

EFFECT OF SOLUTION pH CONDITIONS ON FLUORESCENCE OF SPRAY DEPOSITION TRACERS

H. Zhu, R. C. Derksen, C. R. Krause, R. D. Fox, R. D. Brazee, H. E. Ozkan

ABSTRACT. *Stable analysis of fluorescence is essential to ensure accurate evaluation of pesticide spray application efficiency when fluorescent tracers are used to quantify deposition and drift. The fluorescent intensity of five fluorescent tracers commonly used for the quantitative assessment of spray deposition and off-target loss was investigated with wash solutions over pH conditions from 6.86 to 10.4. The tracers selected in the tests were Acid Yellow 7, Eosin, Fluorescein, Pyranine, and Tinopal. The fluorescence of Pyranine was the most sensitive to the solution pH conditions, followed by Fluorescein and Tinopal. Acid Yellow 7 and Eosin had a nearly constant fluorescent intensity over the pH range from 6.86 to 10.40. The fluorescent strength of Fluorescein increased 1.3 times, Tinopal 1.25 times, and Pyranine 3.0 times as the pH value increased from 6.86 to 8.43, but it became nearly constant when pH value was greater than 8.43. However, Pyranine, Fluorescein, and Tinopal showed much stronger fluorescence than Acid Yellow 7 and Eosin with the same concentrations. A solution containing Fluorescein at pH 8.43 and higher demonstrated 124 times greater fluorescent intensity than the solution containing the same amount of Acid Yellow 7. The fluorescent strength should be examined not only with purified distilled water but also under various wash solution pH conditions during the selection of tracers for pesticide spray deposition and drift trials.*

Keywords. *Dye, Pesticide, Spray drift.*

Pesticides have assured high productivity of agriculture and a quality supply of food and fibers in past decades. Agricultural production and storage consumes about 75% of total pesticide in the United States (Anderson and Magleby, 1997). However, pesticide use can potentially raise concerns about health risks from residues in food and drinking water, create worker hazards, and have negative impacts on wildlife and sensitive ecosystems.

To improve pesticide spray application efficiency and reduce environmental contamination, it is important to measure the spray quality and quantity reaching target areas. Many field tests have been conducted using fluorescent tracers to measure pesticide spray deposition and drift (e.g. Fox et al., 1993; Pergher and Gubiani, 1995; Derksen et al., 2000), evaluate spray penetration and coverage (Carlton et al., 1981; Walker and Huitink, 1989; Farooq and Salyani, 2002), track spray deposits at various locations within canopies (Derksen and Jiang, 1995; Zhu et al., 2002), and improve new sprayer design (Gan-Mor et al., 1996). Fluorescent tracers have advantages in high sensitivity,

relatively low cost, and user safety to quantify pesticide spray deposits and drift in the field.

Fluorescent tracers provide a means of tracking spray movement when either environmental conditions or analytical techniques prohibit the use of active ingredients. While fluorescent tracers are widely used for assessment of spray quantity, many concerns have been raised over their measurement accuracy due to questions of stability of fluorescence exposed to solar radiation during experiments. There has been considerable research on photo degradation of fluorescent tracers that can result in a severe underestimation of the retained deposit (Salyani, 1993; Cross et al., 1997; Zhu et al., 2004). The rate of fluorescent degradation under sunlight varies with the type of fluorescent substances (Cai and Stark, 1997; Pergher, 2001).

Sensitivity of fluorescence is another concern during selection of tracers because the fluorescent strength varies with tracer types and wash solutions. Spray targets are typically washed with water to recover water soluble dyes. Quality of wash solutions used to dissolve tracers is a major element that influences fluorescent measurement accuracy. In field experiments, water and often surfactants are usually used as a carrier to mix with tracers for spray experiments, but the level of minerals and other elements existed in water varies with water sources and locations. For laboratory analysis, distilled water is often used as the wash solution to dissolve spray samples containing tracers. Among various effects, unexpected materials from samples washed into the solution could alter solution pH. The fluorescent strength of some substances appeared to be different in alkaline and acidic solutions (Pringsheim, 1949). However, little information is available on the influence of solution pH conditions on the analysis of fluorescent tracers commonly used in evaluation of pesticide spray application efficiency.

The objective of this research was to determine the influence of wash solution pH conditions on fluorescent

Article was submitted for review in April 2004; approved for publication by the Power & Machinery Division of ASAE in January 2005.

