ENVIRONMENTAL MONITORING RESULTS OF SPINOSAD AERIAL APPLICATIONS FOR THE MEXICAN FRUIT FLY ERADICATION IN VALLEY CENTER, SAN DIEGO COUNTY, 2003

by
Shifang Fan[1], Randy Segawa[1], Dave Kim[1], Johanna Levine[2], Carissa Ganapathy[1], Pamela Wofford[1], Jean Hsu[3], Paul Lee[3]

[1]California Department of Pesticide Regulation
[2]Current address: California Air Resources Board
[3]California Department of Food and Agriculture, Center for Analytical Chemistry

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California Environmental Protection Agency
Department of Pesticide Regulation
1001 I Street
Sacramento, CA 95814
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ABSTRACT

The California Department of Food and Agriculture (CDFA) commenced a series of spinosad (GF-120 NF Naturalyte Fruit Fly Bait) applications in 2003 at Valley Center, San Diego County to eradicate an infestation of the Mexican fruit fly. A total of thirteen aerial treatments were conducted from January 10th to May 30th. The treatment was a spinosad-water mixture applied over 23 square miles. Target application rate was 20 oz/acre of GF-120 NF bait or 3.26 microgram per square foot ($\mu g/ft^2$) of spinosad. The Department of Pesticide Regulation (DPR) monitored applications throughout the aerial treatments. DPR measured the amounts of spinosad sprayed from aircraft and deposited on the ground, and analyzed the concentrations of spinosad residue in surface water, rain runoff, air, and fruit samples.

Mass deposition of spinosad ranged from none detected to 9.63 $\mu g/ft^2$ and the overall average was 1.58 $\mu g/ft^2$, 48.2% of the target application rate. Average spinosad deposition for each application ranged from 0.557 to 2.12 $\mu g/ft^2$ with standard deviations from 0.326 to 2.44 $\mu g/ft^2$. Average over time for each sampling site ranged from 0.544 to 3.90 $\mu g/ft^2$ with standard deviations from 0.581 to 3.56 $\mu g/ft^2$. A two-factor analysis of variance indicated that significant differences existed among applications ($P = 0.005$) and among monitoring sites ($P = 0.007$). Correlation analysis indicated that the average spinosad deposition for each application was not significantly correlated to reported application rate, tank mix concentration, air temperature, relative humidity, wind speed, or site elevation. Sunrise time at Valley Center was significantly correlated to deposition mean ($r = 0.765$). Sunlight affecting spinosad stability was indicated by the results that deposition means of the five samples collected in direct sunlight during three applications were significantly lower than the average of the remaining samples from these three applications ($P = 0.00171$). However, detailed comparisons of mass deposition to sampling time for each sample during each application showed no perceptible relationships. This may be explained by the trees and hilly terrain in Valley Center, and that samples collected after sunrise were not always in direct sunlight.

Of the samples collected in designated non-aerial treatment buffer zones around main water bodies, 59.3% of the samples had positive detections of spinosad ranging from trace amounts to 1.59 $\mu g/ft^2$. The average of the buffer zone samples was 0.162 $\mu g/ft^2$, 10.3% of the average for the aerial treatment area.

Spinosad residues were not detected in the surface water, rain runoff, and ambient air samples. However, these non-detections were ambiguous because storage stability studies for water samples and trapping efficiency studies for air samples were not satisfactory.

Fruit sample results ranged from none detected to 0.162 parts per billion (ppb), with an average of 0.025 ppb spinosad residue. There was no significant difference ($P = 0.208$) between samples collected from the upper portion versus the lower portion of the canopy.

The pesticide GF-120 NF concentrate samples contained 193, 208, and 223 parts per million (ppm) spinosad for each of three DPR sampled applications. The label states this product contains 200 ppm (0.02%) spinosad by weight as the active ingredient and no other active

ingredients. The spinosad concentrations in tank mix samples ranged from 76.3 to 202 ppm, 95.4% to 253% of the target application rate, 80 ppm.

No detectable organophosphate, carbamate, or chlorinated hydrocarbon pesticide residues were found in any samples of the spray materials except for two tank-mix samples. DPR detected 5 ppm of malathion in the tank mixture samples during the third application and 290 ppm of malathion during the fourth application. A subsequent investigation by DPR and CDFA indicated that a manifold used in the mix/load system was the likely source of contamination (Appendix I). Though the 290 ppm malathion was relatively high compared to the spinosad concentration, it was only one to two percent of the normal malathion application rate. Samples of deposition, water, air, and vegetables collected from the treatment area during and following the fourth application showed no detectable malathion residues.

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INTRODUCTION

Background

The Mexican fruit fly (Mexfly), *Anastrepha ludens* (Loew), a destructive pest of fruit, is indigenous to Mexico and has invaded parts of Central America periodically. It has become a serious agricultural pest in these areas and damaged citrus, mango, avocado, peach, pear and other fruit. The female fly attacks ripening fruit, pierces soft skin and lays eggs in its puncture. The eggs hatch into larvae, which feed inside the fruit, ruin the pulp, and damage the fruit quality.

The first eradication of Mexican fruit fly infestation in California occurred in 1954 in San Ysidro. In the last three decades, California has experienced several infestations. The California Department of Food and Agriculture (CDFA) maintains an extensive trapping program, and initiates an eradication measure as soon as an infestation is detected. In November 2003, a major Mexican fruit fly infestation was detected in Valley Center. The CDFA and the United States Department of Agriculture (USDA) established a 117 square mile (mi^2) quarantine zone on December 5, 2003, and a state of emergency was declared on December 18, 2003. The quarantine zone went as far north as the Riverside County line, to the east just outside the community of Pauma Valley, as far south as Mirar De Valley Road near the community of Valley Center, and to the west roughly along Interstate 15.

Aerial Treatment

The CDFA commenced eradication program with a series of aerial applications of the pesticide spinosad in January 10, 2003. The spray area consisted of 28 mi^2 (Figure 1), of which 23 mi^2 were treated using aerial applications and 5 mi^2 were treated using ground applications in CDFA-established buffer zones around water bodies. The buffer zones were excluded from the aerial application. The aerial spray was initially scheduled at intervals of two weeks to last two life cycles of the Mexican fruit fly. After the first five applications, as temperature increased, the spray interval was reduced due to shortened life cycles. With a total thirteen applications, the aerial treatment was completed in May 30, 2003. The eradication project continued with the release of sterile flies.

For the aerial applications, GF-120 NF Naturalyte Fruit Fly Bait (U.S. Environmental Protection Agency Registration Number 62719-498) was used. The bait was diluted with water to a spinosad concentration of 80 ppm (0.0080%) by weight or 0.363 grams per gallon. The bait-water mix was applied at 50 fluid oz/acre with target spinosad application rate of 3.26 $\mu g/ft^2$ (0.142 gram/acre). The applications were made using fixed-wing aircraft with a swath width of 100 feet, sprayed in east and west directions at a height of approximately 500 feet. Seven out of thirteen applications were completed in one night (Table 1). The other six were completed in two nights due to the weather or other incidents.

Pesticide Spinosad

The GF-120 NF Naturalyte Fruit Fly Bait (GF-120 NF) is an organic pesticide under federal and state guideline and contains 0.020% or 200 ppm spinosad as its active ingredient (Dow Agro Sciences, 1999). This product contains no other active ingredients. The bait is a thick yellow brown liquid at 20 ºC. It is non-explosive, non-reactive toward monoammonium phosphate, zinc, and water, and reactive toward potassium permanganate (Kollman, 2003).

Spinosad is a biologically derived insecticide produced via fermentation culture of actinomycete *Saccharopolyspora spinosa*, a bacterial organism isolated from soil. Spinosad is a mixture of two components of the chemical class of 12-membered macrocyclic lactones in a unique tetracyclic ring. Each component, designated spinosyn A or spinosyn D, is an unsaturated tetracyclic ester with two sugar derivatives (forosamine and rhamnose sugars) attached through ether linkages. Commercial formulations contain a spinosyn A to spinosad D ratio of approximately 85:15 (Kollman, 2003). Technical grade spinosad is a light gray to white crystalline solid. Spinosad is soluble in water, and in common organic solvents such as acetone, acetonitrile, methanol, and toluene. It is stable to metal and metal ions for 28 days, but degrades under ultra-violet light. It is non-phytotoxic when used as directed (Kollman, 2003).

Spinosad dissipation and transformation in the environment include photodegradation and biotransformation on plant surfaces, adsorption and photodegradation on soil, biotransformation via soil microorganisms, aqueous photolysis, and abiotic hydrolysis (Kollman, 2003). The low vapor pressure indicates that volatilization of spinosad is not a transport mechanism in the environment (USDA, 2001). Photolysis is the prime route of spinosad dissipation from plant foliar surfaces. After initial photodegradation, residues are available for metabolism by plant biochemical processes. In soil, spinosad adsorbs strongly to soil particles and binds to organic matter. It is unlikely to leach to great depths or ground water. It is photodegraded quickly on soil exposed to sunlight, but the degradation rate decreases at longer exposure times. Soil microorganisms under aerobic condition quickly metabolize spinosad. Half-lives calculated for spinosyn A in silt loam and sandy loam soils were 17.3 and 9.4 days, respectively. The half-life for spinosyn D in silt loam soil was 14.5 days. The degradation rate was slower under anaerobic condition. For both aerobic and anaerobic degradations, two major metabolites detected were formed by mono-N-demethylation of the forosamine sugar of spinosyn A and D (Kollman, 2003; DPR, 1995a). Aqueous photolysis is rapid with half-life less than one day in natural sunlight. It is the predominant mechanism of spinosad degradation in aquatic systems exposed to sunlight. Anaerobic aquatic biodegradation of spinosyn A and D in a water/soil system proceeded slowly with respective calculated half-lives of 161 and 250 days (Kollman, 2003; DPR, 1995b). Because spinosad slowly hydrolyzes under basic conditions and does not hydrolyze significantly under neutral conditions, abiotic hydrolysis is relatively unimportant in natural water bodies and rain, or on moist plant surfaces (Kollman, 2003; Saunders and Bret, 1997). In general, the rapid breakdown and the lack of movement in environment signify the minimal hazards of spinosad to the environment (USDA, 2001). The U.S. Environmental Protection Agency (U.S. EPA) classifies spinosad as a “reduced-risk” compound (U.S. EPA, 2002).

Spinosad acts as a contact and stomach poison against insects and is particularly effective against all stages of flies by intoxicating their nervous system (USDA, 2001; Adan, et al., 1996; Salgado, 1998). The intoxication is caused by persistent activation of nicotinic acetylcholine receptors and prolongation of acetylcholine responses. The receptors affected by spinosyns in insects are

not present or vital to nerve transmission in most other taxa, and toxicity to most other organisms is low (USDA, 2001). The toxicity data revealed that LD_{50} (5-day test) for wildlife of bobwhite quail and mallard duck was >5156 ppm, LC_{50} (96-hour test) for sensitive aquatic species ranged 5.0 ppm in carp to 92.7 ppm in daphnia magna, and EC_{50} ranged 0.107 ppm in freshwater diatom to >105.5 ppm in green algae (Kollman, 2003).

Monitoring Objectives

To support the CDFA's eradication efficacy efforts and provide quality assurance and quality control measures, the DPR Environmental Monitoring Branch conducted environmental monitoring for all aerial treatments. The objectives of the monitoring were to measure the amounts of spinosad in the spray materials and reaching the ground to determine if the proper amount of the pesticide was applied to the target area; to measure spinosad concentrations in ambient air during and after applications, in surface water immediately after applications, and in runoff water after storm events to evaluate environmental safety; and to measure spinosad concentrations and dissipation in fruit and foliage to determine if effective and legal concentrations are achieved. Further details on the objectives were described in the study protocol (Appendix III). Preliminary monitoring results for each individual application have been reported. This final report summarizes all monitoring results with appropriate statistical analysis and discussion.

MATERIALS AND METHODS

Sampling

Mass deposition

Mass deposition of applied spinosad reaching ground was collected for all applications. The sampling sites were selected at 23 locations dispersed throughout the aerial treatment area (Figure 2). Three additional deposition sites (Site 12, 21, and 24) were selected within the designated buffer zones (Figure 2). The criteria for the site selection were one site in each one mi^2 section of the application area, free from interference of trees, buildings, or other obstructions, and accessible and secure for sample collection.

Deposition samples were collected using one square foot mass deposition sheets (MDS) with plastic lined absorbent surface (kimbies). Each deposition sheet was attached using pushpins onto a polyethylene-wrapped cardboard sampling platform. The sampling platform was affixed by a bungee cord to a cinder block on the ground. On each application day, the deposition samplers were set up before the application and collected after the application. Samples were generally collected before sunrise to minimize possible photodegradation. The collected deposition sheet was folded, wrapped with aluminum foil, placed in an envelope, and stored in a freeze-safe with dry ice.

For the first five applications, samples collected at all sites were analyzed for spinosad. Samples collected during the fourth application were also analyzed for malathion to determine potential impact of the malathion contamination. Starting with the fifth application, duplicate deposition

samples were collected, one for spinosad and the other for malathion; but malathion was only analyzed if potential contamination was in question. Based on the monitoring protocol amendment (Appendix IV), depositional sampling sites in aerial treatment area were reduced from 23 to 9 and those in buffer zone were reduced from 3 to 2 for the 6th and later applications. Samples collected during the applications 7, 9, 11, and 13 would be analyzed only if other samples, such as spinosad concentrate, tank mix, and water samples, or application process were in question.

Surface water

Surface water samples were collected to determine spinosad concentration in Keys Creek (Figure 2, Site 28) for all applications. In the treatment area, this creek is the only named water body listed on a U.S. Geological Survey map (1:24,000) where CDFA declared a non-spraying zone. It runs from east to west across the southern part of the treatment area and its flow consists of rain and irrigation runoffs.

For most applications, a set of three water samples were collected, two for spinosad and one for potential malathion analysis, in each sampling period. Background sample sets were collected before application and one or more (if an application took more than one day) application sample sets collected after application.

Water samples were collected using an extended sampling pole. The sampling container was submersed into the center of the creek, rinsed with creek water, and then approximately 1/2 liter water sample was collected. For the first application, an amber glass bottle and a 1-quart canning jar were used for water samples. A discussion between DPR and CDFA laboratory suggested that spinosyns might adhere to, and not be removed by solvent extraction from the amber glass bottles (personal communication between Dow Agro Sciences and the CDFA laboratory). Therefore, the water samples for spinosad analysis were collected in 1-liter clear glass jars with Teflon-lined lids. After pH of the water was measured and recorded, one spinosad sample was stored on ice and the other was frozen on dry ice immediately. The water sample for malathion analysis completely filled a 1-liter amber glass bottle and was stored on ice immediately after collection.

The frozen samples for spinosad were not collected for the first three applications and the malathion samples were not collected for the first four applications. An additional set of spinosad application samples was collected as backup during the first seven applications. The backup samples would be analyzed only if the primary samples were problematical. The potential malathion samples were not analyzed based on no detectable malathion residue in the tank mix samples collected after the fourth application.

Rain runoff

On February 11, a week after the third application, it started raining at 5:00 a.m. and stopped at 2:00 p.m. with an accumulated 0.57 inches of precipitation. On February 12, there were scattered showers from approximately 12:30 a.m. to 1:30 a.m. and steady rain for 8 hours from 9:00 a.m. to 5:00 p.m. with an accumulated 0.84 inches of precipitation at the Valley Center.

During this storm event, seventeen runoff water samples were collected to determine spinosad concentrations at two sites on the Keys Creek. One site was within the treatment area. This was the same site used for the application surface water sampling (Figure 2 and 3, Site 28). Samples were taken at this site approximately every one and half hours from 5 pm to 8:30 pm on February 11 and every hour during the rain on February 12 for a total of 12 samples. The other site was approximately four miles downstream from the treatment area (Figure 3, Site 29). Five samples were taken at this site, approximately every hour from 1:00 pm to 5:00 pm on February 12. The rain runoff samples were collected and analyzed in the same manner as the surface water samples.

Ambient air

Air samples were collected from the second to the fifth applications to determine the ambient air spinosad concentration in the treatment area. Four representative sampling sites (Figure 2, Site 3, 7, 19, and 20) were selected based on spaciousness for airflow and accessibility and security for sampling. At each site, a single sampler was set approximately four to six feet above the ground and shielded from direct spinosad application.

During each of the applications when air samples were collected, an approximate 24-hour background sample was taken before application, an application sample was collected for the duration of application; and a 24-hour post-application sample was collected at each air sampling site. The air sample was collected using a personal air sample pump (SKC#224-PCXR) with a sampling tube (SKC#226-30-16). The sampling tube contained a 2-section XAD-2 sorbent bed, a glass-fiber filter, and a foam separator to adsorb spinosad from ambient air. The tube was installed on the sampler and the pump was set at a flow rate of 3000 ml/minute. The flow rate was measured using a DryCal® Primary flowmeter at the beginning and the end of each sampling interval. The sampled tubes were capped and stored on dry ice. Details of air sampling can be found in SOP EQAI001.00 (Wofford, 2001).

Grapefruit

Fruit samples were collected during the second to the sixth applications at two orchards (Figure 2) in the treatment area. One orchard (Site 3) was north and the other (Site 27) was south of the core infestation area because fruit was not permitted to be taken out of this core area. The site in the north was on a slope with grapefruit and avocado trees planted in alternate rows. The one in the south was on flat ground with only grapefruit trees.

For each application, four background fruit samples were collected before the application and four application samples were collected the morning after application. Two trees in each orchard were randomly selected. The same two trees were sampled for the duration of the monitoring study. From each orchard, two samples were collected representing the upper and lower portion of the canopy. Each sample consisted of two grapefruit from either upper or lower portion of each tree, for a total of four fruit per sample. On each tree, two compass directions were randomly chosen using the second hand on a watch for each sampling period. At each selected direction, one grapefruit was clipped from the upper portion of the tree into a pole fruit picker basket wrapped with aluminum foil; and the lower portion fruit was clipped directly into the sampling container. The four-grapefruit sample was placed into a stainless steel bucket, covered with a stainless steel lid, and stored on dry ice.

Spinosad concentrate and tank mix

The GF-120 NF concentrate was usually transferred and mixed with water in the mixing tanks at airport the day before the scheduled application. The tank mix was loaded into spray tank on each aircraft right before application. Details of mixing-loading system are described in Appendix I. Both GF-120 NF concentrate and tank mix samples were collected to determine spinosad contents and also to screen for potential contamination of organophosphates, carbamates, and chlorinated hydrocarbons.

GF-120 NF concentrate samples for the first application were collected from the holding tank before mixing and no concentrate samples were collected from the second to the fifth applications. From the sixth applications and thereafter, GF-120 NF concentrate samples were collected from original 55-gallon drums into 1-liter brown plastic bottles using a disposable bailer (Enviro-Tech). One composite sample was taken from each of all drums of the same lot used in a single application. More than one composite sample was collected if more than one lot was used for that application. The composite concentrate samples for the eighth and later applications were collected prior to each application and analyzed by the Los Angeles County Department of Agriculture. Their results are not included in this report.

The tank mix samples were collected before spraying on each application day. For the first four applications, the samples were taken from the mixing tanks or loading manifold. From the fifth application, the tank mix sample was a composite of subsamples from the five nozzles on an aircraft. The subsamples were collected using canning jars with a small hole in each lid or aluminum foil cover. The spray nozzle was inserted into the jar and the spray pump was activated for sample collection. The five subsamples were composited into a 1-liter brown Nalgene® plastic bottle and stored on ice.

Investigation of malathion contamination and decontamination

Analysis of tank samples from the third and fourth applications indicated malathion contamination. The chemical analysis result for the third application was not confirmed until after the fourth application was conducted. A subsequent investigation into the source of the contamination and a thorough decontamination were conducted by DPR and CDFA. Between the fourth and the fifth applications, in addition to scheduled regular samples, a total of 12 spray materials and 99 rinsate samples (including 19 backup samples) were collected from the spinosad concentrate drums, mixing tanks, mix/load system, and aircraft. Additional environmental samples, such as lettuce and cabbage were collected from organic farms in the treatment area to determine potential impact of the malathion contamination. Details of the materials and methods for the investigation and decontamination sampling are described in the Appendix I.

Chemical Analysis

All samples were refrigerated or frozen until analyzed by the CDFA Center for Analytical Chemistry. The spinosad analysis in various matrices was reported for spinosyns A and D, as well as the breakdown product spinosyn B. This analysis used a liquid chromatograph with a

tandem mass spectrometer detector (LC/MS/MS). The malathion was quantified using a gas chromatograph with mass selective detector. The organophosphate, carbamates, and chlorinated hydrocarbons were screened using HPLC with a fluorescence detector.

Deposition samples were extracted with methanol and analyzed for spinosyns with a reporting limit of 0.1 $\mu g/ft^2$. The surface water, rain runoff, and rinsate of decontamination samples were extracted with methylene chloride and analyzed for spinosyns with a reporting limit of 0.05 ppb. Air samples were extracted with methanol and methylene chloride, and analyzed with a reporting limit of 0.5 µg/sample (0.1 $\mu g/m^3$ for a 24-hour sampling). Grapefruit samples were extracted with acetonitrile and water, and analyzed with a reporting limit of 1.0 ppb on fresh fruit weight basis. Outer-surface of fruit and inner surface of sample containers were rinsed with methanol and analyzed with a reporting limit of 3 or 5 ng/sample, approximately 0.002 or 0.004 ppb (ng/g fresh fruit). The spinosad concentrate and tank mix samples were extracted with acetone and analyzed using a high-performance liquid chromatograph and ultraviolet detector, providing a reporting limit of 1 ppm (0.0001%).

Malathion reporting limits were approximately 5 ppm for the GF-120 and GF-120/water mixture samples, and approximately 0.04 ppb or 10 ppb for the water and rinsate samples. The difference in detection limits was due to the smaller aliquots and higher concentration of interferences with the GF-120 and GF-120/water mixture analysis.

To screen for organophosphates, carbamates, and chlorinated hydrocarbons, samples were extracted with methylene chloride. After evaporating the solvent, the extracted residues were dissolved in methanol and separated by HPLC. The analyte was derivatized with OPA in a post column reaction and detected by a fluorescence detector.

Weather Information

Temperature, relative humidity, and wind speed at hourly increments during each application period were downloaded from http://cdec.water.ca.gov/queryCSV.html (CDWR, 2003). The Valley Center weather station was located at an elevation of 1370 feet in the San Luis Rey River basin just outside of the spray area. During rain runoff sampling days, precipitation data was also collected from this station. Sunrise time was determined from http://aa.usno.navy.mil/data/docs/RS_OneYear.html (U.S. Naval Observatory 2003). The sunrise data was based on Escondido, the closest city to Valley Center in this database.

RESULTS AND DISCUSSION

In the following results and discussion, each application is numbered in sequence. Also, each site is uniquely numbered and its location is shown on Figures 2 and 3.

Analytical and calculated results were reported to 2 or 3 significant figures. When spinosad residues were below the method detection limits (MDL), none detected (nd) was reported and zero was used in calculations; when less than the reporting limit (RL) was detected, a trace amount (tr) was reported and the quantity of half of the (RL + MDL) was used in calculations.

In calculation and statistical analysis, the quantity of spinosad residues was the sum of quantities of spinosyns A, D, and B. Mean refers to samples' arithmetic mean unless it was denoted differently. The reported spinosad application rates and the mass deposition data were presented in units of $\mu g/ft^2$. The spinosad residues in fruit were reported in unit of ppb on fresh fruit weight basis.

Mass Deposition in Treatment Area

Overall results

Table 1 summarizes the reported aerial application rate and number of mass deposition samples collected and analyzed for each application. The application acreage and gallons of tank mix used for each application were reported by CDFA. The reported application rates were calculated assuming that the tank mix contained the target concentration, 80 ppm. Of 227 mass deposition samples collected, 54 were not analyzed according to the monitoring protocol amendment (Appendix IV) or when the application occurred over two days, the sampled sites were actually out of the spray area that day. If a site was near the edge of the first day's application, it was sampled on both days and both samples were analyzed. The sum of the two results was used to reflect the actual spinosad mass deposition on that site.

The mass deposition of spinosad on each sampling site for each application ranged from none detectable to 9.63 $\mu g/ft^2$ (Table 2). The average of all deposition samples was 1.58 $\mu g/ft^2$, 48.2% of the target application rate. One of the 150 deposition results (0.67%) was below the detection limit; and 15 (10%) results were higher than the target application rate (Figure 4).

Statistical analysis

The 173 mass deposition samples analyzed, including 23 samples collected in multiple application days, were from 23 sites during 9 applications. Samples from all 23 sites were analyzed for spinosad during the first 5 applications. Starting with the sixth application, nine sites continued to be sampled for eight additional applications, but only samples from four applications (Application 6, 8, 10, and 12) were analyzed for spinosad. Site 7 for the first application was missed during sampling. Therefore, a total of 150 deposition results were used for statistical analysis. To test if the continued 9 sites could represent the original 23 sites, a t-test was performed using the mass deposition of the first 5 applications for comparing the 9 continued sites to the 14 discontinued sites, and to the all of 23 sites. Levene's test indicated the variances were homogeneous ($P > 0.05$). The equal-variance, two-tailed t-test was non-significant with $P = 0.199$ between continued and discontinued sites, and $P= 0.376$ between the continued and all sites (Table 3). Therefore, it was concluded that the continued 9 sampling sites still gave a fair representation of the study area.

An ANOVA for two-factor without replication was conducted to gauge the effects of application versus site using the data from 9 continued sites in 9 applications. While Levene's test showed these data satisfied the homogeneity of variance, the Shapiro-Wilk test showed the residuals were not normally distributed ($P < 0.0001$). Therefore, the data were log-transformed for

ANOVA analysis. The one missing data on site 7 during application 1 was estimated to be 2.12 $\mu g/ft^2$ using the least-squares estimate. Hence, the total degree of freedom was 79. The results indicated significant differences among applications (P = 0.00475) and sites (P = 0.00703) (Table 4). Without replication, this ANOVA error term contained possible interaction effects between application and site. Tukey's test for nonadditivity of a single degree of freedom (Steel and Torrie, 1980) was employed to segregate variances between possible interaction effect and random error. With a null hypothesis of additive effect, it was concluded that the interaction between application and site was not indicated (P>0.100), which allowed for use of the interaction term for estimating the mean square error in Table 4.

Factors that affect deposition results

Many factors can affect the deposition results: actual application rate, homogeneity of the tank mix, flight speed and altitude, spraying gaps or overlaps, weather conditions (temperature, wind, humidity, rain, sunlight), site elevation, topography and landscape, sampling technique, analytical methods, and errors and mistakes, etc. Based on available information, reported application rate, measured tank mix concentration, weather conditions, site elevation, and aircraft spray area were analyzed and discussed in this report. Since effects of both the application and site were significant and the interaction was not significant, the factors related to either application or site were considered and analyzed separately.

The average mass deposition of all sites for each application ranged from 0.557 to 2.12 $\mu g/ft^2$. This represented 17.0% to 65.0% of the target application rate, 3.26 $\mu g/ft^2$ (Figure 5). Figure 6 showed that mass deposition was neither correlated with reported spinosad application rate (P=0.713) nor with measured tank mix concentration (P=0.430). However, the deposition mean decreased significantly (P<0.0224) with application date over the spinosad aerial treatment period (Table 5). Therefore factors related to application date were further analyzed.

