams, creek edges, swamps, waste ponds, and dormant rice fields. Do not apply to water intended for irrigation or drinking. Do not use in animal troughs. Use at a rate of one tablet per 100 sq ft per 2 ft of the water column.

#### TARGET SPECIES.

Spinosad adversely affects the larval stages of insect species in the Order Diptera (such as flies and mosquitoes), Thysanoptera, Isoptera and Coleoptera. It is widely used on crops, over 200 types in over 60 countries. Spinosad has a unique mode of action that is different from all other known insect control products. As such, it has its own class, class 5, of insecticide designated by the Insecticide Resistance Action Committee (IRAC).

Spinosad causes excitation of the insect nervous system, leading to involuntary muscle contractions, prostration with tremors, and finally paralysis. These effects are consistent with the activation of nicotinic acetylcholine receptors by a mechanism that is unique among known insecticides. Spinosad also has effects on GABA receptor function that may contribute further to its insecticidal activity. It is an OMRI registered organic pesticide in the US and is widely used in organic agriculture in rotation with other pesticides to reduce building of pesticide resistance.

Based on laboratory studies, Spinosad has been shown to have a persistence of insecticidal action of about 6 weeks when used at the rate prescribed on the label. It breaks down quickly in sunlight and in the presence of microbial activity in soil with a half-life of less than a day in soil and in a water column. In non-targets such as mammals, it is not readily absorbed through the skin and amounts ingested are rapidly metabolized to inactive by-products which are then excreted. It is not toxic to birds, wildlife, fish, and most aquatic invertebrates. Genealogical studies have shown it to not have any mutagenic potential. The oral LD<sub>50</sub> for rats and mice is >2000mg/kg of body weight, the dermal LD<sub>50</sub> is >5000mg/kg of body weight for rabbits and the inhalation LC<sub>50</sub> is >5.18 mg/L of air for rats making it a very safe pesticide for both applicators and non-target organisms.

Spinosad is moderately toxic to aquatic organisms on an acute basis: 96-hr Acute LC<sub>50</sub> 5.0 mg/L, Common Carp (*Cyprinus carpio*) 96-hr Acute LC<sub>50</sub> 5.9 mg/L, Bluegill Sunfish (*Lepomis macrochirus*) 96-hr Acute LC<sub>50</sub> 30 mg/L, Rainbow Trout (*Oncorhynchus mykiss*). 48-hr Acute EC<sub>50</sub> 1.5-14.0 mg/L, Water Flea (*Daphnia magna*) 48-hr LC<sub>50</sub> > 7.9 mg/L, Mysid Shrimp (*Americamysis bahia*). It should be used when mosquito larvae is the dominant species in a body of water to reduce the negative effects on non-targets. It has been shown in field studies that once liquid spray residues have been allowed to dry up to 3 hours, Spinosad is not harmful to foraging bees. Granular and tablet formulations do not pose a threat to bee populations.

\* Criteria used to determine the action threshold are larval density, species significance (nuisance or disease), flight range, dispersal patterns and other environmental and meteorological factors combined with human and domestic animal activity, injury and proximity. Treatment decisions are based on threshold levels of larvae determined for sites by evaluating species, proximity to residents, stage of development and abundance, ecological value, presence, number and type of predators and other aquatic life and other environmental factors.