Mention of proprietary product or company is included for the reader's convenience and does not imply any endorsement or preferential treatment by either USDA-ARS or The Ohio State University.

The authors are **Heping Zhu, ASAE Member Engineer**, Agricultural Engineer, **Richard C. Derksen, ASAE Member Engineer**, Agricultural Engineer, **Charles R. Krause**, Plant Pathologist, **Robert D. Fox, ASAE Member Engineer**, Agricultural Engineer, **Ross D. Brazee, ASAE Member Engineer**, Senior Research Scientist, USDA-ARS, Application Technology Research Unit, Wooster, Ohio; and **H. Erdal Ozkan, ASAE Member Engineer**, Professor, FABE, The Ohio State University, Columbus, Ohio. **Corresponding author:** Heping Zhu, USDA-ARS, Application Technology Research Unit, Ag. Eng. Bldg., OARDC, 1680 Madison Ave., Wooster, OH 44691; phone: 330-263-3871; fax: 330-263-3670; e-mail: zhu.16@osu.edu.

intensity of water soluble tracers, in an effort to minimize analytical errors in the measurement of spray deposition and drift.

MATERIALS AND METHODS

The fluorescent intensity of five different water soluble fluorescent tracers commonly used to quantify agricultural pesticide spray deposition and drift was tested under various wash-solution pH conditions. The five fluorescent tracers were Acid Yellow 7 (Carolina Color and Chemical Co., Charlotte, N.C.), Eosin (Acros Organics, Fisher Scientific), Fluorescein (Aldrich, Milwaukee, Wis.), Pyranine (Acros Organics of Fisher Scientific, Fair Lawn, N.J.), and Tinopal (Ciba-Geigy Chemical Corporation, Toms River, N.J.). The excitation and emission wavelengths, CAS registry number, molecular weight and chemical formula of these tracers are listed in table 1. Rhodamine B is another fluorescent tracer often used in quantification of spray deposition, but it was not selected for this work because some formulations contain a suspected carcinogen (Anonymous, 2002).

The portion of active fluorescent ingredients varied with the five tracers. Fluorescein contained 70% dye content and 30% sodium salt; Pyranine contained 98% pyrenetrisulfonic acid trisodium salt known as Solvent Green 7; Tinopal was a bis-benzenesulfonic acid disodium salt; Acid Yellow 7 contained 20% active fluorescent content known as Brilliant Sulfaflavine; Eosin was a yellowish free acid dye.

Solution samples were prepared in two steps for fluorescent intensity analysis. The first step was to produce an initial tracer solution for representing a spray deposition sample collected from the field. The second step was to dissolve the initial tracer solution in a wash solution with an expected pH value. The initial tracer solution contained a tracer with either purified distilled water or regular tap water. Purified distilled water was used to make initial solutions for all tracers while tap water was used for solutions only containing Pyranine and Tinopal for an additional trial. The pH value was 6.4 for the purified distilled water and 8.7 for the tap water. The concentration of tracers in the initial tracer solution was 0.015 mg/mL for Fluorescein, 0.1 mg/mL for Pyranine, 0.0625 mg/mL for Tinopal, 3.0 mg/mL for Acid Yellow 7, and 0.3 mg/mL for Eosin, respectively. The concentrations were selected based on the pre-trials for fluorescent intensity that fell within the detecting range of the spectrometer used in this research.

In the second step of the sample preparation, 10 μ L of the initial tracer solution was dissolved into a wash solution with one of the five pH values (6.86, 7.41, 8.43, 9.18, and 10.4 at 25°C) to obtain final solutions with known tracer concentration. The wash solutions with pH 6.86, 7.41, 9.18, and 10.4 were prepared by mixing distilled water and sodium carbonate buffer salts (Fisher Scientific, Fair Lawn, N.J.). The solution with pH 8.43 was adjusted from a mixture

consisting of 60% pH 9.0 Fisher buffer solution, 30% distilled water and 10% pH 5.0 Fisher buffer solution. The concentrations of tracers in the final solutions were 0.03, 0.015, and 0.0075 μ g/mL for Fluorescein, 0.05 μ g/mL for Pyranine, 0.0315 μ g/mL for Tinopal, 1.5 and 3.0 μ g/mL for Acid Yellow 7, and 0.03 and 0.3 μ g/mL for Eosin, respectively. For each concentration, three samples were prepared for three replications.