One factor that changed with application date was sunrise time. Spinosad is readily photodegradable. Sunrise time might be expected to affect mass deposition although most samples were collected before sunrise. Over the first 12 applications, sunrise time at Valley Center changed from 6:52 am to 4:56 am Pacific Standard Time (PST) (U.S. Naval Observatory 2003). Mean deposition was significantly correlated (r=0.765) to sunrise time (Figure 7) with P=0.0163. Evidentially, sunlight affecting spinosad stability was indicated by the results of the five samples collected in direct sunlight during applications 4, 6, and 10. The mass deposition of these five samples ranged from trace amount to 0.484 $\mu g/ft^2$ and averaged 0.410 $\mu g/ft^2$. Whereas, the deposition of the remaining samples from these three applications ranged from trace amount to 9.63 $\mu g/ft^2$ and averaged 1.68 $\mu g/ft^2$. The two means were significantly different (P=0.00171) based on a t-test assuming one-tailed distribution and two-sample unequal variance (Table 6). However, detailed comparisons of mass deposition to sampling time for each sample during each application showed no perceptible relationships (Figure 8). This may be explained by given topographic conditions of hilly terrain with many trees in the Valley Center, samples collected after sunrise were not always in direct sunlight.

Other meteorological conditions, average hourly temperature, relative humidity, and wind speed (CDWR, 2003) during each application night (Table 7) showed no statistically significant correlation to the deposition mean (Figure 9).

A measurable factor linked to site effect was elevation. The mass deposition during each application showed no relationship with the site elevation (Figure 10). Mean deposition of all applications was not correlated with elevation for either the nine continued sites (r=0.0442, Figure 11) or all 23 sites (r=0.329, Figure 12). To identify any extremes that might have dominated the correlation analysis, the Tukey's Honestly Significantly Different (HSD) test was applied for multiple sites comparison at each of the nine continued sites. The test was executed using SAS software (SAS 9.1) with unbalanced sample numbers due to missing sample at site 7 during the first application. Because the ANOVA residues of deposition data violated assumption of normality, this test used transformed spinosad deposition values (x) as $\log_{10}(x+1)$ and compared least squares estimated means (LS means) for each pair of sites. The reported deposition means were back-transformed from LS means of the transformed data (Table 8). Among the nine continued sites, the highest and second highest deposition means on sites 7 and 17 were significantly different from the lowest deposition mean on site 1. No other pairs of sites were significantly different (Table 8). In fact, site 7 was the lowest, and site 1 was the second lowest in elevation, while site 17 was the second highest in elevation. Further analyses showed no linear correlations ($r \leq 0.500$) existed between elevation and mass deposition mean for site 7, 17, and 1, or all sites excluding these three extremes, using the data of either the 9 continued sites or all 23 sites.

Another potential factor linked to site effect was flight line. For aircraft operation, the treatment was divided into four areas, A, B, C, and D (Figure 2). Normally, one aircraft sprayed areas A and B; while two other aircraft sprayed areas C and D respectively. Site 1 was in area A and both sites 7 and 17 were in area C (Figure 2); i.e. sites 7 and 17, with the two highest deposition means, were sprayed by one aircraft; and Site 1, with the lowest deposition mean, was sprayed by another aircraft. None of the three sites were in the boundaries of an aircraft's spray area. However, the possibilities of flight line gaps or overlaps still could not be excluded because aircraft swath and plume were not measured. A two-factor ANOVA was executed using data grouped into three areas, A&B, C, and D, sprayed by three individual aircraft to test the effects of aircraft fight line or area on deposition mean. The results showed no significant difference among the three areas (P=0.0767) or among applications (P=0.276) when the continued 9 sites were considered (Table 9). However, this test was confounded because the aircraft assignment for spray area was not consistent throughout the aerial application sequence. In a few occasions, only two aircraft were operated during an application.

Comparison with other aerial applications

Since this study was the first time that GF-120 NF Naturalyte Fruit Fly Bait was aerially sprayed for fruit flies in California, there was no relevant comparison for the quality and efficiency of the applications. In malathion aerial application monitoring studies (Oshima, et al., 1982; Turner, et al., 1991; Ando, et al., 1996; and Bradley, et al., 1997), the average percentage of mass deposition to reported application rate ranged from 75% to 87%. However, only 48.2 % was achieved in this spinosad study. The lower recovery in deposition samples could be due to intrinsic chemical characteristics, such as photodegradability, topography and landscape differences, or spray height and equipment used. Nevertheless, extreme results in this spinosad study were less than those in the previous malathion applications monitored by DPR. Only 0.67% of total deposition results in this study were below the detection limit and 10% were

higher than the target application rate (Figure 4). In the previous malathion studies, approximately 3% - 17% of deposition samples were below the detection limit and 18% - 31% were higher than the application rates (Bradley, et al., 1997).

Mass Deposition in Buffer Zone

Of total 27 analyzed deposition samples collected in designated water body buffer zones, 13 samples (48.1%) detected spinosad ranging from 0.128 to 1.59 $\mu g/ft^2$; 3 samples (11.1 %) contained trace amounts; and 11 samples (40.7%) contained no detectable spinosad. The average of all the buffer zone samples was 0.162 $\mu g/ft^2$, about one-tenth of the 1.58 $\mu g/ft^2$, average of the samples from treatment area.

The mean buffer zone deposition and maximum of the hourly wind speeds during each application period was significantly correlated ($r=0.907$, $P= 0.00185$). This correlation was dominated by the two highest average deposition results (0.666 and 0.296 $\mu g/ft^2$) corresponding to the two highest maximum wind speeds (5 and 3 mph) in the eighth and second applications, respectively (Table 10). However, correlation between the mean buffer zone deposition and average of the hourly wind speeds during each application period was not significant ($P = 0.159$). It was not possible to determine if the detection of spinosad in the buffer zone was due to drifting by wind or shifting of aircraft flight line. The wind speed data was from a regional weather station located outside the spray area, not at each individual sampling site, and the aircraft spray swath was not characterized.

Surface Water and Rain Runoff

Of total 86 surface water samples collected during all 13 applications, 52 primary samples were analyzed for spinosad concentrations. None was detected above the MDL of 0.05 ppb either in 31 refrigerated samples (13 background and 18 application samples) or in 21 frozen samples (9 background and 12 application samples). The storage stability studies indicated approximately 90% to 80% average recoveries in 0 to 2 days and a 1.73% daily loss was statistically significant ($P=0.0132$). After the stability problems were realized, field sample delivery and the lab extraction were expedited. Approximately 65% of water samples were extracted within two days after they were collected. Two application samples, one refrigerated and the other frozen, collected during the second application day of the eighth application were invalid due to laboratory analysis error. Water samples collected for malathion were not analyzed because there was no indication of contamination in tank mix and deposition samples during the later nine applications.

Of total 17 runoff water samples collected during the storm event, no spinosad residues were detected.

Ambient Air

Spinosad is a non-volatile chemical (Kollman, 2003). It can be found in ambient air attached to dust particles or as drift from an aerial application. A total of 46 ambient air samples, 14

background, 16 application, and 16 post application samples, were collected and analyzed. No spinosyns were detected.

Unlike the water samples, spinosad storage was stable for air samples. However, trapping efficiency was a problem. The recoveries of two studies of trapping efficiency were equal to or less than 40% for each spinosyn when 50 µg/tube was spiked. On the other hand, when 2.5-5.0 µg/tube spinosyn A and D were spiked in six blind field spikes, their recoveries ranged from 32.2% to 133% for spinosyn A and 30.8% to 126% for spinosyn D (Appendix II). Before any future spinosad monitoring studies are conducted, analytical and sampling methods need to be improved to achieve acceptable recovery and reproducibility in air samples.

Grapefruit

A Total of 40 fruit samples, 20 background and 20 application samples, were collected and analyzed. Spinosad residues in the background samples ranged from none detected to 0.0667 ppb on fresh fruit weight basis. The application samples ranged from none detected to 0.162 ppb with an average of 0.0250 ppb, without subtraction of their corresponding background samples (Table 11). A t-test for paired background and application samples indicated that post-application concentrations were significantly greater than background (P = 0.0463, Table 12).

The macro effects of site and application sequence on the spinosad applications have been discussed previously in the mass deposition section. A micro effect was analyzed by comparison of the spinosad residue in fruit samples collected from the upper portion and those from the lower portion of the trees. For application samples without background subtraction, there was no statistically significant difference between upper and lower samples (P = 0.208, Table 12).

Spinosad Concentrate and Tank Mix

DPR collected nine samples of spinosad concentrate from the first, sixth and seventh applications. During the first application, two samples were collected from randomly picked 55-gallon drums and contained 206 ppm and 210 ppm of spinosad. During the sixth and seventh applications, three composite drum samples for each application, plus one sample from loading manifold during the sixth application, were collected and analyzed. The concentration ranged from 178 ppm to 258 ppm spinosad. Average concentration was 193 ppm and 223 ppm for the sixth and seventh applications, respectively (Table 13). The labeled concentration is 0.02 % or 200 ppm (Dow AgroSciences, 2000).

A Total of 35 tank mix samples were collected and analyzed for the 13 applications. The first application occurred over two nights. During the first night the tank mix sample contained 202 ppm spinosad, much higher than the expected concentration of 80 ppm. The high concentration was likely due to insufficient mixing and resulted in clogging of the spray nozzle during that application. When the clogging was discovered, the application was stopped and tank material was remixed. The second night tank mix sample contained 90 ppm spinosad, closer to the target concentration. From the second to the thirteenth applications, tank mix concentrations ranged from 76.3 ppm to 122 ppm spinosad, 95.4% to 153% of the target concentration (Table 13). As

discussed previously, there was no significant correlation between tank mix concentration and mass deposition.

Screening tests for the GF-120 concentrate and application tank mix samples showed no detectable organophosphate, carbamate, or chlorinated hydrocarbon pesticides except for the two tank mix samples. Malathion was detected at 5 ppm during the third application and at 290 ppm during the fourth application.

Investigation of Malathion Contamination and Decontamination

When laboratory analysis result of 5 ppm of malathion detected in the tank mix sample collected during the third application was reported on February 20, 2003, the fourth application had already occurred as scheduled on February 18, 2003. DPR expedited the analysis of the tank mix sample for the fourth application, and 290 ppm of malathion was detected and confirmed on February 26, 2003. DPR and CDFA followed an intensive investigation for the source of malathion contamination and concluded that a manifold used in the mix/load system was the likely source of contamination.

After CDFA replaced all parts of the mix/load system and decontaminated all application equipment, aircraft, and other supplies following the fourth application, a total of 111 samples were collected. Of which 92 primary samples, 12 tank samples and 80 rinsate samples, from various locations of the tanks, mix/load system, and aircraft, were analyzed to ensure complete and thorough decontamination. Samples collected after decontamination showed low or no detectable concentrations of malathion. Tank mix samples collected during the fifth and later applications showed no detectable concentrations of organophosphate, carbamate, or chlorinated hydrocarbon pesticides, including malathion.

To evaluate the possible impact of the malathion contamination, 26 deposition samples collected for spinosad analysis during the fourth application were also analyzed for malathion; and the results showed no detectable malathion residue. However, these samples were optimized for spinosad analysis, not for malathion analysis. In addition, three vegetable samples were collected on February 27, 2003 in the treatment area specifically for malathion analysis; and no malathion was detected. However, the treatment area received two inches of rain between February 18 and February 27, 2003. Environmental samples from the third application were not analyzed because their concentrations would have been far below the malathion detection limit.

The highest concentration, 290 ppm, malathion in the tank mix sample was relatively high compared to the spinosad concentration. However, it was one to two percent of the normal malathion application rate and the impacts of the malathion contamination were likely negligible. Detailed description and discussion of the investigation for malathion contamination and decontamination are in the Appendix I.

Quality Control (QC) Summary

Method development and validation were conducted concurrently with sample collection and analysis for this emergency project. Therefore optimizations of sample container selection,

sample collecting and handling procedures, and analytical methods were delayed until results of some field samples collected during early applications had been reported. Continuing quality control and blind spikes in various matrices were analyzed in connection with field samples throughout the study.

Results for spinosad analytical method validation, continuing QC, and blind spikes are summarized in Table 14. Average spinosad recoveries ranged from 70.0% to 97.9% among various matrices for the method validation and the continuing QC. Blind field spikes were only conducted for surface water and air samples. Average recovery was 58.0±7.51% for water and 83.8±33.5% for air samples. For malathion, piperonyl butoxide (PBO) and D-phenothrin screening analysis, recoveries of the continuing QC samples were greater than 92.5% for mass deposition and 90.0% for water samples.

Trapping efficiency study for air sampling resulted in poor recoveries of the spiked spinosad compared to the blind field spikes. The trapping efficiency recoveries ranged from 5.30% to 39.7% when 50 µg/tube of spinosyn A or D was spiked. While the recoveries for the blind field spikes ranged from 30.8% to 133% when 2.5-5.0 µg/tube spinosyn was spiked.

Spinosad storage stability study indicated that MDS and air samples were stable over 21 days and all field samples for MDS and air were analyzed within their stable storage periods. However, water samples, refrigerated or frozen, were not as stable. Two storage stability studies for water samples were conducted. In the first study, spiked water samples were refrigerated at 4 °C. In the second study, the water samples spiked with all spinosyns were stored frozen but only those spiked with spinosyn B were refrigerated. The average recoveries of all spinosyns at 0, 1, 2, 4, 7, 15, 21, and 30 days of storage were 89%, 88%, 82%, 63%, 42%, 36%, 40%, and 38%, respectively, for the first study. The 1.73% daily storage loss was statistically significant (P=0.0132, linear regression). Nevertheless, this regression was not consistent with the results of blind field spikes (Appendix II). In the second study, recoveries of all spinosyns ranged from 61% to 24% in the frozen water samples stored for 4 to 21 days. Average recoveries of spinosyn B in the refrigerated samples were 51%, 58%, 36%, and 46% at 2, 4, 7, and 14 days respectively. After the stability problems were realized, field sample delivery and the lab extraction were expedited. Of 74 field water samples analyzed, 48 samples (64.9%) were extracted within two days after they were collected, 12 samples (16.2%) in three days, 3 samples (4.05%) in four days; and 11 samples (14.9%) collected prior to the completion of stability study, were extracted in 6-7 days. Grapefruit storage stability study showed the recoveries of spinosyn A, B, and D ranged 87.3%-66.4%, 75.5%-65.8%, and 84.3%-80.9%, respectively, in 5-14 days frozen. All grapefruit samples were extracted in 5-14 days after they were collected.

Details of the method development and quality control, including method detection limits determination, method validation, trapping efficiency, storage stability, continuing QC, and blind spikes are described in Appendix II.

CONCLUSIONS

1. Mass deposition of spinosad measured on deposition sheets ranged from none detected to 9.63 μg/ft^2 with an average of overall deposition samples of 1.58 μg/ft^2, 48.2% of the target application rate of 3.26 μg/ft^2. The average deposition of each application ranged from 0.557 to 2.12 μg/ft^2 with standard deviations from 0.326 to 2.44 μg/ft^2. The averages were 17.1% to 65.0% of the target application rate.

2. Both sampling site and spinosad application sequence were statistically significant in explaining the variance in deposition samples. There was no correlation with the reported spinosad application rate, measured tank mix concentration, recorded regional temperature, humidity, wind speed, and sampling site elevation. Sunrise time on the sampling day in Valley Center area showed a significant correlation (r=0.765) to deposition mean of each application. However, there were no accurate sunlight data for each sampling site in the hilly terrain of the Valley Center to further confirm this correlation, and no perceptible relationship between mass deposition on each site and sunrise time in the Valley Center.

3. Thirteen of the 27 analyzed buffer zone deposition samples contained detectable spinosad ranging from 0.128 to 1.59 μg/ft^2. The average buffer zone deposition (0.162 μg/ft^2) was ten-fold lower than the average deposition in the treatment area (1.58 μg/ft^2).

4. No spinosad residues were detected in surface water, rain runoff, and ambient air samples.

5. Spinosad residues in grapefruit samples ranged from none detected to 0.162 ppb and averaged 0.0250 ppb on fresh fruit basis. There was no statistically significant difference between samples collected from the upper portion versus the lower portion of the trees sampled.

6. GF-120 NF concentrate contained 178 to 258 ppm of spinosad among nine samples collected by DPR during three applications. Averages were 208, 193, 223 ppm for applications 1, 6, and 7, respectively, compared to the expected 200 ppm of spinosad. Tank mix concentrations ranged from 76.3 to 202 ppm, 95.4% to 253% of the target tank mix concentration of 80 ppm. The highest concentration was evidently due to incomplete mixing early in the treatment series.

7. The collected samples showed no detectable organophosphate, carbamate, or chlorinated hydrocarbon pesticides in all spray materials except for two tank mix samples during the third and fourth applications where malathion was detected at 5 ppm and 290 ppm, respectively.

8. Malathion contamination was unequivocally identified in several samples collected from the mix/load system and the aircraft during the third and fourth applications. The highest concentration, 290 ppm malathion, in the tank mix sample was relatively high compared to the spinosad concentration. Nevertheless, it was one to two percent of the normal malathion application rate and the impacts of the contamination were likely negligible. Malathion was not detected in any of the environmental samples from the fourth application. However these samples were optimized for spinosad analyses; the reliability of malathion analysis results on these samples was unknown.

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Table 1 Reported application rate and the number of mass deposition samples collected and analyzed for each application

		Application				Deposition sample		
sequence	**# nights**	**start date**	**gallon**	**acre**	***rate (μg/ft^2)**	**collected**	**analyzed**	**not analyzed**
1	2	7-Jan	5250	14720	2.98	45	34	11
2	1	21-Jan	5560	14754	3.14	23	23	0
3	1	4-Feb	5872	14847	3.30	23	23	0
4	1	18-Feb	5802	14847	3.26	23	23	0
5	1	5-Mar	5835	14847	3.28	23	23	0
6	2	18-Mar	5860	15001	3.26	15	15	0
7	1	27-Mar	5852	14981	3.26	9	0	9
8	2	7-Apr	5892	15083	3.26	12	12	0
9	2	21-Apr	5867	15020	3.26	9	0	9
10	2	28-Apr	5914	15140	3.26	11	11	0
11	2	8-May	5828	14920	3.26	16	0	16
12	1	19-May	5917	15158	3.26	9	9	0
13	1	30-May	5861	15004	3.26	9	0	9
total						**227**	**173**	**54**

*Calculated assuming tank mix concentration was 80 ppm of spinosad.

Table 2 Mass deposition of spinosad (μg/ft^2) at each sampling site for each application

Site	Application Sequence*								
	1	**2**	**3**	**4**	**5**	**6**	**8**	**10**	**12**
1	0.17	0.38	1.96	0.05	0.46	0.66	0.78	0.23	0.21
2	1.64	3.68	1.15	1.50	3.22				
3	0.37	0.72	0.81	0.74	1.26	0.71	2.09	1.42	0.22
4	1.59	0.32	1.21	0.94	2.43	0.71	1.61	2.57	1.15
5	2.37	1.32	3.23	0.56	0.94				
6	1.29	2.08	2.56	0.93	1.23				
7	NS**	5.55	1.13	2.08	3.67	1.43	0.70	1.45	0.85
8	4.14	3.83	1.26	9.63	0.63				
9	2.47	1.99	4.41	4.05	2.45				
10	0.89	0.60	3.42	2.41	1.30				
11	1.37	1.78	1.91	2.40	0.53				
13	0.60	2.23	1.09	0.50	0.52	0.48	2.73	0.05	0.52
14	1.24	1.02	1.14	8.37	1.82				
15	2.69	0.93	1.24	1.48	0.58	0.05	1.53	0.45	0.54
16	1.56	3.50	4.16	3.60	0.05				
17	1.91	0.67	1.69	2.96	6.32	1.17	2.48	0.95	0.24
18	1.43	0.90	3.20	0.79	1.44				
19	0.59	1.15	4.68	1.31	1.28	0.48	1.04	0.12	0.82
20	1.59	0.29	1.24	0.12	0.45				
22	ND***	0.25	1.62	2.66	0.10				
23	2.97	0.61	0.58	0.52	0.66				
25	1.51	1.47	3.01	0.41	0.75	0.62	1.17	0.12	0.46
26	2.94	2.54	1.17	0.74	0.23				

*Samples collected during application sequences 7, 9, 11, and 13 were not analyzed per Protocol Amendment (Appendix III)
**not sampled
***none detected

Table 3 t-Test of deposition samples for the 9 continued sites vs 14 discontinued or all 23 sites

Group	Two-Tailed Sample Assuming equal Variances			
	9 continued vs 14 discontinued sites		9 continued vs all 23 sites	
	9 sites	14 sites	9sites	23 sites
Mean	1.53	1.92	1.53	1.77
Variance	1.89	2.87	1.89	2.51
Observations	44	70	44	114
Pooled variance	2.49		2.34	
Hypothesized mean difference	0		0	
df	112		156	
t Stat	-1.29		0.888	
P(T<=t) two-tail	0.199		0.376	
$t_{0.05}$ two-tail	1.98		1.98	

Table 4 Two-factor ANOVA for effects of application and site on spinosad deposition*

Summary	Count	Sum	Mean	Variance
Site 1	9	-4.14	-0.46	0.20
Site 3	9	-1.03	-0.11	0.09
Site 4	9	0.65	0.07	0.08
Site 7	9	2.10	0.23	0.08
Site 13	9	-1.86	-0.21	0.24
Site 15	9	-1.27	-0.14	0.24
Site 17	9	1.43	0.16	0.17
Site 19	9	-0.59	-0.07	0.18
Site 25	9	-1.09	-0.12	0.17
Application 1	9	-0.25	-0.03	0.17
Application 2	9	0.03	0.00	0.15
Application 3	9	1.84	0.20	0.06
Application 4	9	-1.04	-0.12	0.26
Application 5	9	0.98	0.11	0.16
Application 6	9	-2.37	-0.26	0.17
Application 8	9	1.37	0.15	0.04
Application 10	9	-3.41	-0.38	0.35
Application 12	9	-2.94	-0.33	0.07

Source of variation	SS	df	MS	F	P-value
Site	3.10	8	0.39	2.96	0.00703
Application	3.28	8	0.41	3.13	0.00475
Error	8.39	63**	0.13		
Total	14.8	79**			

*Using $\log_{10}$ transformed data

** One degree of freedom lost because one missing data was estimated by the least-squares estimate.

Table 5 Regression analysis of mean deposition and the Julian day of the year* for each application

Regression Statistics				
Multiple R	**R square**	**Adjusted R square**	**Standard error**	**Observations**
0.741	0.549	0.485	0.414	9

ANOVA					
	df	**SS**	**MS**	**F**	**P**
Regression	1	1.46	1.46	8.52	0.0224
Residual	7	1.20	0.171		
Total	8	2.66			

	Coefficients	**Standard error**	**t Stat**	**P-value**	**Lower 95%**	**Upper 95%**
Intercept	2.04	0.262	7.79	0.000108	1.42	2.66
Day of the year	-0.00963	0.00330	-2.92	0.0224	-0.0174	-0.00183

*A method for expressing dates. The Julian days in the year are numbered consecutively from 1 to 365 or 366. For example, the Julian day of June 3, 2006 is 154.

Table 6 t-Test for the means of deposition samples collected under vs not under direct sunlight

Two-sample assuming unequal variances

	Samples collected	
	Under direct sunlight	**Not under direct sunlight**
Mean	0.410	1.68
Variance	0.207	4.26
Observations	5	36
Hypothesized Mean Difference	0	
df	31	
t Stat	3.17	
P(T<=t) one-tail	0.00171	
t Critical one-tail	1.70	

Table 7 Weather conditions during each application*

Application Sequence**	Rain (inches)	Temperature (°F)	Relative humidity (%)	Wind (miles/hour) Average	Range
1	Weather records were not available				
2	0	43.7	97.9	1.08	0-3
3	0	37.3	64.6	0.154	0-1
4	0	41.9	98.5	0.154	0-1
5	0	39.3	98.4	0.154	0-1
6	0	41.5	90.0	0.615	0-1
8	0	53.5	44.5	1.00	0-5
10	0	47.8	88.7	0.769	0-2
12	0	57.0	85.1	0.154	0-1

*Average hourly records for each period from start of spinosad application to start of deposition sample collection. The records were from California Department of Water Resources, Division of Flood Management.
** Weather data for the application sequences 7, 9, 11, and 13 were not presented since the samples collected during these applications were not analyzed per Protocol Amendment (Appendix III)

Table 8 Tukey's HSD for deposition mean comparison between paired sampling sites*

Site	**Site 1**	**Site 3**	**Site 4**	**Site 7**	**Site 13**	**Site 15**	**Site 17**	**Site 19**	**Site 25**
Elevation (ft)	980	1080	1060	800	1240	1340	1640	1560	1660
Deposition Mean (µg/ft^2)	0.544	0.927	1.39	2.11	0.969	1.05	2.04	1.27	1.06
P value for hypothesis of Least Square Mean (i) = Least Square Mean (j)									
Site 1		0.907	0.217	**0.014**	0.944	0.803	**0.023**	0.591	0.823
Site 3			0.951	0.335	1.000	1.000	0.462	1.000	1.000
Site 4				0.960	0.917	0.986	0.991	0.999	0.983
Site 7					0.273	0.473	1.000	0.692	0.449
Site 13						1.000	0.386	0.999	1.000
Site 15							0.617	1.000	1.000
Site 17								0.823	0.592
Site 19									1.000
Site 25									

Group		**Deposition Mean**			**Site**
		Arithmetic mean (µg/ft^2)	**Log$_{10}$ transformed LS mean****	**Back-transformed mean*** (µg/ft^2)**	
	A	2.11	0.448	1.81	7
	A	2.04	0.427	1.67	17
B	A	1.39	0.359	1.29	4
B	A	1.27	0.311	1.05	19
B	A	1.05	0.286	0.93	15
B	A	1.06	0.283	0.92	25
B	A	0.93	0.268	0.85	3
B	A	0.97	0.259	0.82	13
B		0.54	0.167	0.47	1

*Executed with SAS software (SAS 9.1)
**Estimated using transformed deposition data (x) as $\log_{10}(x+1)$.
***Back calculated as 10^ (transformed mean)-1. This mean differs from the arithmetic mean.

Table 9 Two-factor ANOVA for effect of the three aircraft on deposition mean

Summary	Count	Sum	Average	Variance
Spray area A&B	9	8.57	0.95	0.19
Spray area C	9	15.68	1.74	0.88
Spray area D	9	9.90	1.10	0.72
Application 1	3	3.91	1.30	0.76
Application 2	3	4.46	1.49	0.93
Application 3	3	5.61	1.87	0.84
Application 4	3	3.49	1.16	0.77
Application 5	3	5.76	1.92	2.01
Application 6	3	2.10	0.70	0.03
Application 8	3	4.71	1.57	0.01
Application 10	3	2.45	0.82	0.44
Application 12	3	1.67	0.56	0.00

Source of variation	SS	df	MS	F	P-value	$F_{0.05}$
Spray area	3.18	2	1.59	3.03	0.0767	3.63
Application	5.90	8	0.74	1.41	0.267	2.59
Error	8.39	16	0.52			
Total	17.5	26				

Table 10 Average deposition in buffer zone samples vs wind speed for each application

Application Sequence*	Spinosad (µg/ft^2)	Wind speed (miles/hour)	
		Average	Range
1	0.0607	NA**	NA
2	0.296	1.08	0-3
3	0.109	0.154	0-1
4	0.104	0.154	0-1
5	0.149	0.154	0-1
6	0.0135	0.615	0-1
8	0.666	1.00	0-5
10	0.000	0.769	0-2
12	0.083	0.154	0-1

*Samples collected during application sequences 7, 9, 11, and 13 were not analyzed per Protocol Amendment (Appendix IV).
** Data were not available.

Table 11 Spinosad residue in grapefruit samples

Sampling Site	Application Sequence*	Spinosad (ppb)**			
		Background samples		Application samples	
		Lower	Upper	Lower	Upper
27	2	ND***	ND	ND	0.004
27	3	ND	ND	0.023	0.034
27	4	ND	ND	0.011	0.007
27	5	ND	ND	0.002	0.014
27	6	ND	ND	ND	0.006
3	2	ND	ND	0.003	0.002
3	3	ND	0.001	0.023	0.004
3	4	ND	0.001	0.162	0.149
3	5	0.034	0.002	0.003	0.019
3	6	0.005	0.067	0.005	0.028
Average		**0.004**	**0.007**	**0.023**	**0.027**
Standard Deviation		**0.001**	**0.013**	**0.001**	**0.085**
Minimum		**ND**	**ND**	**ND**	**0.002**
Maximum		**0.034**	**0.067**	**0.162**	**0.149**

*No fruit samples were collected during application sequences 1 and 7-13 per Protocol (Appendix III) and Protocol Amendment (Appendix IV).
**fresh fruit weight basis
***None detected.

Table 12 Paired two-sample t-test for spinosad residue (ppb) in grapefruit samples

(Samples from application vs background; application samples from the upper vs lower portion of canopies)

Sample source	**Application**	**Background**	**Upper**	**Lower**
Mean	0.0250	0.00547	0.0267	0.0232
Variance	0.00210	0.000265	0.00197	0.00245
Observations	20	20	10	10
Pearson Correlation	-0.0390		0.968	
Hypothesized Mean Difference	0		0	
df	19		9	
t Stat	-1.77		-0.852	
P(T<=t) one-tail	0.0463		0.208	
t Critical one-tail	1.73		1.83	

Table 13 Average spinosad concentrations of GF-120 concentrate and tank mix samples

Application Sequence	**Spinosad (ppm)**		**Percent of target tank mix concentration**
	GF-120 concentrate	**Tank mix**	
1	208	202	253
1		90.0	113
2		78.0	97.5
3		96.0	120
4		80.0	100
5		84.0	105
6	193	82.4	103
7	223	76.3	95.4
8		88.4	111
9		96.3	120
10		122	153
11		102	128
12		88.5	111
13		102	128

Table 14 Summary of analytical method validation, continuing QC, and blind spike results for spinosad

Matrix	Analytical method validation		Continuing QC		Blind field and trip spike	
	Spike level	Recovery* (%)	Spike level	Recovery (%)	Spike level	Recovery (%)
Deposition	0.3-30 µg/MDS	97.4±5.74	0.3 µg/MDS	97.9±6.16		
Water	0.2-30 ppb	85.3±13.0	0.2 ppb	85.3±11.3	0.25-2.5 ppb	58.1±7.51
Air	1.0-50 µg/tube	97.1±13.0	1.0 µg/tube	87.0±9.68	2.5-5.0 µg/tube	83.8±33.5
Fruit	1.5-400 ppb**	87.0±8.31	3.0 ppb**	85.5±10.4		
Fruit rinse	10-200 ng***	95.1±8.78	30.0 ng***	70.0±9.87		

*average of the average recoveries of spinosyns A, D, and B.
** Fresh fruit basis
***Rinsing water basis

Figure 1 Map of aerial treatment area for Mexican fruit fly in Valley Center, San Diego County, Spring 2003

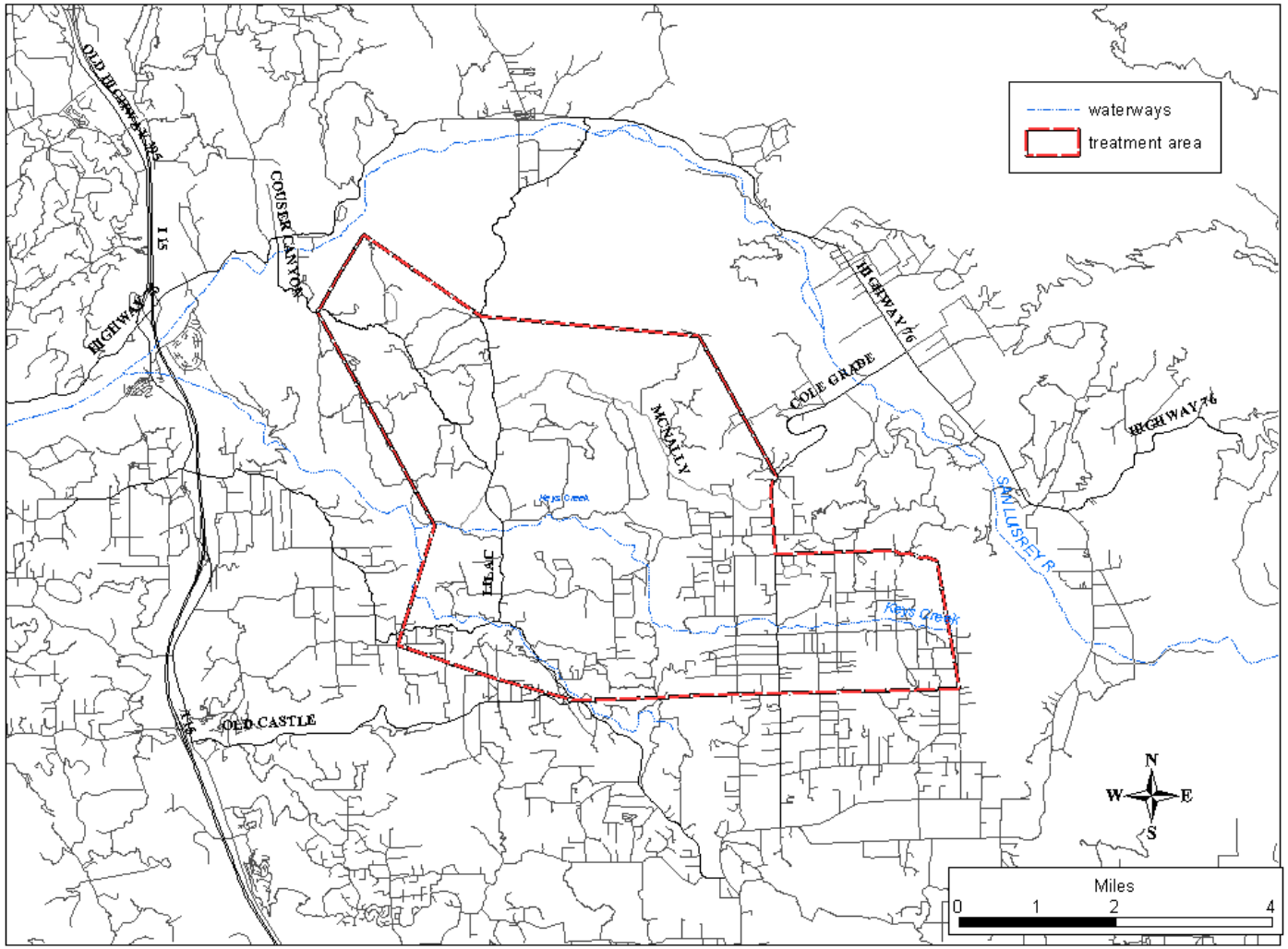


Figure 2 Flight areas and sampling sites for spinosad aerial applications

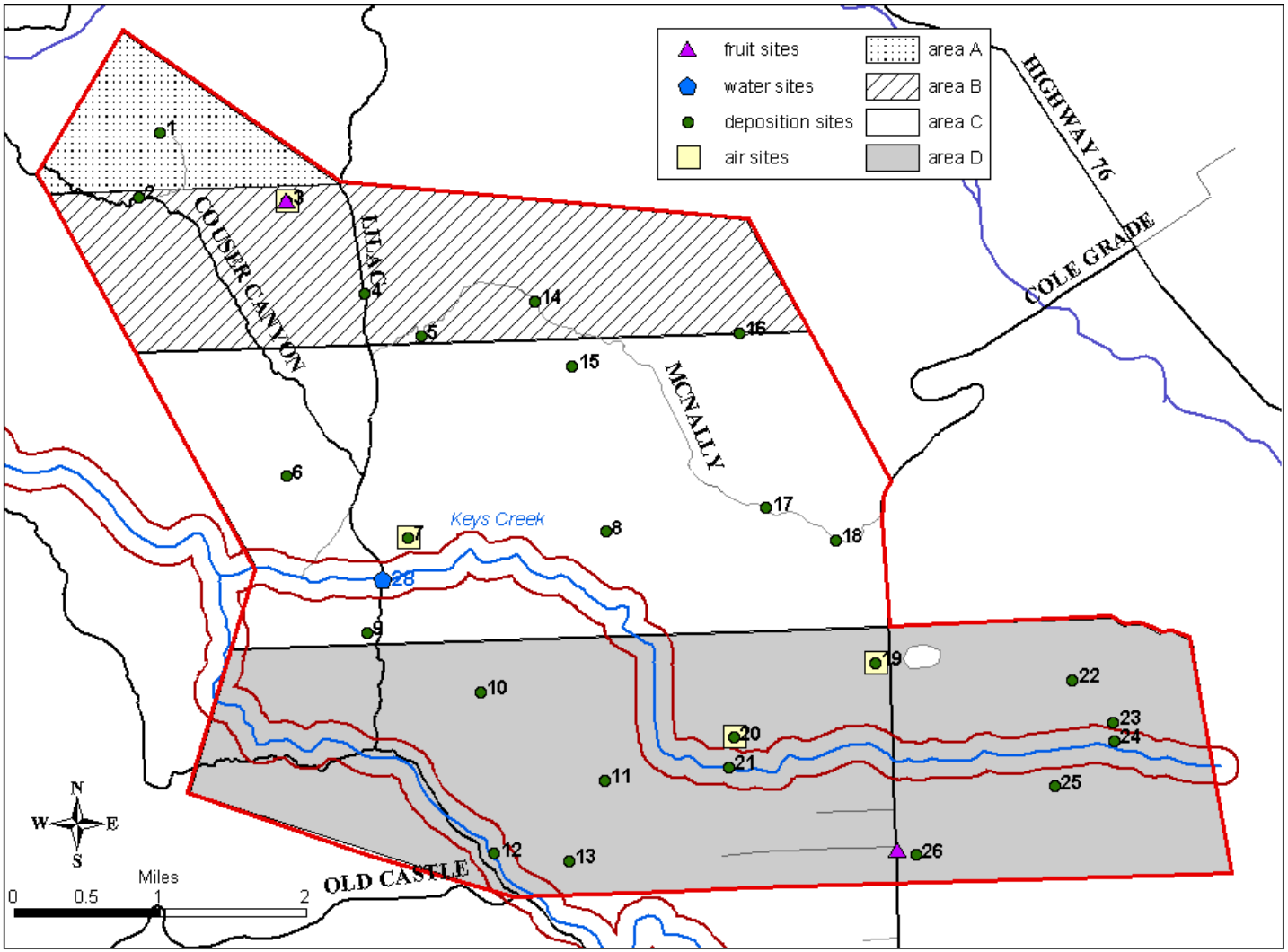


Figure 3 Sampling sites for storm run-off water (February 11-12, 2003)

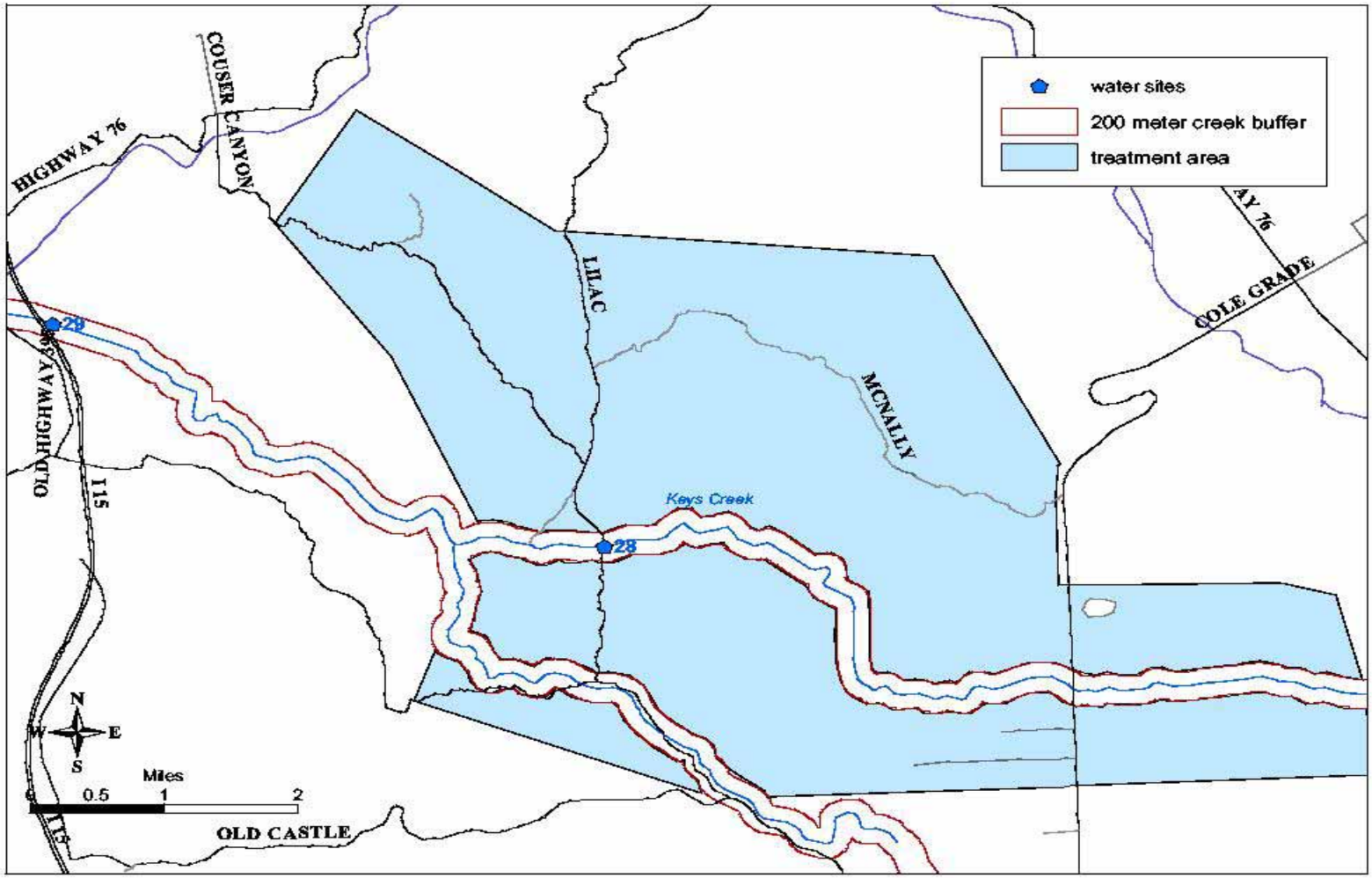


Figure 4 Distribution of the ratio of mass deposition to the target application rate

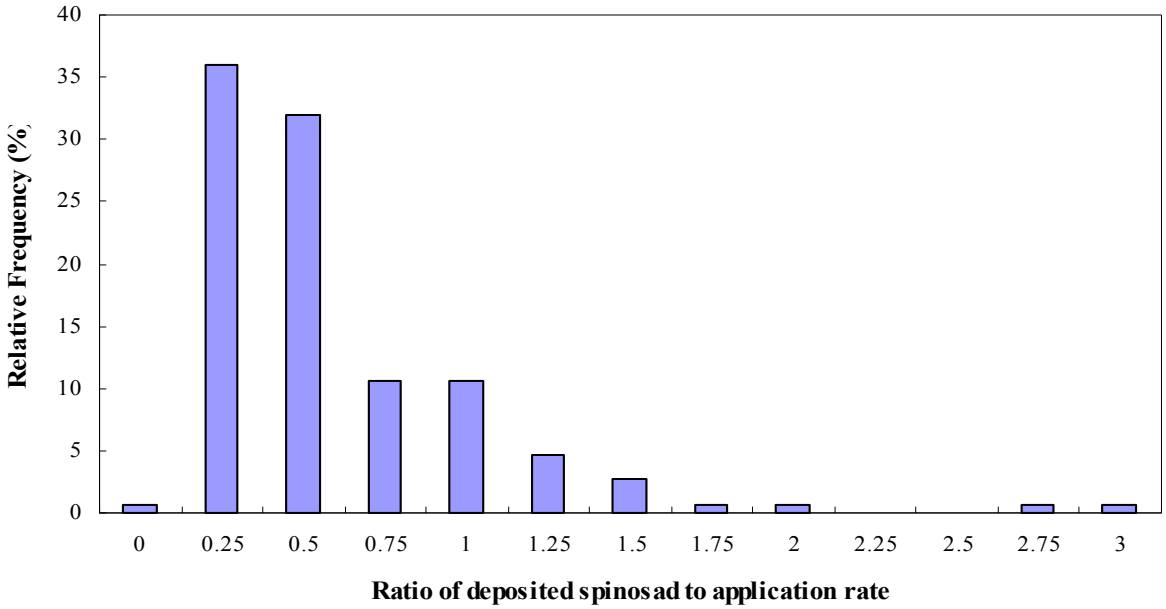


Figure 5 Average (± 1 standard error) spinosad deposition for each application

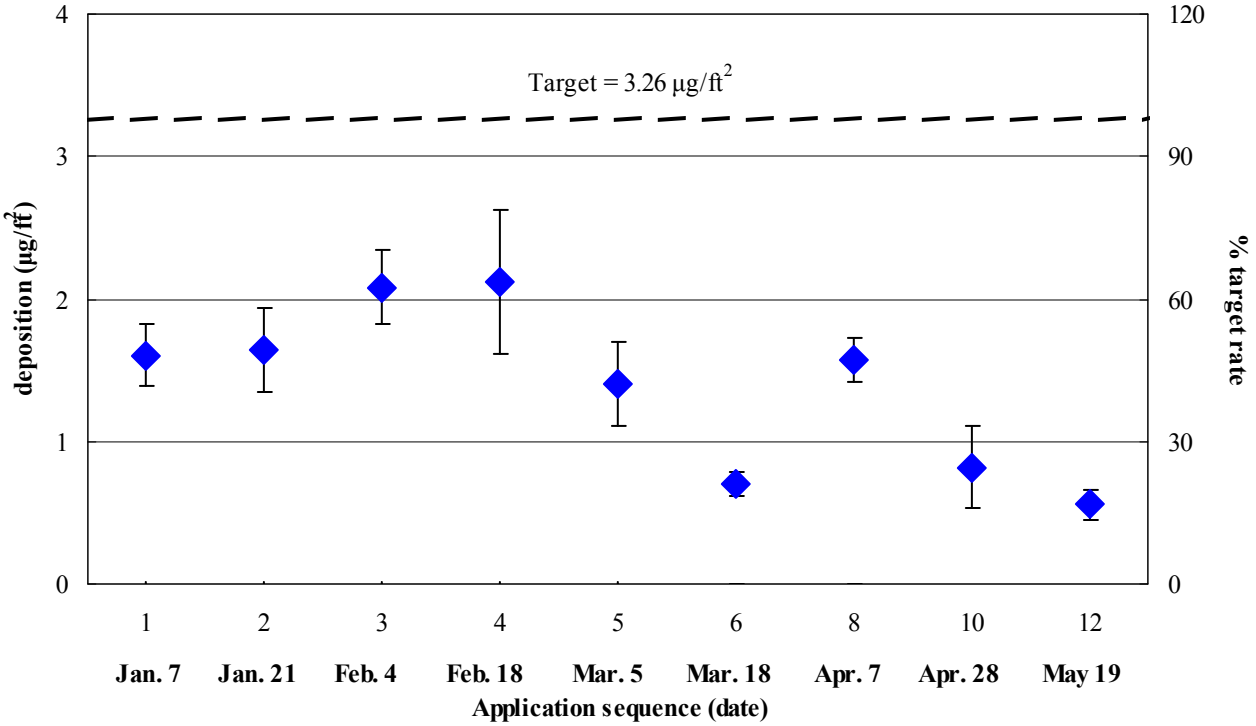
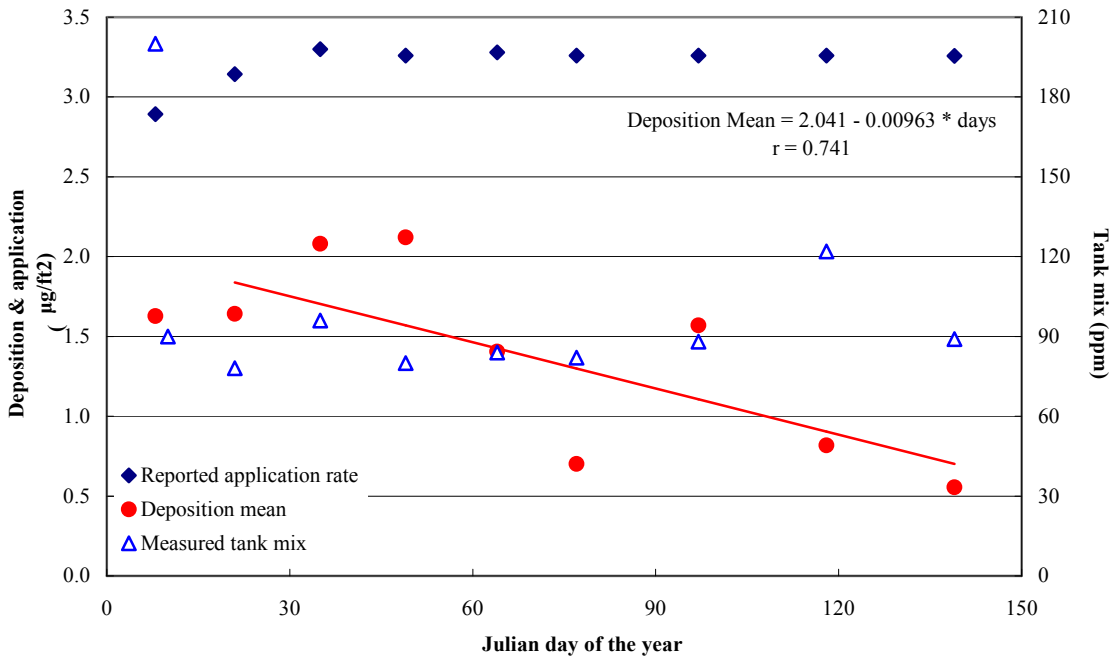


Figure 6 Correlation of the spinosad deposition mean to the reported application rate*, average tank mix concentration**, or the Julian day of the year during each application



*Each reported application rate was calculated as the amount of spinosad used divided by area applied during that application. The amount spinosad used was calculated from the volume of the tank mix used during that application and theoretical tank mix concentration of 80 ppm spinosad.
**Measured spinosad concentration of the tank mix. For the first application, data of the two individual samples were used and detailed explanation is in the "Spinosad concentrate and tank mix" section.