After the final solution with a tracer and a desired pH was achieved, 4 mL of the sample was placed in a cuvette for fluorescent intensity analysis at a given excitation wave length of the tracer, based on peak height ranging from 0 to 1000, with a Model LS 50B luminescence spectrometer (Perkin-Elmer Limited, Beaconsfield, Buckinghamshire, England). The fluorescent intensity of wash solutions without tracers under different pH conditions was also measured at the excitation wave lengths of the five tracers with the spectrometer for background check and was subtracted from the fluorescence intensity of each sample when data was analysis.

Data were analyzed by one way ANOVA, and differences among means were determined with Duncan's New Multiple-Range Test using ProStat version 3.5 for Windows (Poly Software International, Inc., Pearl River, N.Y.). All significant differences were determined at the 0.05 level of significance.

RESULTS AND DISCUSSION

The excitation wave length of wash solutions without tracers at different pH values was 395 nm, which was far away from the excitation wave lengths of the five tracers shown in table 1. The fluorescent intensity of wash solutions was below 10 peak height at all five excitation wave lengths of tracers.

Figure 1 shows the effect of solution pH value ranging from 6.86 to 10.40 on the mean fluorescence recovery rate for solutions containing Fluorescein at concentrations of 0.03, 0.015, and 0.0075 μ g/mL, respectively. The fluorescence recovery rate in this article was based on an assumption that the fluorescence of tracers was recovered 100% at pH 10.40. The fluorescence recovery rate of Fluorescein tracer at concentrations of 0.03 and 0.015 μ g/mL was greater than 96% while it was 89% at 0.0075 μ g/mL when the wash solution pH was 8.43. However, the recovery rate was below 78% for the three concentrations at wash solution pH 6.86. The fluorescent intensity increased as the pH value increased from 6.86 to 8.43, and then became nearly constant when pH value was 9.10 or greater. For example, the average fluorescent intensities of the Fluorescein solutions at 0.03- μ g/mL concentration were 671.3, 766.5, 861.0, 867.0, and 869.9 with pH values of 6.86, 7.10, 8.43, 9.10, and 10.40, respectively. Similarly, at 0.0075- μ g/mL concentration, the average fluorescent intensities of the Fluorescein solutions

Table 1. Fluorescence tracers used in tests.

Fluorescence Tracers	Excitation (nm)	Emission (nm)	CAS Registry Number	Molecular Weight	Formula
Acid Yellow 7	430	500	2391-30-2	404.4	C ₁₉ H ₁₃ N ₂ NaO ₅ S
Eosin	525	545	15086-94-9	647.9	C ₂₀ H ₈ Br ₄ O ₅
Fluorescein	494	520	518-47-8	376.3	C ₂₀ H ₁₀ Na ₂ O ₅
Pyranine	455	508	6358-69-6	524.4	C ₁₆ H ₇ Na ₃ O ₁₀ S ₃
Tinopal	350	430	27344-41-8	562.6	C ₂₈ H ₂₀ Na ₂ O ₆ S ₂

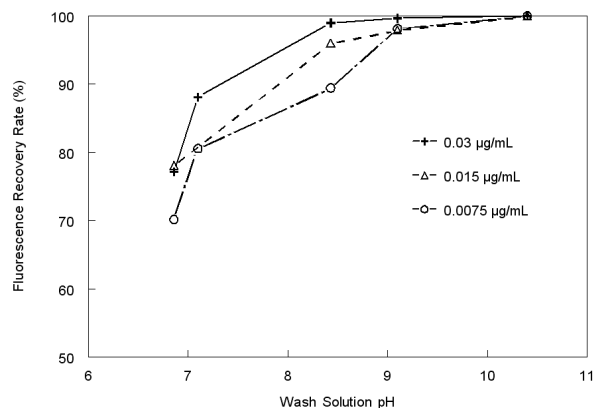


Figure 1. Effect of wash solution pH conditions on fluorescence recovery rate for the Fluorescein tracer at various concentrations.

were 157.2, 180.4, 200.3, 219.8, and 224.0 with pH 6.86, 7.10, 8.43, 9.10, and 10.40, respectively. The fluorescent intensity increased 1.3 times or more for all three concentration solutions when the solution pH increased from 6.86 to 8.43 or higher.