Figure 7 Correlation between mass deposition mean and sunrise time

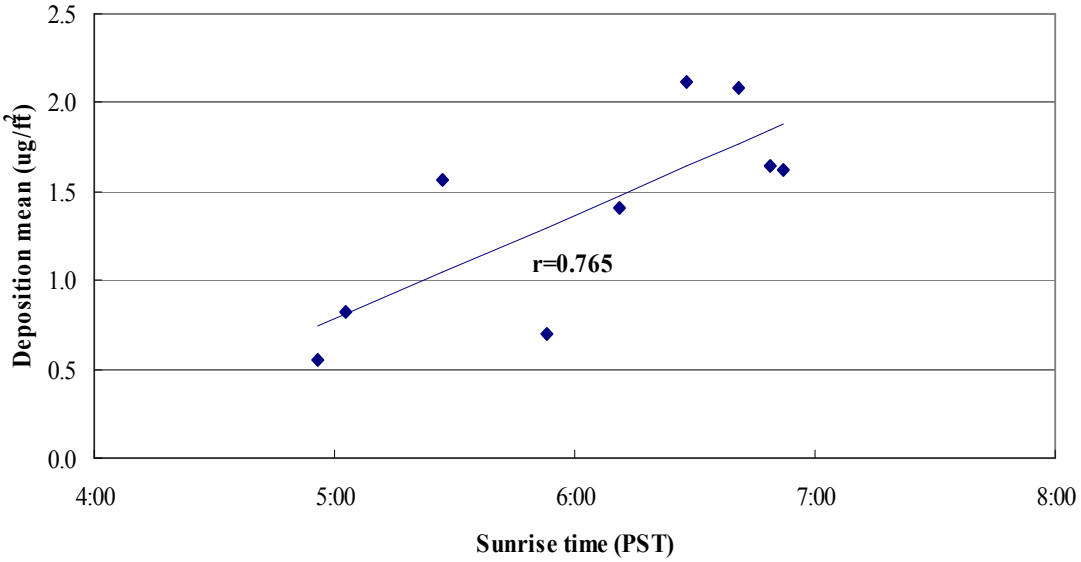


Figure 8 Mass deposition during each application vs. sampling time at each site

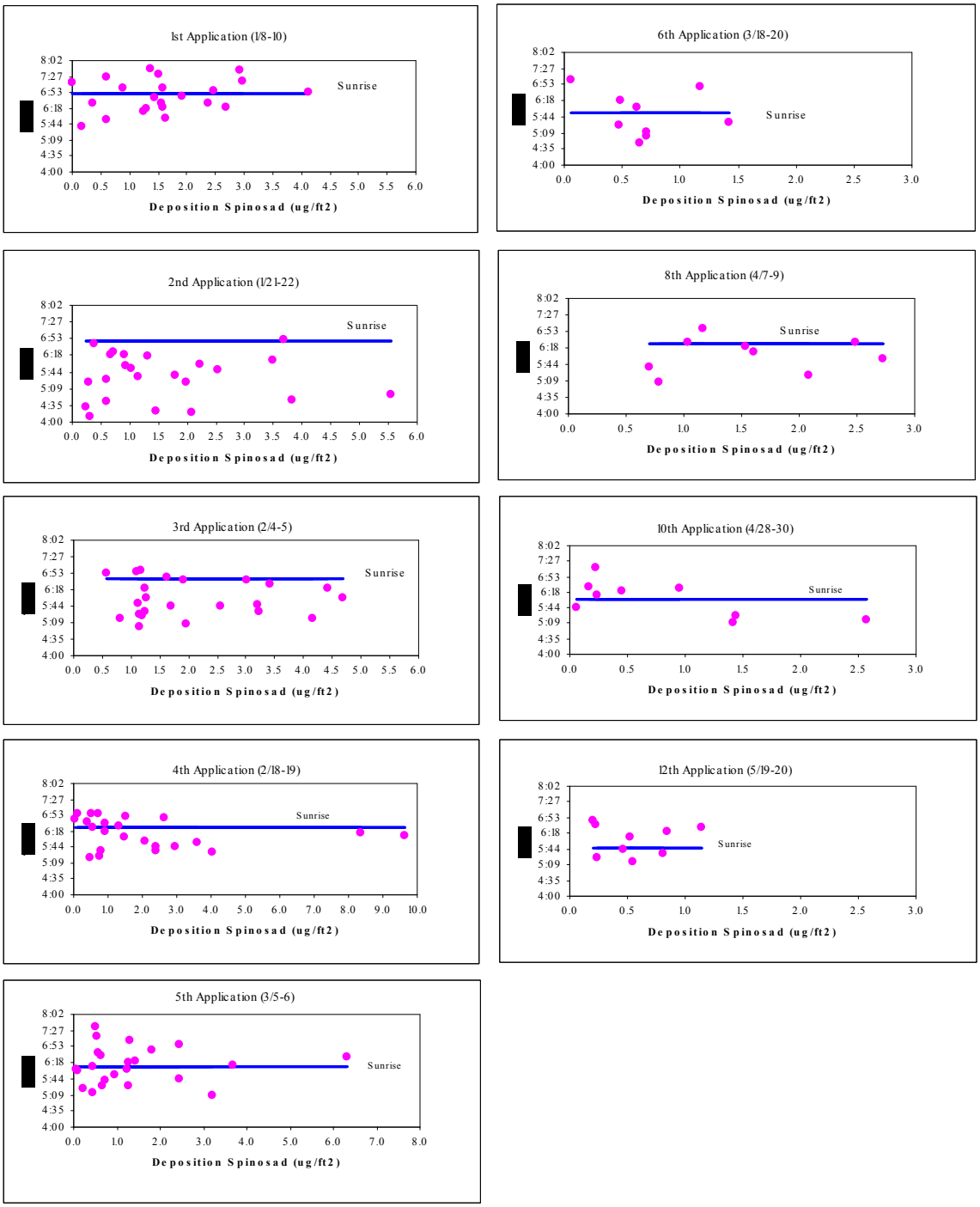


Figure 9 Effects of weather on mass deposition mean for each application

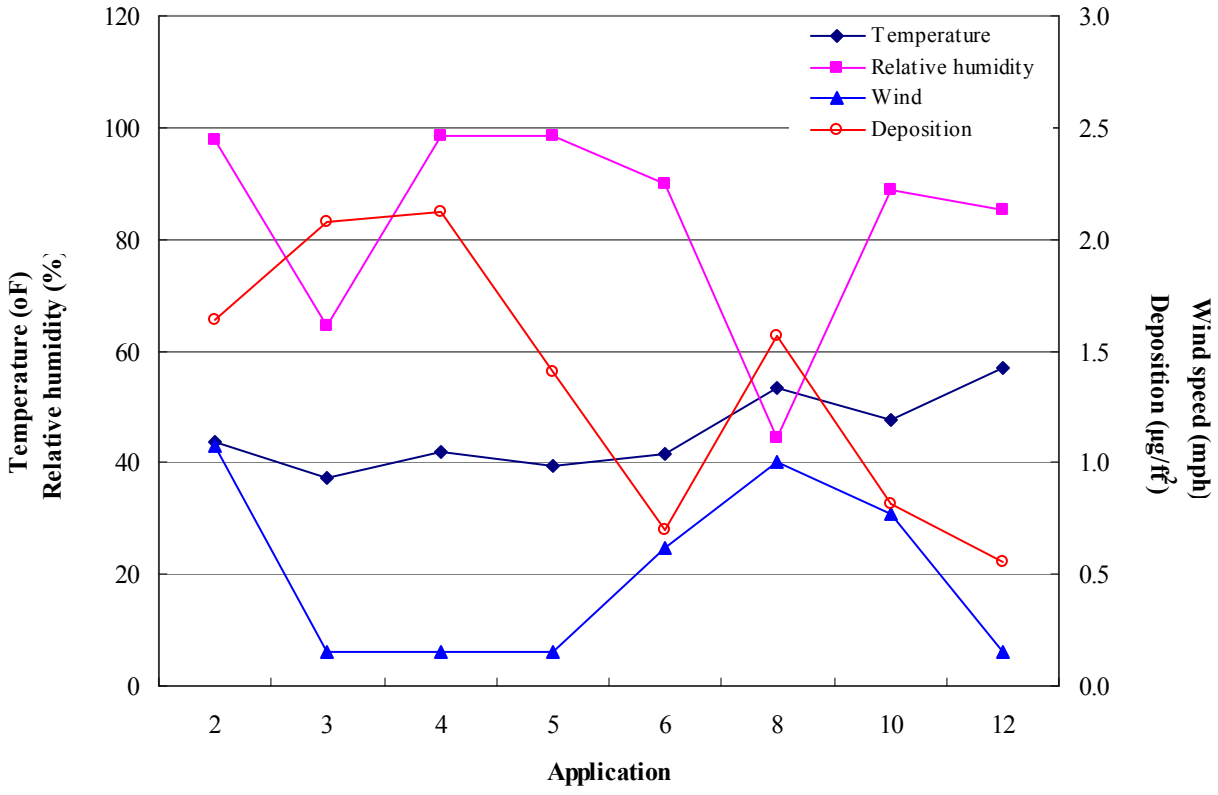


Figure 10 Mass deposition during each application vs. elevation at each site

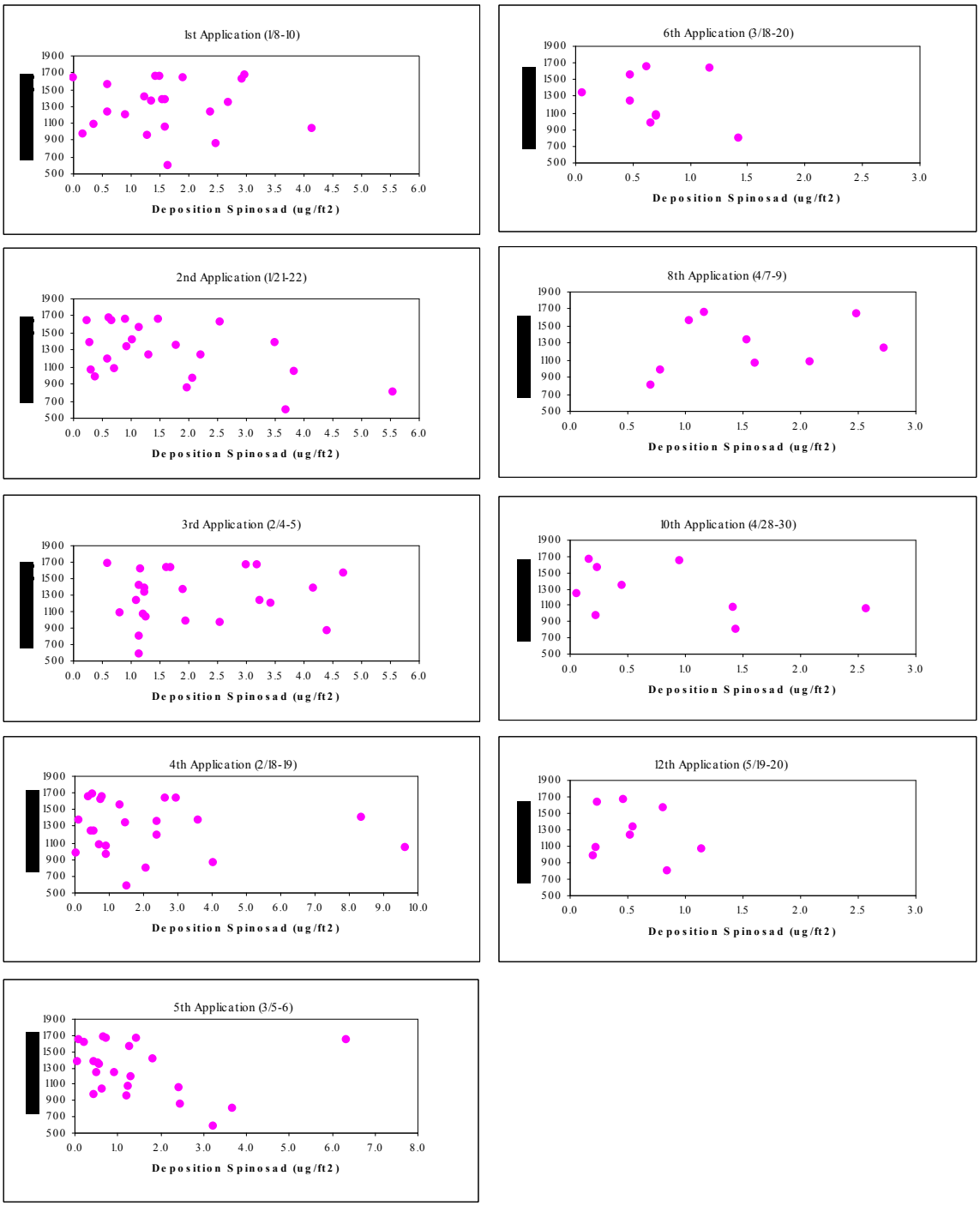


Figure 11 Deposition mean of all applications on each of the 9 continued sites vs. elevation

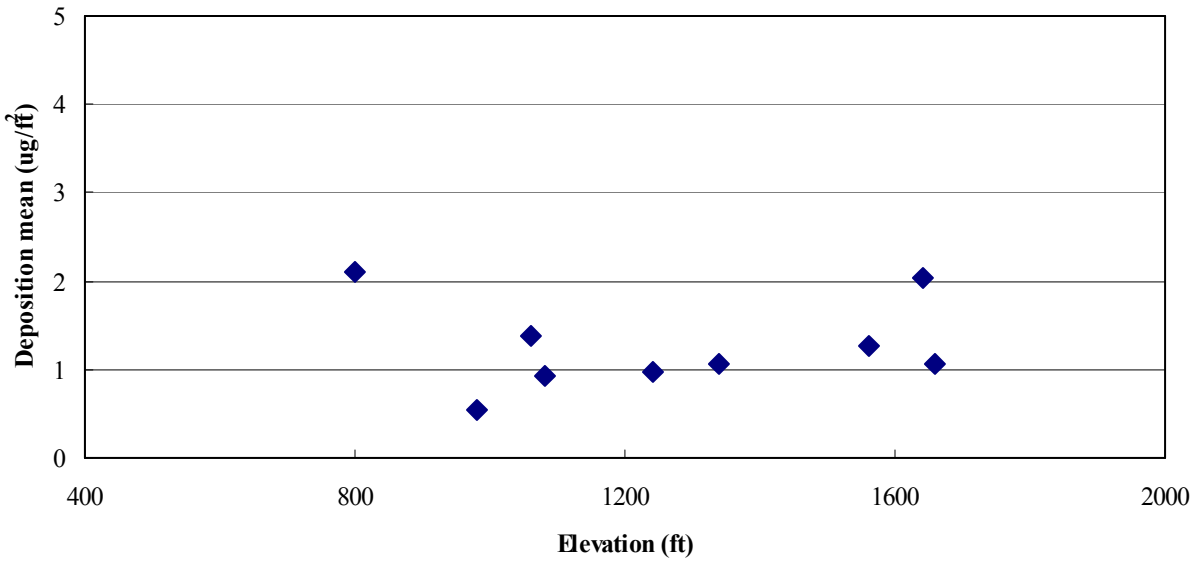
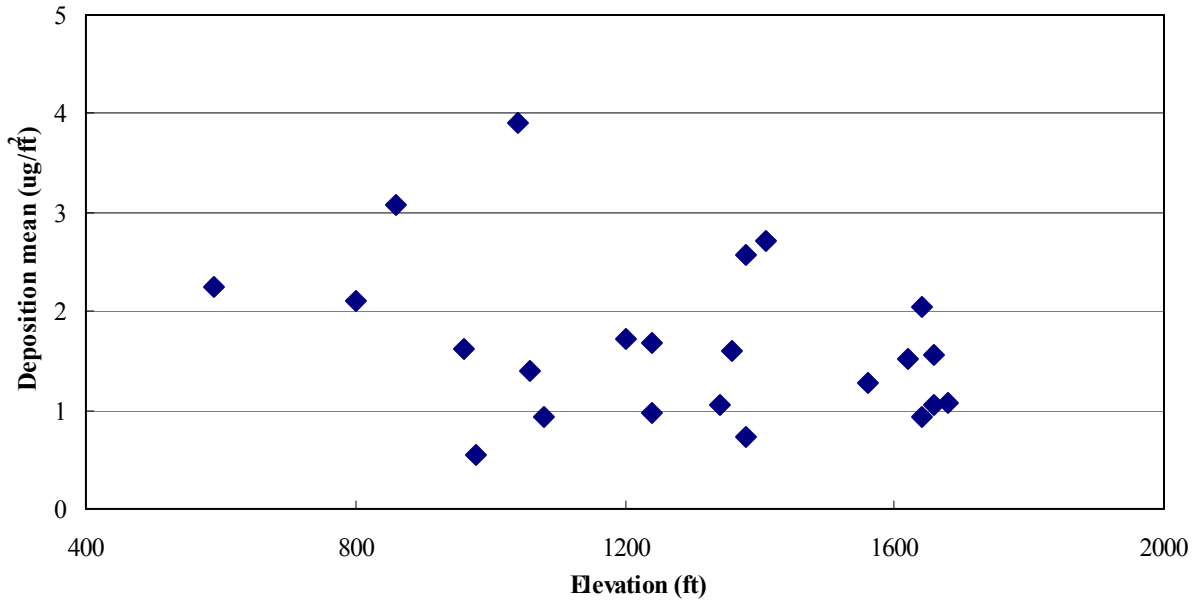


Figure 12 Deposition mean of all applications on each of all 23 sites vs. elevation



APPENDIX I

MALATHION CONTAMINATION IN THE SPRAY MATERIAL USED FOR MEXICAN FRUIT FLY ERADICATION IN VALLEY CENTER, SAN DIEGO COUNTY

TO: John Sanders, Ph. D., Chief
Environmental Monitoring Branch

FROM: Randy Segawa, Senior Environmental Research Scientist (Supervisor)
Dave Kim, Associate Environmental Research Scientist
Pam Wofford, Associate Environmental Research Scientist
Environmental Monitoring Branch
(916) 324-4137

DATE: April 18, 2003

SUBJECT: MALATHION CONTAMINATION IN THE SPRAY MATERIAL USED FOR MEXICAN FRUIT FLY ERADICATION IN VALLEY CENTER, SAN DIEGO COUNTY

The California Department of Food and Agriculture (CDFA) is using a series of aerial applications of spinosad to eradicate a Mexican fruit fly infestation in San Diego County. The pesticide product used for these applications is GF-120 NF Naturalyte Fruit Fly Bait, containing 200 parts per million (ppm) spinosad by weight as the active ingredient; this product contains no other active ingredients. For application, the GF-120 is diluted with water to a tank mix target concentration of 80 ppm of spinosad. The Department of Pesticide Regulation (DPR) detected 5 ppm of malathion in the tank mixture during the third application and 290 ppm of malathion during the fourth application. A subsequent investigation by DPR and CDFA indicates that a manifold used in the mix/load system is the likely source of contamination. Samples of deposition, water, air, and vegetables collected from the treatment area during and following the fourth application showed no detectable residue of malathion. However, the reliability of the malathion methods used for some of these samples is unknown. CDFA decontaminated or replaced all parts of the mix/load system and aircraft following the fourth application. Samples collected after decontamination showed low or no detectable concentrations of malathion. Samples of the mix/load system and aircraft collected during the fifth application showed no detectable concentrations of organophosphate, carbamate, or chlorinated hydrocarbon pesticides, including malathion.

Background

CDFA is using a series of aerial applications of spinosad to eradicate a Mexican fruit fly infestation in the Valley Center area of San Diego County. The pesticide product used for these applications is GF-120 NF Naturalyte Fruit Fly Bait (U.S. Environmental Protection Agency Registration Number 62719-498), containing 200 ppm (0.020 percent) spinosad by weight (mixture of spinosyn A and spinosyn D) as the active ingredient; this product contains no other active ingredients. For application, the GF-120 is diluted with water to a tank mix target concentration of 80 ppm (0.0080 percent) by weight of spinosad or 0.363 grams per gallon (g/gal). The spinosad target application rate is 3.26 $\mu g/ft^2$ (0.142 g/acre, or 35.1 $\mu g/m^2$)

DPR is monitoring these treatments to provide information on the amount of spinosad in the spray material and reaching the ground during application, concentrations in air, concentrations

in surface water, and concentrations in fruit. DPR has completed monitoring of the first four applications. Preliminary results of the monitoring showed some unusual findings, particularly the detection of malathion in samples collected in the spray material during the third and fourth applications. This memorandum describes the results of samples of the spray material, mix/load system, and aircraft, and an investigation into the source of the malathion contamination.

Description of the Mix/Load and Application Systems

CDFA has modified the mix/load system over the course of the applications. The following is a general description of the mix/load and application systems. Detailed diagrams of the mix/load system used for the first four applications are shown in Figures 1 – 4. The GF-120 used for this project is manufactured by Dow AgroSciences and distributed by Western Farm Service. CDFA personnel constructed the mix/load system primarily using CDFA and rented equipment. The aircraft are owned and operated by Dynamic Aviation Group, Inc., under contract to CDFA.

Western Farm Service and CDFA personnel usually transfer and mix the GF-120 with water the day before the scheduled application. Western Farm Service delivers the GF-120 in 55-gallon drums to CDFA's mix/load site at French Valley Airport near Temecula. At the mix/load site, Western Farm Service transfers the GF-120 from 44, 55-gallon drums into one or more 4,000-gallon storage tanks (Tanks 1 – 3, Figures 1 – 4). At the time of transfer, Tanks 1 and 2 each contain approximately 2,000 gallons of water. The GF-120 is then transferred into Tanks 1 and 2 either directly from the 55-gallon drums or from Tank 3. Western Farm Service rinses the 55-gallon drums with water and places the rinsate into a ball tank. CDFA uses the rinsate in the ball tank for the subsequent application. After the GF-120 is placed into Tanks 1 and 2, CDFA adds additional water from a fire hydrant. Applications 1 – 4 each used approximately 2,350 gallons of GF-120 and 3,500 gallons of water, split between Tanks 1 and 2.

During the application, CDFA personnel transfer the GF-120/water mixture from Tanks 1 and 2 into three fixed-wing aircraft through a series of valves, pumps, meters, and other equipment. The three aircraft each contain a tank that holds the 240 gallons used for each load. Each aircraft uses approximately eight loads for each application. The spray material is applied through five nozzles attached to a boom under the wings. At the end of application, the mix/load system and aircraft spray system are rinsed with water. Rinsate from the mix/load system is placed in Tank 2 and used for the subsequent application. The rinsate from the aircraft are placed in a vat and then transferred to Tank 2 and used for the subsequent application.

Description of Sampling and Analysis for Malathion Contamination

DPR staff collected samples from the spray material, mix/load system, and aircraft to determine the source of malathion contamination. Four types of samples were collected: GF-120, GF-120/water mixture, rinsate, and water. The GF-120 and GF-120/water mixture samples were collected from the material in the 55-gallon drums and Tanks 1, 2, and 3. Rinsate samples were collected from the mix/load equipment and aircraft. Water samples were collected from the hydrant. Additional water samples were collected from the mix/load system and aircraft after decontamination. All samples were collected in new brown plastic or amber glass bottles. The samples were kept refrigerated until laboratory analysis.

The CDFA Center for Analytical Chemistry analyzed all samples. Two different methods were used to analyze the samples, one for the GF-120 and GF-120/water mixture samples, and one for the rinsate and water samples. Both methods used gas chromatographs with mass selective detectors for analysis. The GF-120 and GF-120/water mixture samples had a detection limit of approximately 5 ppm for malathion. The rinsate and water samples had a detection limit of approximately 0.0001 ppm for malathion. The difference in detection limits was due to the smaller aliquots and higher concentration of interferences with the GF-120 and GF-120/water mixture analysis.

Dow AgroSciences split several of the DPR samples. In addition, they sampled archived GF-120 spray material. All samples were analyzed for malathion by Dow AgroSciences' laboratory in Indianapolis.

Results of Samples From the Mix/Load System

During the First Four Applications

Monitoring results from the mix/load system for the first application are shown in Figure 1. During the first application, DPR collected samples of the GF-120 from the 55-gallon drums, and the GF-120/water mixture from Tank 1. The GF-120 contained 208 ppm of spinosad, close to the expected concentration of 200 ppm. The first application occurred over two nights. During the first night (January 7, 2003), Tank 1 contained 202 ppm of spinosad, much higher than the expected concentration of 80 ppm. The high concentration was likely due to insufficient mixing of Tank 1. During the second night (January 9, 2003), Tank 1 contained 90 ppm, closer to the expected concentration. None of the samples contained detectable concentrations of organophosphate, carbamate, or chlorinated hydrocarbon pesticides.

Results from the second application on January 21, 2003 are shown in Figure 2. Tank 1 contained 78 ppm of spinosad. No other samples of the mix/load system were collected. No organophosphate, carbamate, or chlorinated hydrocarbon pesticides were detected.
Results from the third application on February 4, 2003 are shown in Figure 3. CDFA made several changes in the mix/load system between the second and third applications. CDFA moved the mix/load site from an area near the tarmac to one of the hangars at French Valley Airport. In addition, a filter and a loading manifold were added to the system. DPR collected a sample of Tank 1 from the output at the end of the loading manifold. This sample contained 96 ppm of spinosad and 5 ppm of malathion, a common insecticide. The laboratory unequivocally identified the malathion using a mass selective detector. DPR received the results of the third application on February 20, 2003.

Results from the fourth application on February 18, 2003 are shown in Figure 4. CDFA made a minor modification in the mix/load system for the fourth application. The GF-120 was transferred directly from the 55-gallon drums into Tanks 1 and 2. For the prior applications, the GF-120 was first transferred into Tank 3. As with the third application, DPR collected a sample of Tank 1 from the loading manifold. Because of the unusual results from the third application, DPR expedited the analysis of the sample for the fourth application, and received the results on

February 26, 2003. This sample contained 80 ppm of spinosad and 290 ppm of malathion. The laboratory unequivocally identified the malathion using a mass selective detector. A second chemist and instrument confirmed the malathion detection.

None of the equipment in the mix/load system or aircraft was used for any other pesticide applications since the start of this program. Therefore, DPR and CDFA initiated an investigation into the cause and potential impact of the malathion contamination.

Malathion Concentrations in Environmental Samples

From the Fourth Application

For the fourth application, DPR collected its normal environmental samples of deposition, water, air, and fruit for spinosad. To determine the potential impact of the malathion contamination, DPR directed the laboratory to attempt to analyze some of the spinosad samples for malathion. None of the 26 deposition samples contained detectable concentrations of malathion. However, these samples were optimized to detect spinosad. The reliability of these samples to detect malathion is unknown. Environmental samples from the third application were not analyzed because the concentrations would have been far below the malathion detection limit.

On February 27, 2003, DPR also collected two lettuce samples and one cabbage sample from organic farms in the treatment area specifically for malathion. No malathion was detected. However, the treatment area received several inches of rain between the fourth application and the date of this sampling.

Results of Samples From the Mix/Load System and Aircraft

Before and After Decontamination

Between February 28, 2003 and March 3, 2003, CDFA decontaminated or replaced all parts of the mix/load system and aircraft. DPR collected additional samples before and after decontamination to determine the source of contamination and to ensure the systems were sufficiently cleaned. GF-120 or GF-120/water mixture remaining after the fourth application was sampled from the 55-gallon drums, and Tanks 1 and 2. The leftover GF-120 from the third application remaining in Tank 3 was also sampled. The remaining samples of the mix/load system consisted of rinsates of the equipment. Except the vat, all of the equipment had been previously rinsed after the fourth application; therefore, these samples consisted of a second rinse. The vat contained the original rinsate from the fourth application.

Results of the samples collected prior to decontamination are shown in Figure 5 and Table 1. Forty-three of the 55-gallon drums and Tanks 1, 2, and 3, containing the leftover GF-120 or GF-120/water mixture contained no detectable malathion, with a detection limit of approximately 5 ppm. Only 43 of the 44 drums used for the fourth application could be located. Tanks 1 and 2 contained leftover material from the fourth application, as well as approximately 2,000 gallons of water, in preparation for the fifth application, but the tanks

had not been mixed. The samples from Tanks 1 and 2 contained 20 ppm and 33 ppm of spinosad, respectively. If Tanks 1 and 2 originally contained 80 ppm of spinosad and 290 ppm of malathion during the fourth application on February 18, 2003 and the proportion stayed consistent, the expected concentration of malathion at the time of sampling on February 28, 2003 was 70 – 120 ppm, well above the 5 ppm detection limit.

Several rinsates from the remaining parts of the mix/load system and the aircraft contained detectable concentrations of malathion (Figure 5 and Table 1). The highest malathion concentrations were detected in samples from the loading manifold and the vat containing the aircraft rinsate. The sample from the vat is the only one containing the original rinsate from the fourth application.

Samples of rinsates from the mix/load system and aircraft after decontamination showed low or no detectable concentrations of malathion (Figure 6). Rinsate samples from two of the aircraft showed higher malathion concentrations after decontamination than before. Samples collected after decontamination may have inadvertently contained malathion from the surface of the spray booms or nozzles. Additionally, the rinse water was transferred from aircraft to aircraft in series, in which cross contamination between the aircraft may have occurred. Tank 2 had no detectable malathion before contamination, but 0.0001 ppm of malathion after decontamination. This is likely due to differences in detection limit. The GF-120/water mixture analyzed before decontamination had a detection limit of approximately 5 ppm. The rinsate from Tank 2 after decontamination had detection limit of approximately 0.0001 ppm

Dow AgroSciences did not detect malathion in any of the archived GF-120 product lots, with a detection limit of approximately 1 ppm.

Results of Samples From the Mix/Load System and Aircraft

During the Fifth Application

Results from the fifth application on March 5, 2003 are shown in Figures 7 and 8. CDFA made a minor modification in the mix/load system for the fifth application. Rinsate from the 55-gallon drums of the fourth application was not used for the fifth application. Before the fifth application, a composite sample from each of the four lots of GF-120 was collected and analyzed. During the fifth application, DPR collected GF-120/water mixture samples from Tanks 1 and 2, the loading manifold, and the three aircraft. Spinosad concentrations ranged from 78 to 98 ppm. No organophosphate, carbamate, or chlorinated hydrocarbon pesticides, including malathion were detected in any of the samples.

Discussion and Conclusions

Malathion was unequivocally identified in several samples collected from the mix/load system and the aircraft. It's likely that malathion was applied during the third and fourth applications because malathion was detected in samples from the aircraft nozzles before and after decontamination, as well as in the aircraft rinsate from the fourth application. The level of malathion contamination may be high relative to the amount of spinosad, particularly during the

fourth application where the malathion concentration exceeded the spinosad concentration. However, the 290 ppm of malathion detected in the sample from the fourth application may not be representative of the entire 5800 gallons of spray mixture applied because malathion was not detected in the leftover spray material of Tanks 1 and 2.

Even if the all of the spray mixture from the fourth application contained 290 ppm of malathion, the impacts were likely negligible. Malathion was not detected in any of the environmental samples from the fourth application, although the environmental samples may not be reliable for malathion. Malathion is a common pesticide, and has been used for fruit fly eradication programs on several occasions. The malathion used for fruit fly eradications is normally applied at a rate of 950 $\mu g/ft^2$. If the spray mixture contained 290 ppm of malathion, the application rate would have been approximately 12 $\mu g/ft^2$, or one to two percent of the normal rate. DPR has monitored previous fruit fly eradication programs using malathion (Ando, et al. 1996; Bradley, et al. 1997; Segawa, et al. 1991). A risk assessment conducted by the Office of Environmental Health Hazard Assessment concluded that the aerial application of malathion posed no significant risk to public health at the normal application rate of 950 $\mu g/ft^2$ (Russell, et al., 1991).

From the available information, the source of contamination is not clear. There are five possible sources of contamination: the GF-120 product, the water, the mix/load system, the aircraft, or sampling/laboratory analysis.

The GF-120 product is an unlikely source of the contamination, but it cannot be completely eliminated. Malathion was not detected in any of the archived GF-120 lots used for the third or fourth applications. Analysis of 43 of 44 drums used for the fourth application did not detect malathion. Unfortunately, the last drum could not be located, so the GF-120 cannot be completely eliminated as a source of contamination.

The water used in the mix/load system is an unlikely source of contamination. The mix/load system uses water from a fire hydrant. Malathion was not detected in samples of the water from the hydrant. Malathion would need to occur intermittently in the water hydrant for it to be the source of contamination.

The mix/load system is a likely source of contamination, but there are inconsistencies in the information. This is a likely source of contamination because the original samples containing malathion from the third and fourth applications were collected from the loading manifold of the mix/load system. Samples from the loading manifold had one of the highest malathion concentrations in comparison to the other samples. The loading manifold and filter were first used for the third application, and the loading manifold was first sampled during the third application.

There are two main inconsistencies with the loading manifold as the source of contamination. First, malathion was detected in parts of the mix/load system “upstream” of the loading manifold. However, it is possible for a small amount of backflow to occur in the system. It is also possible that malathion contamination from the third application was recycled through the entire mix/load system via the aircraft rinsate. The second inconsistency is that the sample from the fourth application had a much higher malathion concentration (290 ppm) than the third

application (5 ppm). If the manifold was the source of malathion, one would expect dilution to reduce the malathion concentration from the third to the fourth application since the loading manifold was not used for anything else between the third and fourth application.

Other parts of the mix/load system are less likely sources of contamination, although there are several vulnerable points for contamination. The other parts of the mix/load system had lower malathion concentrations compared to the loading manifold. Tanks 1 and 2 are among the most vulnerable points for contamination. However, if Tanks 1 and 2 were the source of contamination, the samples collected just before decontamination should have contained 70 – 120 ppm malathion. Since no malathion was detected, either the tanks did not contain malathion or the malathion degraded during the 10-day period between samples. Malathion degrades rapidly under alkaline conditions (pH >7), but the material in Tanks 1 and 2 were slightly acidic (pH 4). The ball tank is another vulnerable point of contamination. It contained the rinsate from the drums of the third application, but this sample also had no detectable malathion.

The aircraft are a possible, but less likely source of contamination. Samples from the nozzles of all three aircraft had detectable malathion concentrations. In addition, the vat containing the rinsate from the aircraft had the highest malathion concentration. The vat had the highest concentration probably because it is the one piece of the system that still contained the original rinsate from the fourth application. These results are consistent with the loading manifold as the source of contamination. In order for the aircraft to be the source of contamination, significant backflow in the mix/load system would need to occur. The backflow would need to contaminate the loading manifold on a continuous basis, since this was the point of the original sampling for the third and fourth applications.

Errors in the sampling or laboratory analysis are a possible, but less likely source of contamination. All materials used for the sampling were new, used once, and discarded. Each sample was collected with new disposable gloves. The laboratory analyzed quality control samples concurrently with the field samples, including blanks. None of the blanks contained malathion. If the contamination was due to sampling or laboratory errors, it's likely that either all samples would contain malathion, or the malathion would be detected randomly. Most parts of the system have been sampled more than once. The same parts are consistently positive or negative for malathion. The DPR staff assigned and materials used for this project have not been used to monitor malathion applications for several years.

Future Activities

DPR and CDFA will expand its monitoring of the mix/load system and aircraft for future applications. DPR or CDFA will collect samples and analyze samples of each lot of GF-120 prior to its use. DPR or CDFA will collect samples of the mix/load system and aircraft for each application.

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Segawa, R.T., J.A. Sitts, J.H. White, S.J. Marade, and S.J. Powell. 1991. Environmental monitoring of malathion aerial applications used to eradicate Mediterranean fruit flies in Southern California, 1990. Report EH 91-03. California Environmental Protection Agency, Department of Pesticide Regulation, Sacramento, California.

Attachment

Table 1. Results of sampling after the fourth application, prior to decontamination. See Figure 5 for a diagram of the sample locations.

Sample Location	Sample Type	Malathion (ppm)	Approximate Detection Limit (ppm)
55-gallon Drums*	GF-120	ND**	5
Ball Tank	GF-120 Rinsate (3rd applic)	ND	0.0001
Transfer Pump	2nd Rinsate	ND	0.0001
Hydrant	Water	ND	0.0001
Mixing Pump	2nd Rinsate	ND	0.0001
Tank 1	GF-120/Water Mix	ND	5
Tank 2	GF-120/Water Mix	ND	5
Tank 3	GF-120 (3rd applic)	ND	5
Loading Pump	2nd Rinsate	0.0023	0.0001
Filter	2nd Rinsate	0.0007	0.0001
Flow Meter	2nd Rinsate	~0.01	0.0001
Loading Manifold	2nd Rinsate	1.4	0.0001
Nozzles of N7198Y	2nd Rinsate	0.027	0.0001
Nozzles of N70U	2nd Rinsate	ND	0.0001
Nozzles of N7136M	2nd Rinsate	ND	0.0001
Vat	1st Rinsate	2.3	0.0001

*GF-120 from 43 of the 44 55-gallon drums were analyzed. The last drum could not be located.
** None Detected

Table 2. Results of sampling after the fourth application, after decontamination. See Figure 6 for a diagram of the sample locations.

Sample Location	Sample Type	Malathion (ppm)	Approximate Detection Limit (ppm)
Transfer Pump	Water	ND*	0.0001
Tank 1	Water	ND	0.0001
Tank 2	Water	0.0001	0.0001
Filter	---	Not Sampled**	---
Loading Manifold	---	Not Sampled	---
Nozzles of N7198Y	Water	0.017	0.0001
Nozzles of N70U	Water	0.0003	0.0001
Nozzles of N7136M	Water	0.0061	0.0001

* None Detected
**A new filter and loading manifold were installed.

Figure 1
Mexican Fruit Fly Spinosad Mix/Load System
Monitoring Results During Application 1 (1/7/03 and 1/9/03)

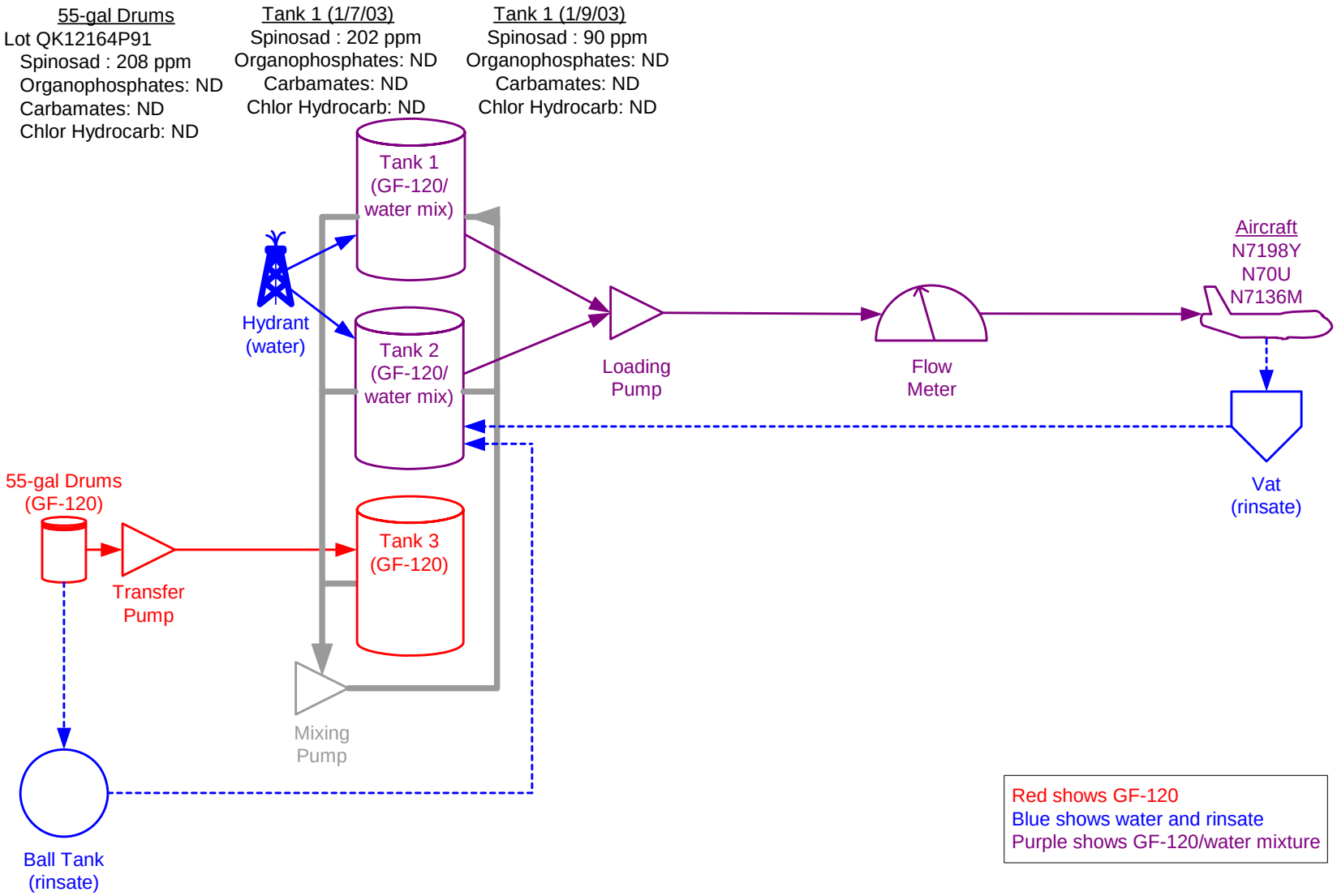


Figure 2
Mexican Fruit Fly Spinosad Mix/Load System
Monitoring Results During Application 2 (1/21/03)

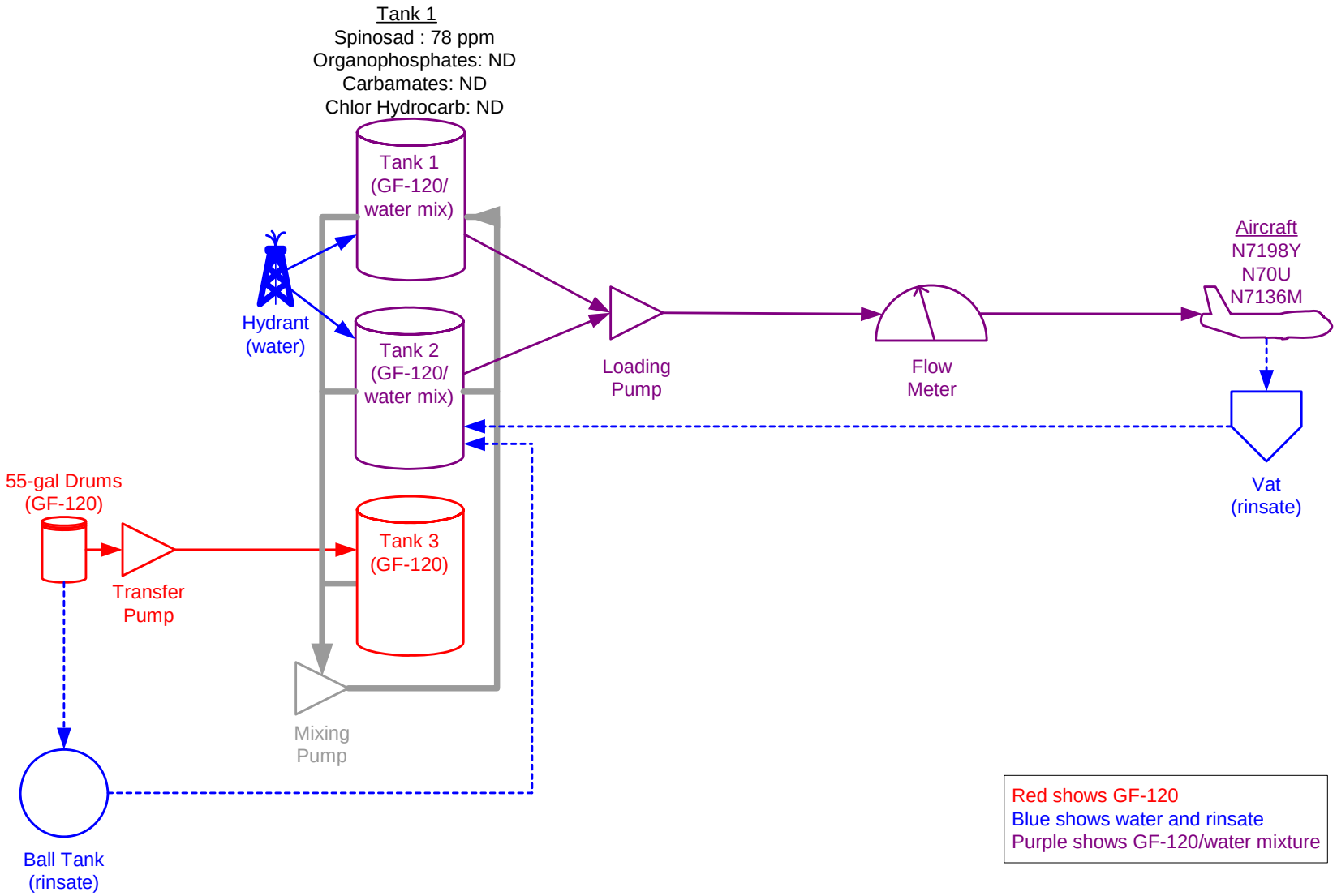


Figure 3
Mexican Fruit Fly Spinosad Mix/Load System
Monitoring Results During Application 3 (2/4/03)

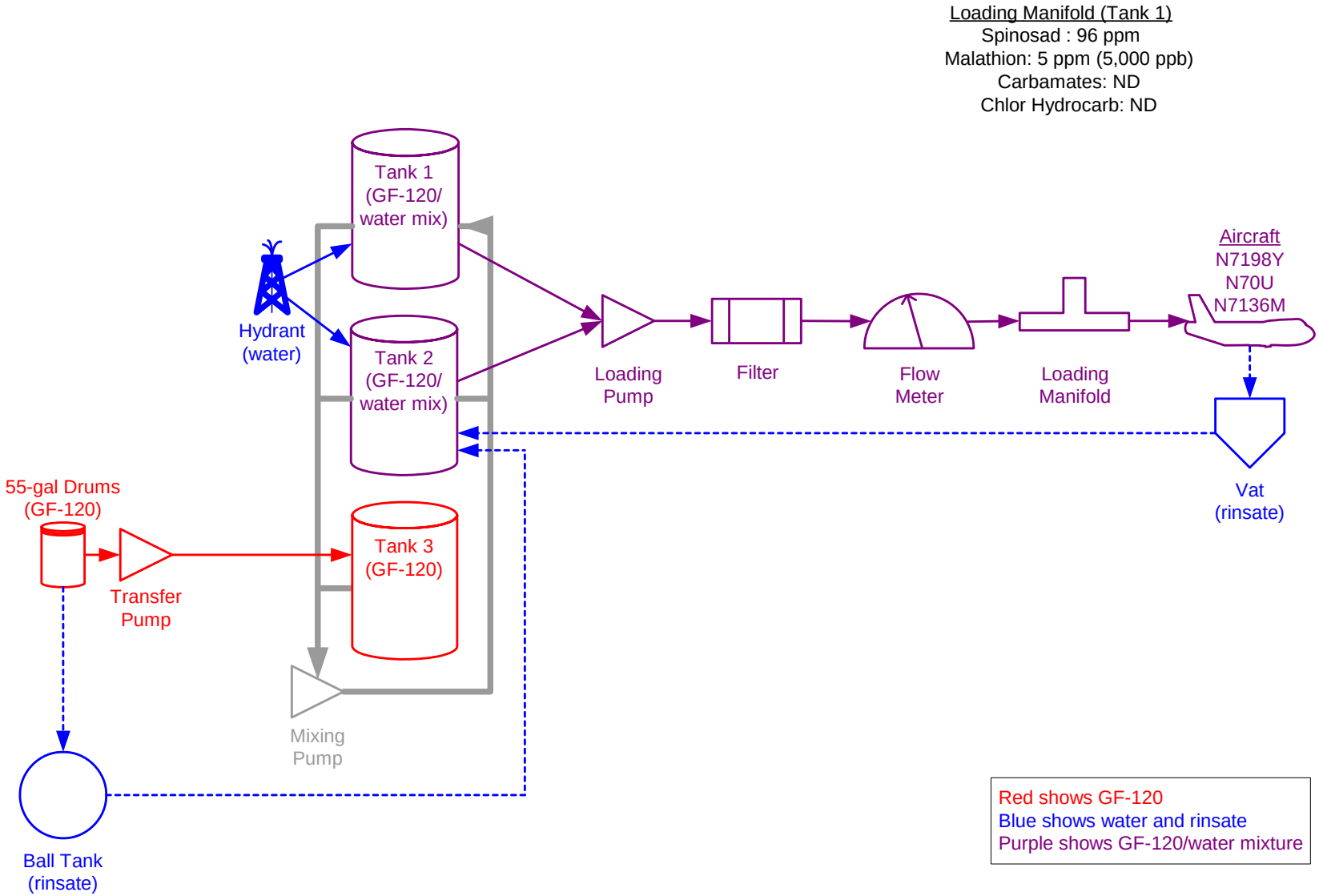


Figure 4
Mexican Fruit Fly Spinosad Mix/Load System
Monitoring Results During Application 4 (2/18/03)

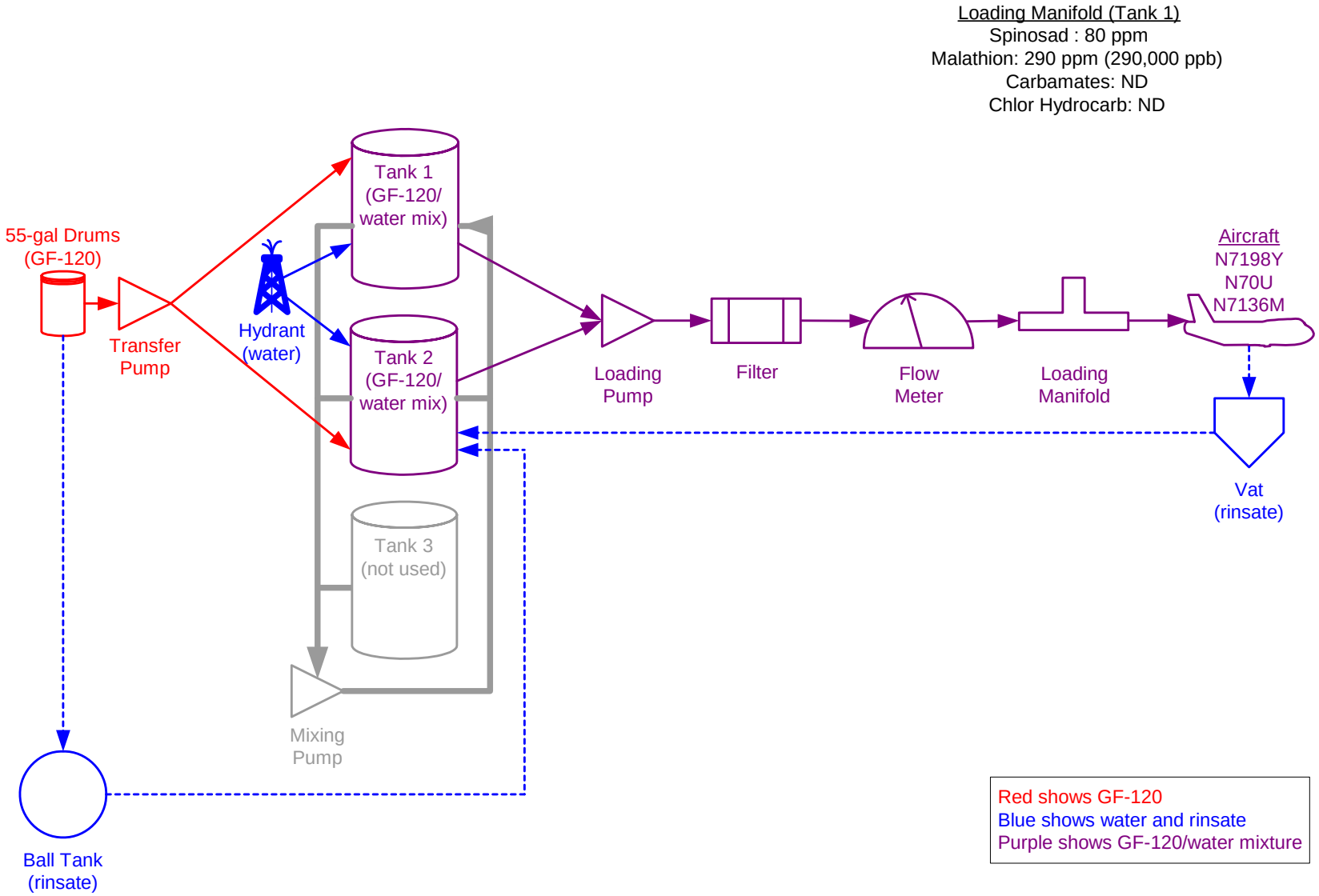


Figure 5
Mexican Fruit Fly Spinosad Mix/Load System
Malathion Monitoring Results After Application 4; Prior to Decontamination (2/27/03 - 3/1/03)

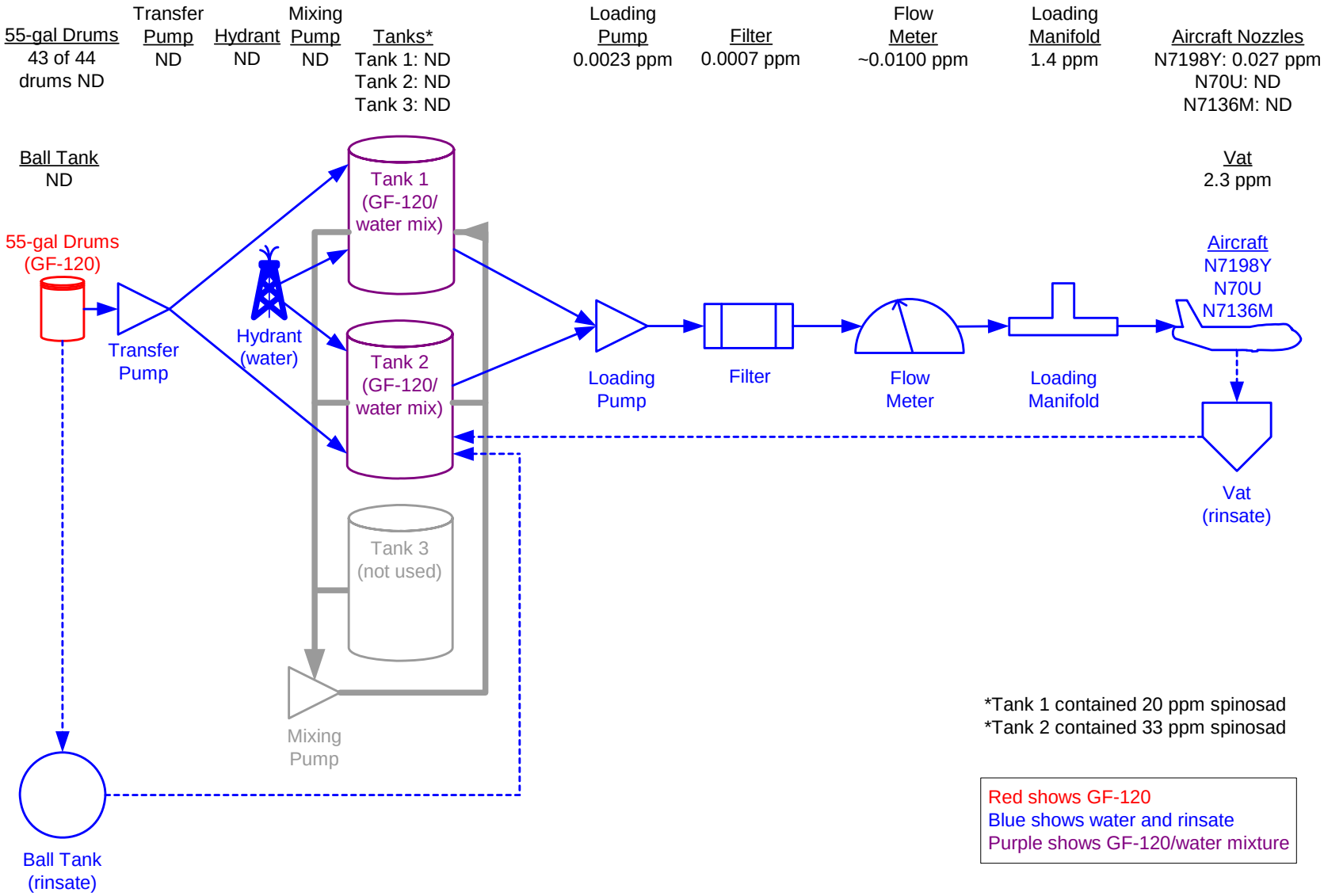


Figure 6
Mexican Fruit Fly Spinosad Mix/Load System
Malathion Monitoring Results After Decontamination (3/1/03)

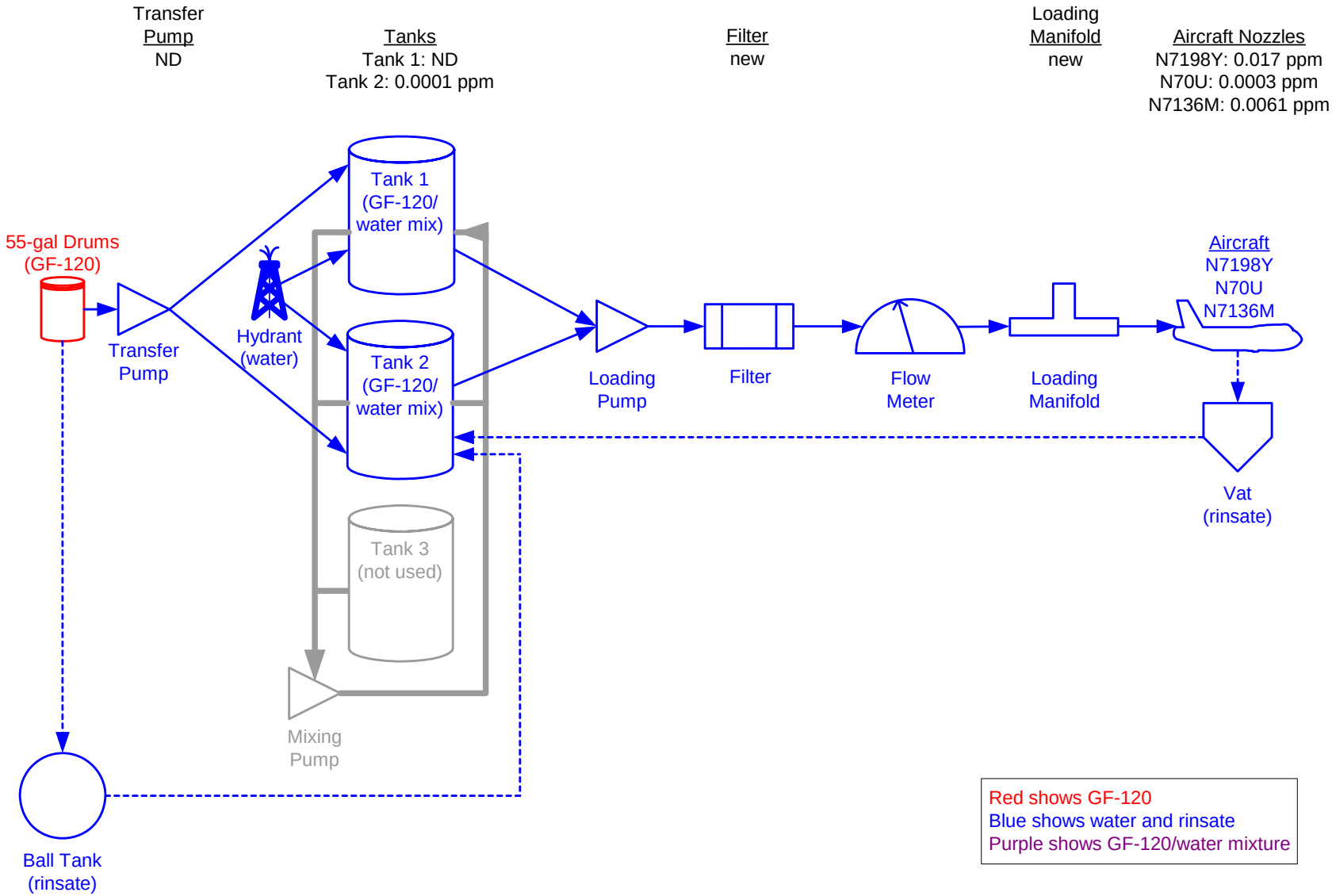


Figure 7
Mexican Fruit Fly Spinosad Mix/Load System
Monitoring Results for Organophosphates, Carbamates, and Chlorinated Hydrocarbons
Prior to Application 5 (2/28/03)

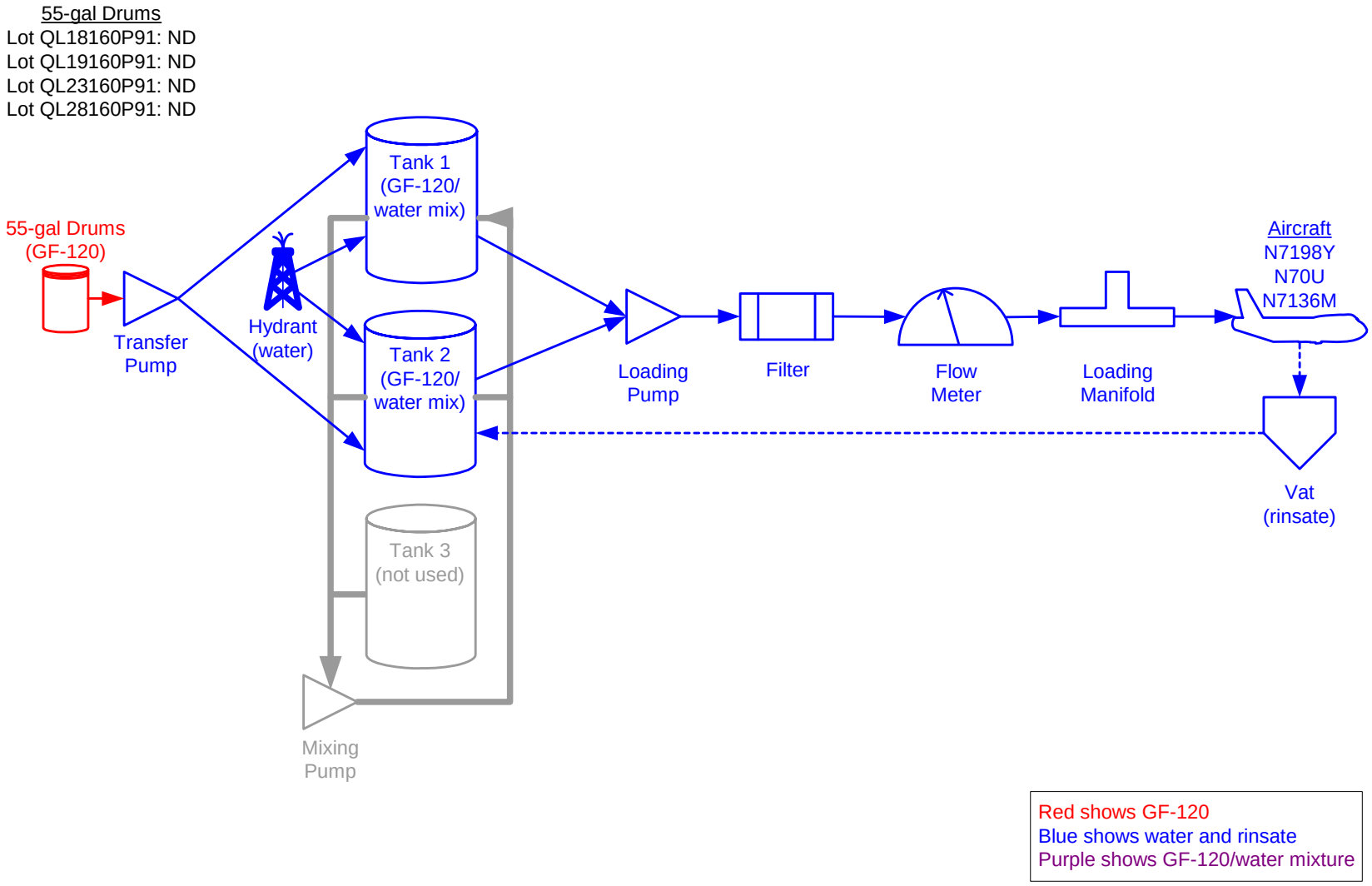


Figure 8
Mexican Fruit Fly Spinosad Mix/Load System
Monitoring Results for Spinosad During Application 5 (3/5/03)

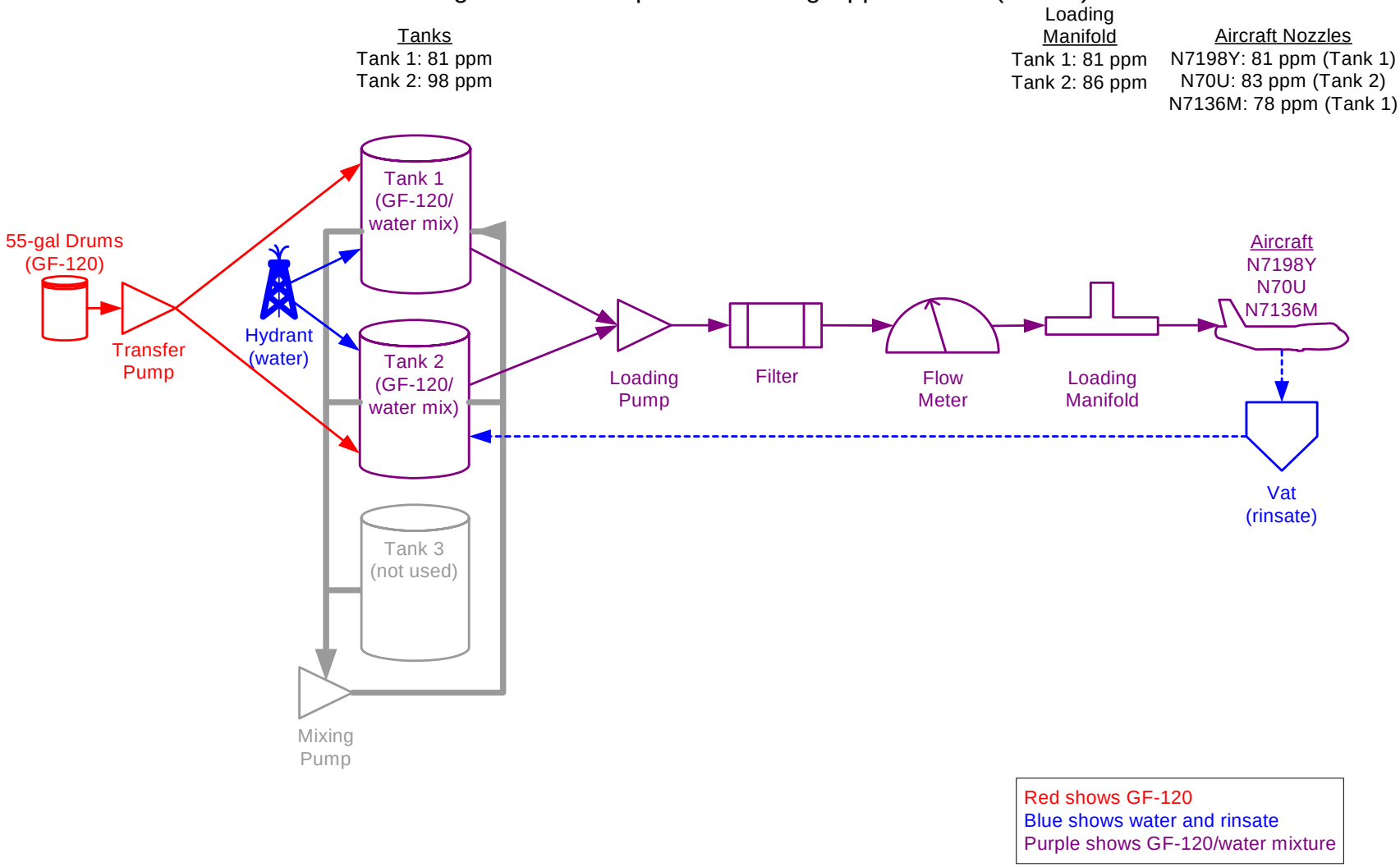
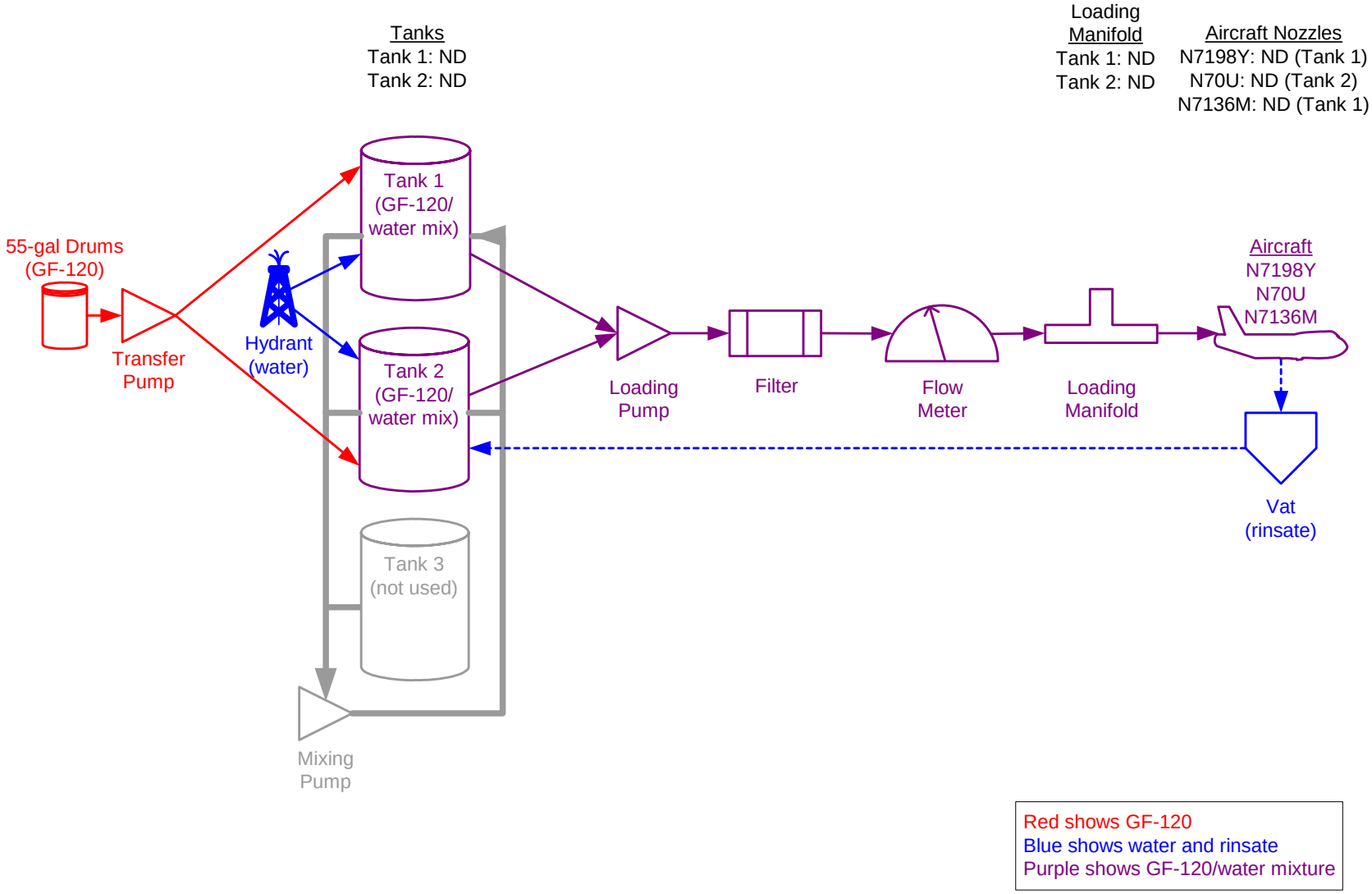


Figure 9
Mexican Fruit Fly Spinosad Mix/Load System
Monitoring Results for Organophosphates, Carbamates, and Chlorinated Hydrocarbons During Application 5 (3/5/03)



APPENDIX II

QUALITY CONTROL DATA REPORT FOR MEXFLY STUDY #216

Method Detection Limit (MDL) Determination, Method Validation, Stability Testing, Continuing Quality Control (QC), and Blind Spike Data

The analytical methods for the Mexican Fruit Fly Eradication Project were developed and validated according to EM SOP QAQC001.00. Since this was an emergency project method development and validation had to be conducted concurrently with sample collection and analysis. This delayed adjustments and adaptation to the analytical methods, sampling methods, selection of sample containers, and handling sample transportation until some field samples were collected and analyzed. Once the need for adjustment was discovered, changes were promptly made.

In this data report, upper and lower control limits and warning limits for QC were determined by adding or subtracting 2X and 3X standard deviations (SD) from the average percent recovery of the method validation. The procedures and definitions for the EM QC program are listed in SOP QAQC001.00.

MDL Determination and Method Validation

Mass Deposition Sheets (MDS)

The MDL was determined by spiking seven MDS with 0.2 µg/MDS of spinosyn A, B, and D. Table 1 shows the results of the recoveries. The MDLs for spinosyn A, B, and D were 0.077, 0.0286 and 0.205 µg/MDS, respectively. The reporting limit (RL) was set at 0.1µg/MDS for each spinosyn.

The MDS method was validated by spiking five individual MDS with each spinosyn at five different levels ranging from 0.3 µg to 30.0 µg (Table 2). This procedure was repeated three times. The average recoveries for Spinosyn A, B, and D were 97.6%, 94.7%, and 99.8%, respectively. The SD for all three spinosyns were 6.70 % or less (Table 2).

Surface Water

The MDL was determined by spiking seven 1-liter water samples with 0.05 ppb spinosyn A, B, and D. Table 3 shows the results of the recoveries. The MDL was 0.025 ppb and the RL was set at 0.05 ppb for the three individual spinosyns.

The surface water method was validated by spiking five 1-liter amber bottles of water with each spinosyn at five different levels ranging from 0.20 ppb to 30 ppb (Table 4). This procedure was repeated three times and the average recoveries were 85.7%, 82.8 %, and 87.3% for spinosyn A, B, and D, respectively. The SD was 13.5% for spinosyn A, 12.9% for B, and 12.7% for D.

Ambient Air

The MDL for air samples was determined by spiking seven OSV XAD resin tubes with 0.05 µg/tube of spinosyn A, B, and D. Air was pumped through for a short time and the tubes were extracted and analyzed. Table 5 shows the results of the recoveries. The MDLs were 0.119, 0.134, and 0.148 µg /tube for spinosyns A, B, and D, respectively, and the RL was set at 0.5 µg/tube for each spinosyn.

The air method was initially validated using an acetonitrile extraction and it was not acceptable due to data variability. The method validation was conducted again using a methylene chloride extraction. Four resin tubes were spiked with spinosyns at four different levels, ranging from 1.0 µg/tube to 50 µg/tube (Table 6). This procedure was repeated three times. The average recoveries were 96.4%, 102%, and 92.8 % with SD of 12.0%, 14.3%, and 12.5% for Spinosyn A, B, and D, respectively.

Grapefruit

Grapefruit was analyzed two different ways. One method was originally developed to analyze whole grapefruit. However, the RL for this method was not low enough to detect spinosad at the application rate in the treatment area. Subsequently, another method was developed to include analyzing a composite rinse of grapefruit surface and a rinse of inner surface of the sample container. The rinsing method improved the RL by 300 times (Lee, draft CDFA method). As a consequence, two sets of MDL determination and method validation were conducted for the whole grapefruit and the rinsing solution (Tables 7 through 10). The grapefruit used in the method development was purchased from various grocery stores.

MDL for the whole grapefruit was determined by spiking seven subsamples of ground whole grapefruit with 2.5 ppb of each spinosyn. The resulting MDLs were 0.903 ppb, 0.959 ppb, and 0.719 ppb for spinosyn A, B, and D, respectively (Table 7). The RL was set at 1.0 ppb for all three spinosyns. The method validation consisted of three sets of samples (Table 8). For each set, the whole grapefruit samples were spiked with each spinosyn at seven levels ranging from 1.5 to 400 ppb. The recoveries averaged 88.8% with a SD of 9.56% for spinosyn A, 80.4% with a SD of 7.15% for spinosyn B, and 91.7% with a SD of 8.22% for spinosyn D.

To create rinsing solution for method development, four grapefruits were rinsed with methanol. MDL was determined by spiking seven aliquots of the rinsing solution with 7.5 ng of each spinosyn. The results are in the Table 9. The MDLs were 2.83 ng, 2.03 ng, and 3.02 ng per sample for spinosyn A, B, and D, respectively. The RL was set at 5.0 ng/sample for all three spinosyns.

The first validation for the grapefruit rinsing method (rep 1) was difficult due to the matrix effects of wax, resin and polymers applied to grapefruit sold at grocery stores. This problem was not noticed during MDL determination. Therefore, further validation (reps 2-4) was performed using organic grapefruit with no coatings. The rinse solution was spiked with each spinosyn at three different levels, 10, 50, and 200 ng/sample. The average recoveries were 103%, 86.6%, and 95.8% with SD of 5.27%, 14.9%, and 6.17% for spinosyn A, B, and D, respectively (Table 10).

Storage Stability

Storage stability tests were conducted on all matrices to determine if there would be any dissipation of spinosyns when samples were stored. All tests were conducted by CDFA Center for Analytical Chemistry under similar conditions as the field samples were stored. Usually a QC blank and a QC spike were created and analyzed on the day the storage sample was removed

and tested. All blanks were none detected and the QC spikes are shown on the Tables 11 through 16.

MDS

Storage stability was tested by placing fifteen replicates of MDS spiked with 1 µg /MDS of spinosyn A, B, and D in a freezer. Three replicates were removed from the freezer for analysis on days 0, 3, 7, 14, and 21. A QC sample was tested on day 3. The study results indicated no apparent dissipation of any spinosyn (Table 11). All field MDS samples were analyzed within 20 days after samples were collected.

Surface Water

Storage stability for water was tested twice concurrently with field sampling. The first storage study began on January 29, 2003. Twenty-four QEC jars filled half-full of American River water were spiked with 0.20 ppb of each spinosyn and refrigerated at 4 °C. Three replicates were taken from the refrigerator and extracted on days 0, 1, 2, 4, 7, 15, 21, and 30. It was found that the spinosyns were not stable under these conditions (Table 12).

Another stability test began on March 18, 2003. Eighteen QEC jars half-full with river water, nine spiked with 0.20 ppb of spinosyns A and D, and the other nine spiked with spinosyn B, were frozen. Fifteen additional jars spiked with 0.20 ppb spinosyn B were refrigerated. The frozen samples were extracted on days 4, 11,and 21 and the refrigerated were extracted on days 2, 4, 7, 14, and 28. The samples extracted on day 28 were contaminated and all other reported recoveries were less than 60% (Tables 13).

A regression analysis indicated a significant linear reduction of the recovery mean with the storage days for all spinosyns at P = 0.00326, 0.0165, and 0.00243 for spinosyn A, B, and D, respectively (Table 14). The slope indicated a reduction rate of approximately 1.86%, 1.34%, and 1.99% per day for spinosyn A, B, and D, respectively.

Since spinosyns were not stable under both storage stability studies, two field spikes were prepared concurrently at the lab timed with the collection of two replicate field samples. One of the pair was frozen immediately and the other kept at 4 °C. Both sets of samples and spikes were transported, extracted directly from the jars, and analyzed as soon as possible.

Ambient Air

Air sample stability was determined by spiking 5.0 µg of spinosyn A, B, and D directly into 15 OSV XAD resin tubes, and placing the tubes in a freezer. Three replicate resin tubes were taken from the freezer and analyzed on days 0, 1, 8, 15 and 22. The results showed no apparent dissipation of any of the three spinosyns in resin tubes when kept frozen (Table 15). All air samples were extracted within 6 days after samples were collected.

Grapefruit

Stability tests were conducted on frozen grapefruit matrix. Twenty-one replicate grapefruit samples were spiked with 100 ppb spinosyn A, B, and D. Three replicates were removed from the freezer and extracted on days 0, 1, 5, 7, 11, 14, and 21. Residues of the spinosyns A and D decreased slowly, with some variation, over time. Spinosyn B decreased at a slightly more rapid

rate (Table 16). Data showed that within 5-14 days average recoveries of spinosyn A, B, and D were 87.3%-66.4%, 75.5%-65.8% and 84.3%-80.9%, respectively. All fruit samples were extracted in 5-14 days after samples were collected.

Trapping Efficiency for Air Sampling

To determine if the SKC OSV XAD resin tube can trap and hold the spinosyns, trapping efficiency was tested according to SOP FSAI003.00. Three replicate tubes were directly spiked with spinosyns A and D at 50 µg/tube. Spinosyn B was spiked into other three tubes. Air was pumped through for 24 hours at 3 liters/minute. Results showed poor recoveries with an average of 28.3%, and 5.30% for spinosyn A and D, plus 3.70% conversion to metabolite spinosyn B. The average recovery for spiked spinosyn B was 40.4%.

The trapping efficiency was attempted again using the same sampling method by spiking only spinosyn A and D into 3 tubes since spinosyn B is not in the formulation of the chemical spinosad. The laboratory separated each part of the OSV tube, filter, front resin bed, front foam, rear resin bed, and rear foam, and analyzed each part separately. The results were not improved much with average recoveries of 39.7% and 25.9% for spinosyn A and D, plus 5.71% conversion to metabolite spinosyn B (Table 17). In two of the replicates, the majority, 80.0% in average, spinosyns were trapped in the front resin bed. Approximately 8.80% was found in the filter and 10.5% in the front foam. In the third replicate, very little (7.16%) was recovered. For the best recovery achievable, it is recommended to analyze the whole tube including all parts in future studies.

Continuing QC and Blind Spikes

The Continuing QC program is documented in SOP QAQC001.00. CDFA laboratory conducts continuing QC samples with every set of field samples. Continuing QC samples generally consist of a blank matrix and a matrix spiked with the analyte. All continuing QC samples are spiked the day the field samples are to be extracted. The results of all continuing QC with blank matrices for this study were none detected.

A blind spike is a matrix sample spiked with analyte by a chemist other than the chemist extracting and analyzing that matrix. The blind spike is then given to field staff to relabel and disguise as a field sample. Blind spike samples were prepared and submitted for water and air analyses in this study. In general, the blind spike samples are made in advance of field sampling, however once the results of the stability study indicated problems for spinosad in water, the water blind spikes were prepared on the same day as the water samples were taken in the field.

MDS

Twenty-eight continuing QC samples for MDS sample analysis were spiked with spinosyn A, D, and B at 0.30 µg/MDS. Average recoveries were 98.8%, 95.3% and 100% for spinosyn A, B, and D, respectively. SDs were under 7.36% for all spinosyns. Only two spinosyn D spikes were beyond control limits (Table 18).

Surface Water

Twenty-one continuing QC samples for surface water were spiked with 0.20 ppb of the three spinosyns. All results, except for one, were within control limits (Table 19). Average recoveries were 89.9%, 79.0% and 87.1% with SD of 14.0%, 10.7% and 9.11% for spinosyns A, B, and D, respectively.

Five sets of duplicate blind spikes for surface water were spiked with 0.25 to 2.5 ppb spinosyns A and D. Their recoveries were generally lower than continuing QC spikes. The average recoveries of spinosyn A and D were 62.0% and 54.1% with SD of 7.61% and 7.40%. Out of ten blind spikes, recoveries of five for spinosyn A and of nine for spinosyn D were beyond warning limits (Table 20). In addition, analyses showed conversion of spinosyn A to spinosyn B in four of ten spikes. The four spikes were the ones spiked at the highest levels of spinosyn A, either 2.0 or 2.5 ppb. The conversion ranged from 2.50% to 4.64% (Table 20).

The blind spikes were extracted 1 to 18 days after spiking. Initially they were spiked prior to field water sampling, sent to and carried with the field sampling team, and then resubmitted to the lab with the field water samples. After the stability problems were discovered, DPR expedited the field sample delivery to the CDFA Lab for prompt extraction. However, the results were still not satisfactory. Considering the stability was a major issue for water samples, we decided to have the lab spike the blind spikes on the day when the field samples were collected. When the field samples arrived at the laboratory, the field staff then relabeled the blind spikes and submitted them back to the lab blindly with the regular field water samples. Days to extraction were reduced to 1 or 2 days on the last two sets of blind spikes. The results of a regression analysis of the recovery mean with the days from spiking to extraction for spinosyn A and D were different from those of the storage stability study. The regression was significant with P=0.0120 for spinosyn A, but not for spinosyn D, P=0.252 (Table 21). The regression slope for spinosyn A was 0.727% per day, less than half of that (1.86%/day) in storage stability study.

Ambient Air

Ten continuing QC samples for air sample analysis were spiked with 1.0 µg/tube spinosyn A, B and D just prior to extraction. Average recoveries of spinosyn A, B, and D were 82.2%, 94.6%, and 84.2% with SD of 8.97%, 8.58%, and 11.5%, respectively. There were only two incidences beyond the warning limit of 120% for spinosyn A and one for spinosyn D beyond the warning limit of 118% (Table 22).

Six blind field spikes for air samples were spiked by the lab with 2.5-5.0 µg/tube spinosyn A and D, transported to the field, placed on an air pump during field sampling by EM Staff, then disguised as regular field air samples and submitted back to the lab for analysis. Average recoveries for spinosyn A and D were 84.4% and 83.1%. However, the recoveries ranged from 32.2% to 133% with SD 33.6% for spinosyn A and 30.8% to 126% with SD 33.4% for spinosyn D (Table 23). Due to time and other limitations there was no opportunity to improve the recovery and reproducibility by optimizing either sampling or analytical methods.

Grapefruit

Six continuing QC samples for the whole grapefruit were made by spiking an aliquots of grounded whole grapefruit at 3.0 ppb spinosyn A, B, and D. Average recoveries were 90.3%, 76.8% and 90.0% with SD of 9.83%, 11.8%, and 9.58% for spinosyn A, B, and D, respectively. Only two samples were below warning limits of spinosyn B (Table 24).

Three continuing QC samples for grapefruit and container rinse were made by spiking aliquots of methanol rinse of clean grapefruit and containers with 30 ng/sample of spinosyn A, B, and D. Average recoveries were 70.7%, 70.7%, and 68.6% with SD of 6.98%, 18.2%, and 4.44% for spinosyn A, B, and D, respectively. The recoveries of all three samples were beyond lower control limits of spinosyn A and D; and one sample was lower than the lower warning limit of spinosyn B (Table 25). Because of a time constraint to develop a new lab method for the fruit rinse samples, the method was not fully validated until the field and QC samples had been analyzed. The low recoveries might be due to the matrix effects of wax, resin and polymers applied to the grapefruit purchased from grocery stores and used for QC samples.

Continuing QC for Malathion, Piperonyl butoxide (PBO)and D-phenothrin Analysis

Three sets of duplicate continuing QC samples were spiked with 0.1 ppb malathion for analysis of water rinsate samples collected during investigation of malathion contamination and decontamination. All malathion QC samples were within the established control limits (Table 26) with average recovery 101% and SD 12.4%.

MDS and water samples were collected in the treatment area and analyzed for malathion, PBO, and D-phenothrin during the malathion contamination investigation. Duplicate continuing QC samples were spiked with 2.0 µg each of the three analytes on MDS or 0.2 ppb in water. The average recoveries of malathion, PBO, and D-phenothrin were, respectively, 98.8%, 103%, and 95.0% for MDS and 100%, 95.0%, and 92.5% for water samples (Table 27). Malathion QC samples were within the control limits. The control limits were not established for PBO or D-phenothrin since the samples were analyzed for an impromptu contingency of contamination.

References

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Table 1. Method detection limit (MDL) determination for spinosad in mass deposition sheets*

Spike Number	Spike Level	Recovery					
		Spinosyn A		Spinosyn B		Spinosyn D	
	µg/MDS	µg/MDS	%	µg/MDS	%	µg/MDS	%
1	0.2	0.190	95.0	0.157	78.5	0.203	101.5
2	0.2	0.193	96.5	0.162	81.0	0.201	100.5
3	0.2	0.190	95.0	0.164	82.0	0.202	101.0
4	0.2	0.188	94.0	0.158	79.0	0.197	98.5
5	0.2	0.189	94.5	0.167	83.5	0.185	92.5
6	0.2	0.185	92.5	0.181	90.5	0.198	99.0
7	0.2	0.187	93.5	0.176	88.0	0.192	96.0
Mean % recovery			**94.4**		**83.2**		**98.4**
Standard Deviation			**1.27**		**4.52**		**3.21**

*Report limit was 0.1 µg/MDS

Table 2. Method validation for spinosad in mass deposition sheets*

Spinosyn	Spike Level	Recovery (%)					QC Limits Settings** (%)			
		Replicate			Mean	SD	UCL	UWL	LWL	LCL
	µg/MDS	1	2	3						
A	0.3	95.3	94.3	94.0	94.5	0.681	116	110	85.4	79.3
	1.0	94.2	97.8	82.8	91.6	7.83				
	3.0	99.9	101	101	101	0.651				
	10.0	97.2	103	94.0	98.2	4.72				
	30.0	96.1	110	104	103	6.86				
		Average			**97.6**	**6.10**				
B	0.3	90.7	96.7	89.3	92.2	3.93	115	108	81.2	74.5
	1.0	85.0	102.0	81.2	89.4	11.1				
	3.0	91.5	97.5	102.5	97.2	5.51				
	10.0	90.6	99.3	92.0	94.0	4.67				
	30.0	98.2	105	98.7	101	3.56				
		Average			**94.7**	**6.70**				
D	0.3	100	99.0	104	101	2.59	113	109	81.2	86.5
	1.0	96.8	98.6	88.7	94.7	5.27				
	3.0	97.9	102	102	101	2.57				
	10.0	100	106	94.0	99.9	5.75				
	30.0	99.8	102	106	102	2.93				
		Average			**99.8**	**4.42**				

*All matrix blanks were none detected.
** UCL - upper control limit, UWL - upper warning limit,
LWL - lower warning limit, LCL - lower control limit

Table 3. Method detection limit (MDL) determination for spinosad in water*

Spike Sample Number	Spike Level ppb	Recovery Spinosyn A ppb	Recovery Spinosyn A %	Recovery Spinosyn B ppb	Recovery Spinosyn B %	Recovery Spinosyn D ppb	Recovery Spinosyn D %
1	0.05	0.041	82.0	0.045	90.0	0.044	88.0
2	0.05	0.041	82.0	0.038	76.0	0.045	90.0
3	0.05	0.040	80.0	0.034	68.0	0.040	80.0
4	0.05	0.036	72.0	0.037	74.0	0.045	90.0
5	0.05	0.043	86.0	0.040	80.0	0.045	90.0
6	0.05	0.042	84.0	0.038	76.0	0.047	94.0
7	0.05	0.032	64.0	0.035	70.0	0.037	74.0
Average			**78.6**		**76.3**		**86.6**
Standard Deviation			**7.81**		**7.25**		**7.00**

*Report limit was 0.05 ppb

Table 4. Method validation for spinosad in surface water*

Spinosyn	Spike Level ppb	Recovery (%) Replicate 1	Replicate 2	Replicate 3	Mean	SD	QC Limits Settings** (%) UCL	UWL	LWL	LCL
A	0.2	78.5	90.9	124.0	97.8	23.5	126	113	58.8	45.3
	2.0	81.7	78.8	93.1	84.5	7.56				
	5.0	77.9	77.6	65.2	73.6	7.25				
	10.0	87.1	82.6	90.5	86.7	3.96				
	30.0	72.4	89.9	95.8	86.0	12.2				
		Average			**85.7**	**13.5**				
B	0.2	75.0	85.5	119.5	93.3	23.26	122	109	56.9	43.9
	2.0	82.4	73.3	76.0	77.2	4.67				
	5.0	89.0	71.5	62.7	74.4	13.39				
	10.0	74.6	88.0	86.2	82.9	7.27				
	30.0	78.6	91.3	88.3	86.1	6.64				
		Average			**82.8**	**12.9**				
D	0.2	81.5	94.5	117	97.5	17.7	125	113	61.8	49.1
	2.0	85.1	93.8	88.1	89.0	4.42				
	5.0	81.1	79.6	70.2	77.0	5.91				
	10.0	77.6	92.0	89.8	86.5	7.76				
	30.0	66.9	86.5	106	86.5	19.6				
		Average			**87.3**	**12.7**				

*All matrix blanks were none detected.
**UCL - upper control limit, UWL - upper warning limit,
LWL - lower warning limit, LCL - lower control limit

Table 5. Method detection limit (MDL) determination for spinosad in air*

Spike Sample Number	Spike Level µg/tube	Recovery Spinosyn A µg/tube	%	Spinosyn B µg/tube	%	Spinosyn D µg/tube	%
1	0.5	0.384	76.8	0.372	74.4	0.391	78.2
2	0.5	0.392	78.4	0.397	79.4	0.367	73.4
3	0.5	0.479	95.8	0.458	91.6	0.452	90.4
4	0.5	0.449	89.8	0.468	93.6	0.474	94.8
5	0.5	0.455	91.0	0.396	79.2	0.387	77.4
6	0.5	0.476	95.2	0.480	96.0	0.490	98.0
7	0.5	0.426	85.2	0.402	80.4	0.427	85.4
Average			**87.5**		**84.9**		**85.4**
Standard Deviation			**7.62**		**8.53**		**9.42**

*Extracted with acetonitrile. Report limit was 0.5 µg/tube

Table 6. Method validation for spinosad in air sampling tube*

Spinosyn	Spike Level µg/tube	Recovery (%) Replicate 1	2	3	Mean	SD	QC Limits Settings** (%) UCL	UWL	LWL	LCL
A	1.0	96.0	103	102	100	3.61	132	120	72.5	60.6
	5.0	92.2	100	109	100	8.49				
	20	119	79.0	78.0	92.1	23.5				
	50	98.9	84.5	95.5	93.0	7.50				
		Average			**96.4**	**12.0**				
B	1.0	109	111	124	115	8.21	145	131	73.4	59.1
	5.0	108	7605	113	99.0	19.6				
	20	110	82.3	85.0	92.3	15.1				
	50	108	93.9	106	102	7.44				
		Average			**102**	**14.3**				
D	1.0	101	103	112	105	6.10	130	118	67.7	55.1
	5.0	82.6	71.9	106	86.7	17.3				
	20	99.2	74.4	85.6	86.4	12.4				
	50	93.4	87.5	96.6	92.5	4.65				
		Average			**92.8**	**12.5**				

*Extracted with methylene chloride. All matrix blanks were none detected.
**UCL - upper control limit, UWL - upper warning limit,
LWL - lower warning limit, LCL - lower control limit

Table 7. Method detection limit (MDL) determination for spinosad in whole grapefruit*

Spike Sample Number	Spike Level ppb	Recovery Spinosyn A ppb	Spinosyn A %	Spinosyn B ppb	Spinosyn B %	Spinosyn D ppb	Spinosyn D %
1	2.5	2.23	89.2	2.28	91.2	1.96	78.4
2	2.5	1.76	70.4	1.55	62.0	1.81	72.4
3	2.5	1.50	60.0	1.41	56.4	1.44	57.6
4	2.5	2.13	85.2	1.97	78.8	1.68	67.2
5	2.5	2.29	91.6	2.10	84.0	2.08	83.2
6	2.5	2.05	82.0	1.96	78.4	1.97	78.8
7	2.5	2.19	87.6	1.95	78.0	2.03	81.2
Average			**80.9**		**75.5**		**74.1**
Standard Deviation			**11.5**		**12.2**		**9.10**

*Report limit was 1.0 ppb

Table 8. Method validation for spinosad in whole grapefruit*

Spinosyn	Spike Level ppb	Recovery (%) Replicate 1	2	3	Mean	SD	QC Limits Settings** (%) UCL	UWL	LWL	LCL
A	1.5	106	81.0	99.1	95.5	13.1	117	108	69.7	60.1
	2.5	118	91.0	89.8	99.7	16.2				
	10.0	84.3	91.5	90.9	88.9	3.99				
	50.0	86.3	76.5	85.2	82.8	5.54				
	100	83.1	79.5	91.3	84.6	6.05				
	200	82.8	84.1	81.7	82.9	1.20				
	400	90.7	87.1	83.6	87.1	3.55				
		Average			**88.8**	**9.56**				
B	1.5	87.3	79.1	101	89.0	10.9	102	95.0	66.1	59.0
	2.5	92.9	83.7	79.2	85.3	6.98				
	10.0	78.8	73.1	78.8	76.9	3.26				
	50.0	81.4	67.7	80.9	76.7	7.77				
	100	80.8	72.8	86.2	79.9	6.74				
	200	76.8	73.6	77.8	76.1	2.19				
	400	80.6	76.3	80.7	79.2	2.51				
		Average			**80.4**	**7.15**				
D	1.5	104	93.9	110	102	8.10	116	108	75.3	67.0
	2.5	107	97.5	89.3	98.0	9.01				
	10.0	84.5	96.6	93.7	91.6	6.32				
	50.0	92.6	85.0	88.7	88.8	3.80				
	100	85.7	80.4	94.6	86.9	7.18				
	200	83.6	90.8	80.3	84.9	5.37				
	400	96.1	87.0	84.9	89.3	5.95				
		Average			**91.7**	**8.22**				

*All matrix blanks were none detected.
** UCL - upper control limit, UWL - upper warning limit,
LWL - lower warning limit, LCL - lower control limit

Table 9. Method detection limit (MDL) determination for spinosad in rinse of grapefruit and containers*

Spike Sample Number	Spike Level ng	Recovery Spinosyn A ng	Spinosyn A %	Spinosyn B ng	Spinosyn B %	Spinosyn D ng	Spinosyn D %
1	7.5	6.57	87.6	4.06	54.1	6.01	80.1
2	7.5	5.40	72.0	4.18	55.7	4.91	65.5
3	7.5	5.74	76.5	3.61	48.1	6.15	82.0
4	7.5	4.69	62.5	4.73	63.1	4.16	55.5
5	7.5	5.21	69.5	4.82	64.3	5.43	72.4
6	7.5	4.98	66.4	5.59	74.5	6.48	86.4
7	7.5	3.67	48.9	4.19	55.9	4.10	54.7
Average			**69.1**		**59.4**		**70.9**
Standard Deviation			**12.0**		**8.64**		**12.8**

*Report limit was 5.0 ng

Table 10. Method validation for spinosad in rinse of grapefruit and containers*

Spinosyn	Spike Level ng	Recovery (%) Replicate 1	2	3	4	Mean	SD	QC Limits Settings** (%) UCL	UWL	LWL	LCL
A	10.0	105	101	106	98.4	103	3.55	119	113	92.3	87.0
	50.0	106	99.9	108	111	106	4.54				
	200	108	97.3	92.2	103	100	6.66				
		Average				**103**	**5.27**				
B	10.0	112	95.8	88.8	76.2	93.2	14.9	131	116	56.9	42.0
	50.0	103	65.4	84.4	94.8	86.8	16.1				
	200	96.3	64.5	71.6	86.8	79.8	14.4				
		Average				**86.6**	**14.9**				
D	10.0	79.7	105	95.6	93.5	93.3	10.3	114	108	83.5	77.3
	50.0	97.6	97.0	98.1	99.4	98.0	10.2				
	200	101	96.1	90.5	96.9	96.1	4.28				
		Average				**95.8**	**6.17**				

*All matrix blanks were none detected.
**UCL - upper control limit, UWL - upper warning limit,
LWL - lower warning limit, LCL - lower control limit

Table 11. Results of the storage stability study for mass deposition sheets

Spinosyn	Days	Recovery (%) Replicate* 1	2	3	Mean	Lab QC**
A	0	101	94.0	93.6	96.2	100
	3	99.8	98.5	98.3	98.9	
	7	88.4	100	84.1	90.8	
	14	93.1		109	101	
	21	99.8	103	104	102	
B	0	97.4	94.0	96.9	96.1	93.3
	3	97.6	97.7	95.2	96.8	
	7	86.6	94.7	75.8	85.7	
	14	85.2		102	93.6	
	21	87.1	90.8	96.7	91.5	
D	0	102	98.3	98.1	99.5	105
	3	102	102	95.5	99.7	
	7	91.8	97.1	88.3	92.4	
	14	82.6		100	91.3	
	21	97.3	98.0	96.8	97.4	

*Spike level was 1 µg/MDS and rep#2 for day 14 was lost during extraction.
**QC spike level was 0.30 µg/MDS and only conducted on day 3.

Table 12. Results of the first storage stability study for surface water (refrigerated)

Spinosyn	Days	Recovery* (%) Replicate 1	2	3	Mean	Lab QC
A	0	87.0	89.3	92.5	89.6	90.3
	1	97.3	83.8	94.8	92.0	103
	2	80.5	79.0	76.8	78.8	82.8
	4	57.3	64.8	60.8	61.0	87.3
	7	38.8	41.8	38.0	39.5	93.8
	15	32.5	31.3	29.5	31.1	91.5
	21	34.8	33.3	44.3	37.5	82.8
	30	28.0	35.3	34.8	32.7	85.3
B	0	84.8	79.3	90.8	85.0	89.5
	1	76.5	75.3	81.5	77.8	90.3
	2	99.3	106	93.3	99.6	99.3
	4	62.8	66.0	68.8	65.9	81.5
	7	57.3	58.0	51.0	55.4	86.8
	15	63.5	46.5	56.5	55.5	83.5
	21	59.5	44.3	51.0	51.6	67.3
	30	35.5	40.0	48.0	41.2	89.5
D	0	90.8	85.5	82.5	86.3	90.5
	1	85.3	81.0	87.5	84.6	95.8
	2	77.8	78.3	76.5	77.5	91.8
	4	60.0	59.3	60.0	59.8	86.5
	7	33.0	36.8	37.8	35.9	94.8
	15	26.8	26.5	22.3	25.2	89.0
	21	22.3	19.5	23.5	21.8	84.3
	30	27.0	28.0	31.3	28.8	93.8

*Spike level was 0.2 ppb of each spinosyn.

Table 13. Results of the second storage stability study for surface water

Spinosyn	Days	Recovery (%) Replicate* 1	2	3	Mean	Lab QC
			Frozen			
A	4	48.8	49.5	50.8	49.7	
	11	56.0	61.0	57.3	58.1	86.5
	21	27.8	37.3	27.0	30.7	80.5
B	4	40.5	42.0	52.5	45.0	
	11	32.3	39.0	48.8	40.0	88.8
	21	23.5	24.3	25.0	24.3	64.5
D	4	40.3	47.3	46.8	44.8	
	11	55.5	55.3	55.5	55.4	92.0
	21	23.8	36.3	24.5	28.2	96.0
			Refrigerated			
B	2	48.5	52.8	52.8	51.4	79.3
	4	61.5	62.8	49.8	58.0	80.8
	7	40.0	42.0	26.0	36.0	80.0
	14	41.8	54.0	41.8	45.9	81.3
	28		contaminated			

*Spike level was 0.2 ppb of each spinosyn. Spinosyn A and D were spiked together and B separately.

Table 14. Regression Analysis for surface water storage stability studies

Spinosyn	Multiple R	R Square	Adjusted R^2	Standard Error	Observations
			Regression Statistics		
A	0.798	0.636	0.596	14.8	11
B	0.607	0.368	0.319	16.3	15
D	0.812	0.659	0.621	15.0	11

ANOVA

		df	SS	MS	F	Significance F
A	**Regression**	1	3443	3443	15.8	0.00326
	Residual	9	1967	219		
	Total	10	5410			
B	**Regression**	1	2009	2009	7.57	0.0165
	Residual	13	3450	265		
	Total	14	5460			
D	**Regression**	1	3923	3923	17.4	0.00243
	Residual	9	2034	226		
	Total	10	5957			

		Coefficients	Std. error	t Stat	P-value	Lower 95%	Upper 95%
A	**Intercept**	74.3	6.66	11.1	0.00000144	59.2	89.3
	Slope	-1.86	0.470	-3.97	0.00326	-2.93	-0.802
B	**Intercept**	68.3	6.27	10.9	0.0000000661	54.8	81.8
	Slope	-1.34	0.488	-2.75	0.0165	-2.40	-0.288
D	**Intercept**	70.8	6.78	10.5	0.00000247	55.5	86.2
	Slope	-1.99	0.478	-4.17	0.00243	-3.07	-0.909

Table 15. Results of the storage stability study for air sampling tubes

Spinosyn	Days**	Recovery* (%) Replicate* 1	2	3	Mean	QC
A	0	98.4	89.9	95.3	94.5	78.9
	1	100	103	97.5	100	97.8
	8	129	112	144	128	121
	15	112	113	109	112	93.6
	22	120	134	131	128	121
B	0	112	96.8	82.4	97.2	78.9
	1	93.8	96.5	87.7	92.7	101
	8	105	98.0	119	107	128
	15	98.8	103	119	107	93.8
	22	105	135	115	118	128
D	0	114	98.2	102	105	77.5
	1	99.3	98.1	92.1	96.5	92.0
	8	106	105	128	113	119
	15	107	110	109	109	85.5
	22	112	132	119	121	119

*Spike level was 5.0 µg/tube.
**Day 8 was re-extracted due to water contamination during sonication

Table 16. Results of the storage stability study for grapefruit

Spinosyn	Days	Recovery (%) Replicate* 1	2	3	Mean	QC**
A	0	95.0	92.9	86.0	91.3	98.0
	1	83.9	76.6	88.6	83.0	93.0
	5	88.1	89.0	84.8	87.3	106
	7	78.1	76.0	77.8	77.3	100
	11	73.4	78.4	75.0	75.6	99.3
	14	65.5	62.5	71.3	66.4	96.0
	21	83.2	78.4	80.1	80.6	93.3
B	0	88.1	90.4	88.9	89.1	70.7
	1	86.0	82.4	90.6	86.3	63.7
	5	76.9	77.5	72.2	75.5	99.3
	7	72.3	68.8	69.4	70.2	88.3
	11	69.3	66.5	63.3	66.4	93.7
	14	67.8	60.0	69.5	65.8	77.7
	21	67.0	64.3	63.4	64.9	83.7
D	0	88.1	99.8	89.7	92.5	92.3
	1	85.1	87.4	86.4	86.3	110
	5	81.5	83.5	87.9	84.3	93.7
	7	81.3	80.9	86.0	82.7	105
	11	74.9	79.6	75.1	76.5	102
	14	82.5	72.7	87.6	80.9	93.3
	21	80.2	75.7	80.2	78.7	87.4

*Spike level was 100 ppb on fresh fruit basis.
**QC spike level was 3.0 ppb on fresh fruit basis.

Table 17. Trapping efficiency (2nd study) for spinosad in air sampling tube*

Sample	Run Time Minute	Spinosyn A Spike µg	Spinosyn A Recovery µg	Spinosyn A Recovery %	Spinosyn D Spike µg	Spinosyn D Recovery µg	Spinosyn D Recovery %	Conversion to spinosyn B Spike µg	Conversion to spinosyn B Recovery µg	Conversion to spinosyn B Recovery %
Blank	1439	0.0	ND**	ND	0.0	ND	ND	0.0	ND	ND
Rep 1	1438	50.0	30.3	60.6	50.0	18.6	37.1	0.0	4.2	8.24
Rep 2	1439	50.0	3.5	7.05	50.0	2.8	5.56	0.0	0.9	1.71
Rep 3	1440	50.0	25.7	51.4	50.0	17.6	35.1	0.0	3.6	7.17
Average				**39.7**			**25.9**			**5.71**
Standard deviation				**28.6**			**17.7**			**3.50**

*Air flow of all pumps were close to 3 liters/minute.
**None detected

Table 18. Continuing quality control for spinosyns in mass deposition samples*

Date	Spinosyn A Recovery µg	Spinosyn A Recovery %	Spinosyn A Exceed control limits	Spinosyn B Recovery µg	Spinosyn B Recovery %	Spinosyn B Exceed control limits	Spinosyn D Recovery µg	Spinosyn D Recovery %	Spinosyn D Exceed control limits
01/13/03	0.283	94.3	ok	0.272	90.7	ok	0.298	99.3	ok
01/14/03	0.275	91.7	ok	0.274	91.3	ok	0.278	92.7	ok
01/14/03	0.275	91.7	ok	0.280	93.3	ok	0.288	96.0	ok
01/15/03	0.306	102	ok	0.312	104	ok	0.314	105	ok
01/21/03	0.262	87.3	ok	0.249	83.0	ok	0.233	77.7	LCL
01/23/03	0.294	98.0	ok	0.301	100.3	ok	0.306	102	ok
01/24/03	0.291	97.0	ok	0.258	86.0	ok	0.319	106	ok
01/27/03	0.318	106	ok	0.292	97.3	ok	0.320	107	ok
01/28/03	0.301	100	ok	0.279	93.0	ok	0.326	109	UWL
02/06/03	0.291	97.0	ok	0.301	100	ok	0.322	107	ok
02/07/03	0.288	96.0	ok	0.286	95.3	ok	0.304	101	ok
02/07/03	0.299	99.7	ok	0.295	98.3	ok	0.314	105	ok
02/21/03	0.335	112	UWL	0.280	93.3	ok	0.339	113	UCL
02/21/03	0.306	102	ok	0.312	104	ok	0.306	102	ok
02/21/03	0.274	91.3	ok	0.254	84.7	ok	0.291	97.0	ok
03/07/03	0.315	105	ok	0.281	93.7	ok	0.328	109	UWL
03/10/03	0.296	98.7	ok	0.266	88.7	ok	0.279	93.0	ok
03/11/03	0.284	94.7	ok	0.282	94.0	ok	0.286	95.3	ok
03/21/03	0.317	106	ok	0.315	105	ok	0.315	105	ok
03/21/03	0.290	96.7	ok	0.284	94.7	ok	0.282	94.0	ok
04/14/03	0.297	99.0	ok	0.278	92.7	ok	0.294	98.0	ok
04/14/03	0.288	96.0	ok	0.290	96.7	ok	0.310	103	ok
04/14/03	0.315	105	ok	0.304	101	ok	0.286	95.3	ok
04/14/03	0.308	103	ok	0.271	90.3	ok	0.270	90.0	LWL
05/05/03	0.286	95.3	ok	0.302	101	ok	0.292	97.3	ok
05/05/03	0.311	104	ok	0.295	98.3	ok	0.290	96.7	ok
05/22/03	0.292	97.3	ok	0.289	96.3	ok	0.301	100	ok
05/22/03	0.306	102	ok	0.300	100	ok	0.327	109	ok
Average	**0.297**	**98.8**		**0.286**	**95.3**		**0.301**	**100**	
Standard deviation	**0.0161**	**5.38**		**0.0172**	**5.75**		**0.0221**	**7.36**	
UCL-Upper Control Limit			116			115			113
UWL-Upper Warning Limit			110			108			109
LWL-Lower Warning Limit			85.4			81.2			91.0
LCL-Lower Control Limit			79.3			74.5			86.5

*spike level was 0.3 µg of each spinosyn.

Table 19. Continuing quality control for spinosyns in surface water samples*

Date	Spinosyn A Recovery ppb	Spinosyn A Recovery %	Spinosyn A Exceed control limits	Spinosyn B Recovery ppb	Spinosyn B Recovery %	Spinosyn B Exceed control limits	Spinosyn D Recovery ppb	Spinosyn D Recovery %	Spinosyn D Exceed control limits
01/13/03	0.143	71.5	ok	0.148	74.0	ok	0.145	72.5	ok
01/23/03	0.180	90.0	ok	0.191	95.5	ok	0.191	95.5	ok
02/06/03	0.187	93.5	ok	0.159	79.5	ok	0.164	82.0	ok
02/13/03	0.180	90.0	ok	0.166	83.0	ok	0.206	103	ok
02/14/03	0.190	95.0	ok	0.166	83.0	ok	0.190	95.0	ok
02/14/03	0.185	92.5	ok	0.177	88.5	ok	0.173	86.5	ok
02/14/03	0.182	91.0	ok	0.146	73.0	ok	0.183	91.5	ok
02/14/03	0.172	86.0	ok	0.149	74.5	ok	0.162	81.0	ok
02/26/03	0.171	85.5	ok	0.179	89.5	ok	0.188	94.0	ok
03/12/03	0.175	87.5	ok	0.146	73.0	ok	0.174	87.0	ok
03/12/03	0.287	144	UCL	0.196	98.0	ok	0.208	104	ok
03/21/03	0.164	82.0	ok	0.160	80.0	ok	0.166	83.0	ok
03/21/03	0.183	91.5	ok	0.169	84.5	ok	0.173	86.5	ok
04/01/03	0.171	85.5	ok	0.171	85.5	ok	0.183	91.5	ok
04/04/03	0.161	80.5	ok	0.129	64.5	ok	0.156	78.0	ok
04/04/03	0.189	94.5	ok	0.161	80.5	ok	0.192	96.0	ok
04/23/03	0.178	89.0	ok	0.164	82.0	ok	0.175	87.5	ok
05/01/03	0.187	93.5	ok	0.148	74.0	ok	0.160	80.0	ok
05/12/03	0.159	79.5	ok	0.160	80.0	ok	0.167	83.5	ok
05/12/03	0.185	92.5	ok	0.134	67.0	ok	0.166	83.0	ok
06/02/03	0.145	72.5	ok	0.100	50.0	LWL	0.136	68.0	ok
Average	**0.180**	**89.9**		**0.158**	**79.0**		**0.174**	**87.1**	
Standard deviation	**0.0280**	**14.0**		**0.0214**	**10.7**		**0.0182**	**9.11**	
UCL-Upper Control Limit			126			122			125
UWL-Upper Warning Limit			113			109			113
LWL-Lower Warning Limit			58.8			56.9			61.8
LCL-Lower Control Limit			45.3			43.9			49.1

*spike level was 0.2 ppb of each spinosyn.

Table 20. Blind spike recovery for spinosad spiked in surface water

Sample number	Date		Days	Spinosyn	Spike level	Recovery		Exceed control limits*	Comments
	Spiked	Extracted	Spiked to extracted		ppb	ppb	%		
				A	0.25	0.146	58.4	LWL	Refrigerated
13				D	0.25	0.133	53.2	LWL	
	2/3	2/21	18	B**	0	Nd***	0		
				A	0.25	0.144	57.6	LWL	Frozen
14				D	0.25	0.128	51.2	LWL	
				B	0	nd	0		
				A	2.0	1.03	51.5	LWL	Refrigerated
132				D	2.0	0.91	45.5	LCL	
	2/27	3/12	13	B	0	0.09	4.50		
				A	2.0	1.17	58.5	LWL	Frozen
133				D	2.0	1.13	56.5	LWL	
				B	0	0.05	2.50		
				A	2.5	1.59	63.7	ok	Refrigerated
1105				D	2.5	1.36	54.3	LWL	
	4/3	4/9	6	B	0	0.116	4.64		
				A	2.5	1.29	51.7	LWL	Frozen
1106				D	2.5	1.14	45.5	LWL	
				B	0	0.074	2.96		
				A	0.35	0.247	70.6	ok	Frozen
633				D	0.45	0.251	55.8	LWL	
	4/29	5/1	2	B	0	nd	0.0		
				A	0.35	0.243	69.4	ok	Refrigerated
634				D	0.45	0.216	48.0	LCL	
				B	0	nd	0.0		
				A	0.35	0.231	66.0	ok	Frozen
430				D	0.25	0.153	61.2	LWL	
	5/20	5/21	1	B	0	nd	0.0		
				A	0.35	0.254	72.6	ok	Refrigerated
134				D	0.25	0.174	69.6	ok	
				B	0	nd	0.0		
Average				**A**			**62.0**		
				D			**54.1**		
Standard deviation				**A**			**7.61**		
				D			**7.40**		

*UCL - upper control limit, UWL - upper warning limit,
LWL - lower warning limit, LCL - lower control limit
**Spinosyn B was not spiked. Detected spinosyn B was considered as a conversion from spiked spinosyn A and calculation of conversion percentage was based on the amount of spinosyn A spiked.
***none detected

Table 21. Regression Analysis for surface water blind spikes

Regression Statistics					
Spinosyn	**Multiple R**	**R Square**	**Adjusted R Square**	**Standard Error**	**Observations**
A+B*	0.753	0.566	0.512	4.65	10
D	0.400	0.160	0.0551	7.20	10

ANOVA						
		df	**SS**	**MS**	**F**	**Significance F**
A+B	**Regression**	1	225	226	10.4	0.0120
	Residual	8	173	21.6		
	Total	9	399			
D	**Regression**	1	79.0	79.0	1.53	0.252
	Residual	8	414	51.8		
	Total	9	493			

		Coefficients	**Standard Error**	**t Stat**	**P-value**	**Lower 95%**	**Upper 95%**
A+B	**Intercept**	69.3	2.32	29.8	0.0000000106	63.9	74.6
	Slope	-0.727	0.225	-3.23	0.0120	-1.25	-0.208
D	**Intercept**	57.57	3.59	16.0	0.000000233	49.2	65.8
	Slope	-0.430	0.348	-1.24	0.252	-1.23	0.373

*Spinosyn B was not spiked. Detected spinosyn B was considered as a conversion from spiked spinosyn A and calculation of conversion percentage was based on the amount of spinosyn A spiked.

Table 22. Continuing quality control for spinosyns in air samples*

Date	**Spinosyn A**			**Spinosyn B**			**Spinosyn D**		
	Recovery		**Exceed control limits**	**Recovery**		**Exceed control limits**	**Recovery**		**Exceed control limits**
	µg	**%**		**µg**	**%**		**µg**	**%**	
01/23/03	0.856	85.6	ok	0.920	92.0	ok	1.04	104	ok
01/24/03	0.842	84.2	ok	0.917	91.7	ok	0.875	87.5	ok
02/07/03	0.840	84.0	ok	0.904	90.4	ok	0.904	90.4	ok
02/07/03	0.904	90.4	ok	0.960	96.0	ok	0.912	91.2	ok
02/20/03	0.888	88.8	ok	1.06	106	ok	0.912	91.2	ok
02/21/03	0.926	92.6	ok	1.03	103	ok	0.843	84.3	ok
03/07/03	0.788	78.8	ok	0.971	97.1	ok	0.656	65.6	LWL
03/07/03	0.643	64.3	LWL	0.865	86.5	ok	0.682	68.2	ok
03/13/03	0.698	69.8	LWL	0.787	78.7	ok	0.767	76.7	ok
04/04/03	0.832	83.2	ok	1.05	105	ok	0.831	83.1	ok
Average	**0.822**	**82.2**		**0.946**	**94.6**		**0.842**	**84.2**	
Standard deviation	**0.090**	**8.97**		**0.0862**	**8.58**		**0.115**	**11.5**	
UCL-Upper Control Limit			132			145			130
UWL-Upper Warning Limit			120			131			118
LWL-Lower Warning Limit			72.5			73.4			67.6
LCL-Lower Control Limit			60.6			59.1			55.1

*spike level was 1.0 µg of each spinosyn.

Table 23. Blind spike recovery for spinosad spiked in air sampling tubes

Sample number	Date		Days	Spinosyn*	Spike level	Recovery		Exceed control limits**	Comments
	Spiked	Extracted	Spiked to extracted		µg/tube	µg/tube	%		
467	1/30	2/7	8	A	5.0	5.10	102	ok	trip spike
				D	5.0	5.42	108	ok	
468				A	5.0	1.61	32.2	LCL	field spike
				D	5.0	1.54	30.8	LCL	
669	2/13	2/20	7	A	3.0	2.10	70.0	ok	field spike
				D	3.0	1.92	64.0	LWL	
673				A	3.0	4.00	133	UWL	field spike
				D	3.0	3.78	126	UWL	
426	2/27	3/7	8	A	2.5	2.20	88.0	ok	field spike
				D	2.5	2.20	88.0	ok	
677				A	2.5	2.03	81.2	ok	field spike
				D	2.5	2.03	81.2	ok	
Average				**A**			**84.4**		
				D			**83.1**		
Standard deviation				**A**			**33.6**		
				D			**33.4**		

*None conversion of spinosyn B was detected.
**UCL - upper control limit, UWL - upper warning limit,
LWL - lower warning limit, LCL - lower control limit

Table 24. Continuing quality control for spinosyns in grapefruit samples*

Date	Spinosyn A			Spinosyn B			Spinosyn D		
	Recovery		Exceed control limits	Recovery		Exceed control limits	Recovery		Exceed control limits
	ppb	%		ppb	%		ppb	%	
01/27/03	2.98	99.3	ok	2.81	93.7	ok	3.05	102	ok
01/28/03	2.73	91.0	ok	2.62	87.3	ok	2.64	88.0	ok
02/13/03	2.19	73.0	ok	2.37	79.0	ok	2.3	76.7	ok
02/26/03	2.91	97.0	ok	2.09	69.7	ok	2.9	96.7	ok
03/14/03	2.88	96.0	ok	2.00	65.3	LWL	2.9	95.0	ok
03/24/03	2.56	85.3	ok	2.00	66.0	LWL	2.5	82.0	ok
Average	**2.71**	**90.3**		**2.305**	**76.8**		**2.70**	**90.0**	
Standard deviation	**0.295**	**9.83**		**0.355**	**11.8**		**0.285**	**9.58**	
UCL-Upper Control Limit			117			102			116
UWL-Upper Warning Limit			108			94.7			108
LWL-Lower Warning Limit			69.7			66.1			75.3
LCL-Lower Control Limit			60.1			59.0			67.0

*spike level was 3.0 ppb of each spinosyn

Table 25. Continuing quality control for spinosyns in rinse of grapefruit and container *

Date	Spinosyn A Recovery ppb	Spinosyn A Recovery %	Spinosyn A Exceed control limits	Spinosyn B Recovery ppb	Spinosyn B Recovery %	Spinosyn B Exceed control limits	Spinosyn D Recovery ppb	Spinosyn D Recovery %	Spinosyn D Exceed control limits
02/11/03	23.5	78.3	LCL	26.9	89.7	ok	22.1	73.7	LCL
02/26/03	20.7	69.0	LCL	20.7	69.0	ok	19.9	66.3	LCL
03/24/03	19.4	64.7	LCL	16.0	53.3	LWL	19.7	65.7	LCL
Average	**21.2**	**70.7**	**LCL**	**21.2**	**70.7**	**ok**	**20.6**	**68.6**	**LCL**
Standard deviation	**2.10**	**6.98**		**5.47**	**18.2**		**1.33**	**4.44**	
UCL-Upper Control Limit			119			131			114
UWL-Upper Warning Limit			113			116			108
LWL-Lower Warning Limit			92.3			56.9			83.5
LCL-Lower Control Limit			87.0			42.0			77.3

*spike level was 30.0 ng of each spinosyn

Table 26. Continuing quality control for malathion spikes in rinse samples

Date	Spike level ppb	Recovery* Replicate 1 ppb	Recovery* Replicate 1 %	Recovery* Replicate 2 ppb	Recovery* Replicate 2 %	Normalized Difference** %
3/1/03	0.1	0.093	93.0	0.0887	88.7	2.37
3/2/03	0.1	0.113	113	0.111	111	0.89
3/4/03	0.1	0.090	90.3	0.103	107	8.46

*Recoveries for all spikes were within the control limits (66.0%-126%) and warning limits (76.0%-116%).
**Calculated by absolute((rep1-rep2)/(rep1+rep2)*100).

Table 27. Continuing quality control for malathion, piperonyl butoxide, and D-phenothrin spikes in MDS and water samples

Date	Matrix	Spike replicate	Spike level	Recovery Malathion µg or ppb	Recovery Malathion %	Recovery Piperonyl Butoxide µg or ppb	Recovery Piperonyl Butoxide %	Recovery D-phenothrin µg or ppb	Recovery D-phenothrin %
3/21/03	MDS	1	2.0 µg	1.95	97.5	2.1	105	1.95	97.5
		2		2.00	100	2.0	100	1.85	92.5
3/24/03	Water	1	0.2 ppb	0.19	95.0	0.19	95.0	0.180	90.0
		2		0.21	105	0.19	95.0	0.190	95.0

APPENDIX III

PROTOCOL FOR MONITORING SPINOSAD (GF-120 Fruit Fly Bait) IN FRUIT FLY AERIAL TREATMENT PROGRAMS

California Environmental Protection Agency
Department of Pesticide Regulation
P.O. Box 4015
Sacramento, California 95812-4015

PROTOCOL FOR MONITORING SPINOSAD (GF-120 Fruit Fly Bait) IN FRUIT FLY AERIAL TREATMENT PROGRAMS
STUDY 216

December 2002

I. INTRODUCTION

The California Department of Food and Agriculture (CDFA) in cooperation with the San Diego County Department of Agriculture may use ground and aerial treatments of spinosad to eradicate Mexican fruit fly infestations in the Valley Center area of San Diego County. The Department of Pesticide Regulation (DPR) will conduct monitoring of these treatments to provide information on the concentrations of the insecticide in various matrices that may include mass deposition, surface water, storm runoff, air, foliage, fruits, and spray tanks. Additionally, DPR may monitor dissipation of the insecticide in foliage and fruit. Previous fruit fly eradication programs monitored by DPR depended on aerial treatments of malathion bait (Ando et al., 1996; Bradley et al., 1997; Segawa et al., 1991; and Oshima et al., 1982). The monitoring program described in this protocol is based on the previous protocols developed for the malathion bait monitoring programs.

The current treatment area consists of approximately 25 square miles in San Diego County with avocado and citrus as the predominant crops. GF-120 Fruit Fly Bait, with a 0.02% active ingredient of spinosad, will be used a rate of 20 fluid ounces per acre and may be used on organically grown crops. Twelve aerial application events are scheduled beginning in late December 2002 with sequential applications every 14 to 21 days.

II. PERSONNEL

This study will be conducted by the Division of Enforcement, Environmental Monitoring, and Pest Management & Licensing, under the general direction of Doug Okumura (Assistant Director). Key personnel are listed below.

Project Leader: Randy Segawa
Senior Scientist: Bruce Johnson
Field Coordinator: Dave Kim
Laboratory Liaison: Carissa Ganapathy
Analyzing Laboratory: CDFA, Center for Analytical Chemistry

All questions from the media should be directed to Glenn Brank, (916) 445-3974, e-mail gbrank@cdpr.ca.gov. Other questions concerning this monitoring program should be directed to Randy Segawa, (916) 324-4137, fax (916) 324-4088, e-mail rsegawa@cdpr.ca.gov.

III. OBJECTIVES

The objectives of this monitoring are to: 1) provide quality control for the applications by measuring the amount of spinosad in the spray material and reaching the ground during application; 2) measure concentrations in air; 3) determine concentrations in surface water immediately after application and in runoff water following storm events; and 4) determine concentrations and dissipation in fruit.

IV. MONITORING PLAN

This monitoring plan will be followed for 6 to 12 of the aerial application events. Some matrices may not be sampled for each application event.

OBJECTIVE 1: To measure the amount of spinosad in the spray material and reaching the ground (mass deposition). These results will be used to determine if the proper amount of spinosad is applied.

Spray Material – GF-120 contains 0.02 percent (by weight) of spinosad active ingredient. For this eradication program, CDFA will mix GF-120 with water so that the diluted material will contain 0.008 percent (by weight) of spinosad. DPR or County Agricultural Commissioner staff will collect samples of GF-120 and the dilute spray mixture to ensure each contains the proper amount of spinosad. Samples will be collected in glass or plastic bottles and kept on ice or refrigerated until transported to the laboratory for analysis. DPR will collect one sample of each lot of GF-120 used for this eradication program. DPR or the County Agricultural Commissioner will collect the following number of samples of the dilute spray material for each application event monitored.

1 to 2 samples

Mass Deposition – CDFA will apply GF-120 at a rate of 20 fluid ounces per acre, equivalent to 0.005 avoirdupois ounces per acre or 35.1 micrograms per square meter or 3.26 micrograms per square foot of spinosad active ingredient. Deposition sheets (measuring 1 ft^2) will be set out prior to application at 10 – 30 sites within the treatment area. Sites will be located in open areas away from obstructions to reduce the interference with spinosad droplet deposition. Additionally, final site selection will be reviewed to avoid locations on the edges of the treatment area. The cards will be collected approximately 30 minutes following application and will be used to determine the amount of spinosad

reaching the ground. Samples will be stored on dry ice until transported to the laboratory for analysis. DPR will collect the following number of samples for each application event monitored.

10 to 30 sites x 1 sample/site = 10 to 30 samples

OBJECTIVE 2: To measure the amount of spinosad in outdoor ambient air. There are no health standards for pesticides in air. DPR uses screening levels to evaluate the possible health effects of exposure to a chemical, based on a chemical's toxicity. A concentration that is below the screening level is not considered to represent a significant health concern and would not generally undergo further evaluation, but also should not automatically be considered "safe." Similarly, a concentration that is above the screening level does not necessarily indicate a significant health concern, but indicates the need for a further and more refined evaluation. DPR's screening level for spinosad is 160 micrograms per cubic meter ($\mu g/m^3$).

Air – Spinosad is a non-volatile pesticide, so little or no spinosad should be detectable in air once the spray settles. Spinosad's lack of volatility makes it difficult to develop a method to detect it in air. DPR will monitor air concentrations if a suitable method can be developed. Two to six sites located throughout the treatment area will be sampled to measure outdoor ambient air concentrations of spinosad. Sites must be accessible at all hours, protected from any direct spray, and have electrical power to run the samplers. The samples will be collected for a period prior to application (background), during application, and from 24 to 48 hours after application in 24-hour intervals. All air samples are stored on dry ice until transported to the laboratory for analysis. If a suitable method can be developed, DPR will collect the following number of samples for each application event monitored.

2 to 6 sites x 3 to 4 sample periods x 1 sample/site = 6 to 24 samples

OBJECTIVE 3: To measure the concentrations of spinosad in surface water immediately following application and in runoff water following storm events. These results will be used to determine if spinosad may adversely affect aquatic organisms. Spinosad is slightly toxic to moderately toxic to aquatic organisms, with lethal concentrations ranging from 0.1 to 100 parts per million (ppm), depending on the organism.

Surface Water – Flowing waterways that traverse the spray zone will be monitored to determine the concentrations due to aerial deposition. A background water sample will be collected prior to each monitored application at an inflow site upstream of the treatment area boundary and at an outflow site immediately below the application boundary. After application, a sample will be collected from the outflow site. If an impounded water body (i.e., ponds, lakes, and reservoirs) is located within the spray zone, a sample will be taken from the impounded water body prior to and after the application. Water samples are collected and stored in one liter amber bottles with Teflon® lined lids. Samples

are stored on wet ice or in a 4° C refrigerator until transported to the appropriate laboratory for analysis. DPR will collect the following number of samples for each application event monitored.

2 to 6 sites x 1 to 2 sample periods/site = 3 to 12 samples

Surface Water Runoff – Surface water will be monitored during storm runoff events to determine spinosad concentrations due to wash off from exposed surfaces. The first storm event will be sampled, and samples will be collected at points of discharge and/or at areas of concern for aquatic organisms. The California Department of Fish and Game (CDFG) will assist in the selection of sites. The number and frequency of samples collected will depend on intensity and duration of the runoff. When practical, automatic samplers will be used to collect runoff water samples. DPR will collect the following number of samples for each application event monitored.

20 to 50 samples (estimated)

OBJECTIVE 4: To determine concentrations and dissipation of spinosad residue in fruit. These results will be used to determine if effective and legal concentrations are achieved. The application rate for spinosad is low. Current monitoring methods may not detect spinosad concentrations lethal to fruit fly larvae. The maximum legal concentration (tolerance) for spinosad in most commodities is 0.3 ppm.

Fruit - Fruit samples will be collected from one or two species (e.g., grapefruit and/or avocado) at two to five sites within the treatment area to determine efficacy of the spray program. Background samples will be collected prior to application events. Samples will be collected after applications with a minimum of two samplings: day after application and day prior to the next application. Each sample will be a composite of several fruit collected from a single field or orchard. DPR will collect and analyze whole (unpeeled) fruit and identify the sampled fruit as ripe or unripe. DPR will collect the following number of samples for each application event monitored.

1 or 2 species x 2 to 5 sites x 2 to 5 sample periods = 4 to 50 samples

IV. CHEMICAL ANALYSIS/QUALITY CONTROL

CDFA's Center for Analytical Chemistry will perform the laboratory analysis for spinosad (spinosyns A and D). The laboratory will attempt to analyze for breakdown products if standards can be obtained. Quality control measures will include analysis of samples containing known amounts of spinosad (spikes) to check the accuracy and precision of the methods, and samples containing no spinosad (blanks) to check for contamination, as described in Segawa et al. (1995). The quality control samples will comprise approximately 10% of the field samples.

V. DATA ANALYSIS

Mass deposition on deposition cards will be presented as micrograms of spinosad per area ($\mu g/m^2$); concentrations of spinosad in air will be reported as both micrograms per cubic meter ($\mu g/m^3$) and parts per trillion (ppt), water concentrations will be reported as both micrograms per liter ($\mu g/L$) and parts per billion (ppb), fruit samples will be reported as parts per million (ppm). When sample size permits, means, percentiles and frequency histograms will be presented. The mass deposition results will be compared to the target application rate. Air concentrations will be compared to the screening level. Water concentrations will be compared to aquatic toxicity levels. Fruit concentrations will be compared to effective levels and tolerances. Samples used for tolerance purposes must be at the harvest stage, and in its unpeeled, natural form.

VI. REFERENCES

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APPENDIX IV

AMENDMENT OF PROTOCOL FOR MONITORING SPINOSAD (GF-120 Fruit Fly Bait) IN FRUIT FLY AERIAL TREATMENT PROGRAMS

California Environmental Protection Agency
Department of Pesticide Regulation
P.O. Box 4015
Sacramento, California 95812-4015

PROTOCOL FOR MONITORING SPINOSAD (GF-120 Fruit Fly Bait) IN FRUIT FLY AERIAL TREATMENT PROGRAMS
STUDY 216

AMENDMENT 1

March 2003

The California Department of Food and Agriculture (CDFA) is using a series of aerial applications of spinosad to eradicate a Mexican fruit fly infestation in the Valley Center area of San Diego County. The pesticide product used for these applications is GF-120 NF Naturalyte Fruit Fly Bait (U.S. Environmental Protection Agency Registration Number 62719-498), containing 0.020% spinosad by weight (mixture of spinosyn A and spinosyn D) as the active ingredient. For application, the GF-120 is diluted with water to a tank mix target concentration of 0.0080% by weight of spinosad or 0.363 grams per gallon (g/gal). The spinosad (active ingredient) target application rate was 3.26 $\mu g/ft^2$ (0.142 g/acre, or 35.1 $\mu g/m^2$)

The Department of Pesticide Regulation (DPR) has completed monitoring of the first four applications. Preliminary results of the monitoring have shown some unusual findings, particularly the detection of malathion in samples collected from the mix/load system during the third and fourth applications.

As a result of the preliminary findings, DPR will implement the following changes to the monitoring protocol, effective with the sixth application.

<u>Mix/Load System</u> – Prior to the fifth application, a single GF-120/water mixture sample was collected for each application. Additional samples will be collected at various points in the mix/load system. Figure 1 shows the current mix/load system. For each application, DPR will collect the following samples:

one composite sample of GF-120 from the 55-gallon drums of each GF-120 lot prior to application (Figure 1, sampling point #1),

one sample of rinsate that flows through the system between Tank 1 and the Loading Manifold (Figure 1, sampling point #2),

one sample of rinsate that flows through the system between Tank 2 and the Loading Manifold (Figure 1, sampling point #2), and

one sample of the rinsate from each aircraft (Figure 1, sampling point #3), and

one sample of the GF-120/water mixture from the nozzles of each aircraft (Figure 1, sampling point #3).

DPR will collect 9 to 11 samples of the mix/load system for each application monitored, depending on the number of lots.

All samples of the mix/load system will be analyzed for spinosad (spinosyns A, D, and breakdown product B), organophosphates, carbamates, and chlorinated hydrocarbons. For the samples from the 55-gallon drums, the system rinsate, and the aircraft rinsate, DPR will collect samples and report preliminary results prior to each application.

Preliminary results show low or no detectable concentrations in all environmental media. Intensive monitoring is no longer necessary. DPR will make the following changes to the monitoring.

Mass Deposition – For each of the first five applications, DPR collected mass deposition samples from 23 sites. Beginning with the sixth application, DPR will collect samples from 10 – 15 sites for each application monitored.

Air – For the second through fifth applications, DPR collected air samples from four sites. This sampling will be discontinued.

Fruit – For the second through fifth applications, DPR collected fruit samples from two sites. This sampling will be discontinued.

Water – For the first five applications, DPR collected water samples from one surface water site (Keys Creek). This sampling will not change. DPR will collect two water samples for each application monitored.

In addition, DPR will skip all monitoring except the mix/load system for Applications 7, 9, 11, 13, and 15.

All samples will be analyzed for spinosad (spinosyns A, D, and breakdown product B).

All other protocol elements will remain the same.
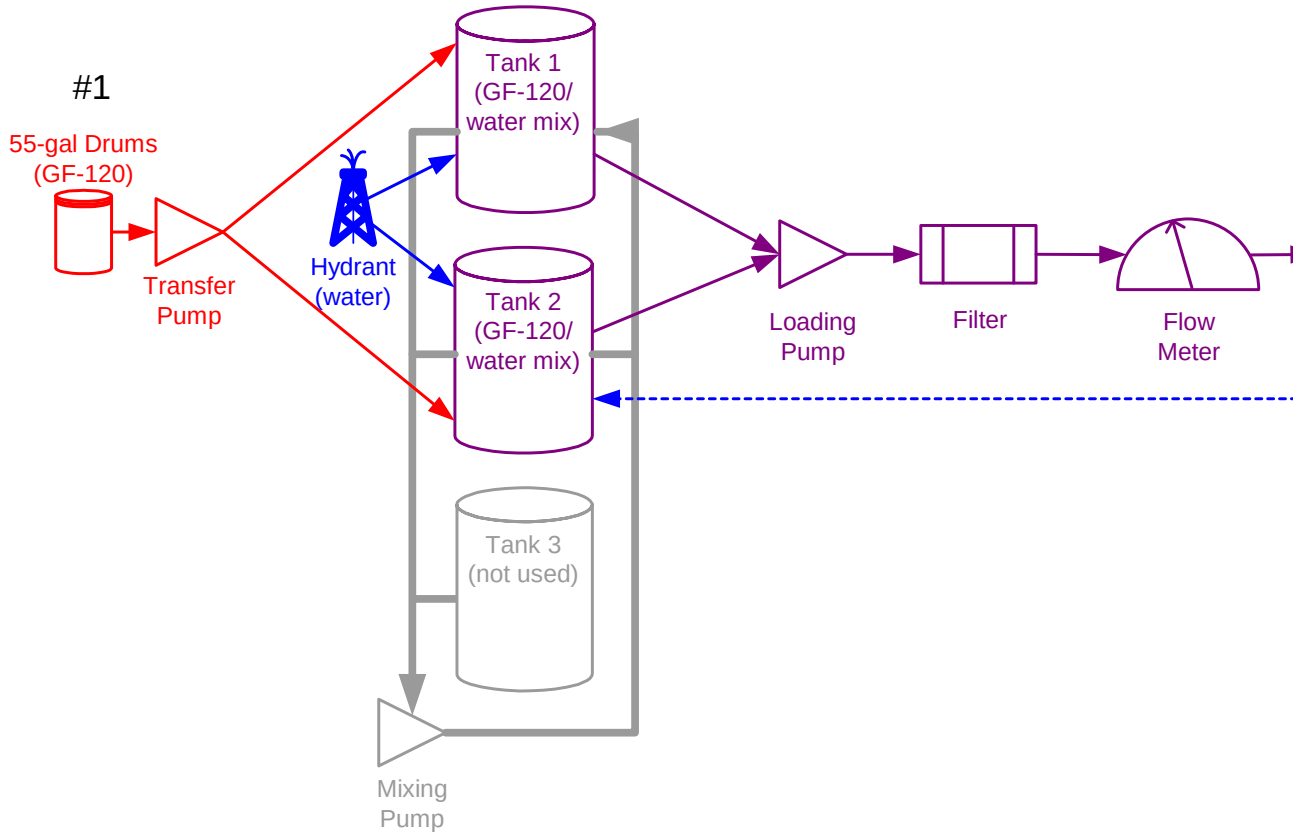
Figure 1. Mix/load system for the fifth application and later. Sampling points are labeled #1, #2, and #3.