Similar to Fluorescein, the fluorescent intensity of both Tinopal and Pyranine substances increased as the solution pH increased from 6.86 to 8.43 while the fluorescent intensity became nearly constant when the pH was greater than 8.43 (fig. 2). The fluorescence recovery rate increased from 32.4% to 96.4% (or nearly 3.0 times) for the 0.05-µg/mL Pyranine solution and from 85.6% to 97.6% for the 0.0315-µg/mL Tinopal solution when wash solution pH increased from 6.86 to 8.43. When the wash solution pH was greater than 8.43, the mean fluorescent intensity was 633.5 with 2.7% coefficient of variation for Pyranine and was 838.7 with 1.6% coefficient of variation for Tinopal. Therefore, the fluorescence for the Pyranine, Fluorescein, and Tinopal tracers became weaker when solution was more acidic and became more intense when the solution became more alkaline.

The fluorescence of Pyranine, Fluorescein, and Tinopal in solutions with different pH conditions varied with the states of ionization formed in either alkaline or acidified solutions. The density of ions in solutions might strengthen the

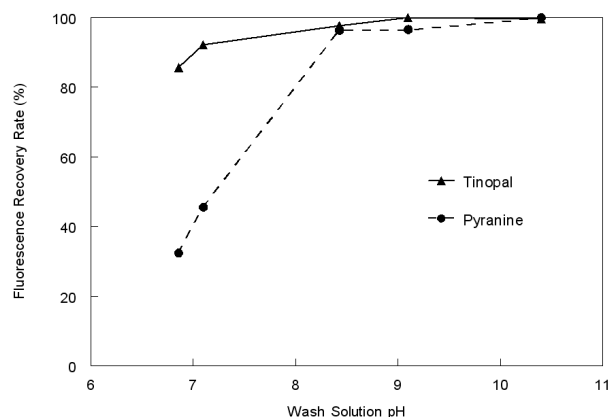


Figure 2. Effect of wash solution pH conditions on fluorescence recovery rate for solutions containing 0.0315-µg/mL Tinopal and 0.05-µg/mL Pyranine, respectively.

absorbing power for the three tracers. Many previous studies reported that purified distilled water was used to dissolve spray samples containing fluorescent tracers. However, the purified distilled water always has pH lower than 7 because of dissolved carbon oxide (CO₂), appearing as an acidified solution. Some tests indicated that pH of the plastic container-stored, purified, distilled water from the same laboratory as used in this experiment decreased from 6.7 to 5.6 after it was periodically opened and closed. When solution pH was close to 7 or below, fluorescent intensity of the three tracers was very sensitive to changes in pH conditions (figs. 1 and 2). To minimize analytical error, a wash solution instead of purified distilled water with a pH value above 8.43 is suggested for analyze Pyranine, Fluorescein, and Tinopal tracers.

The fluorescent intensity of Acid Yellow 7 and Eosin had little variation with the solution pH conditions. Figure 3 illustrates that Acid Yellow 7 and Eosin at two different concentrations had a nearly constant fluorescence over the pH range from 6.86 to 10.40. The mean fluorescent intensity was 871.4, 96.2, 691.3, and 362.2 with 1.3%, 14.7%, 4.6%, and 5.3% coefficient of variation for Eosin at 0.3 µg/mL, Eosin at 0.03 µg/mL, Acid Yellow 7 at 3.0 µg/mL, and Acid Yellow 7 at 1.5 µg/mL, respectively. The fluorescent intensity of Acid Yellow 7 and Eosin was not affected by solution pH conditions. The fluorescent intensity of different dyes responded differently to solution pH conditions.

Statistical analysis indicated the tracer concentration in wash solutions under different pH conditions did not significantly influence the tracer recovery rate ($p < 0.05$). When wash solution pH changed from 6.86 to 10.40, the ratio of fluorescent intensities varied from 1.8 to 1.9 for the Fluorescein solution between concentrations of 0.03 and 0.015 µg/mL, and from 3.9 to 4.3 for the solution between concentrations of 0.03 and 0.0075 µg/mL (table 2). Similarly, the ratio of fluorescent intensities varied from 1.8 to 2.1 for the Acid Yellow 7 solution between concentrations of 3.0 and 1.5 µg/mL, and it was from 7.8 to 10.9 for the Eosin solution between concentrations of 0.3 and 0.03 µg/mL, respectively.

Table 3 shows the fluorescent intensity of solutions containing 0.0315-µg/mL Tinopal and 0.05-µg/mL Pyranine with the initial tracer solution containing either distilled

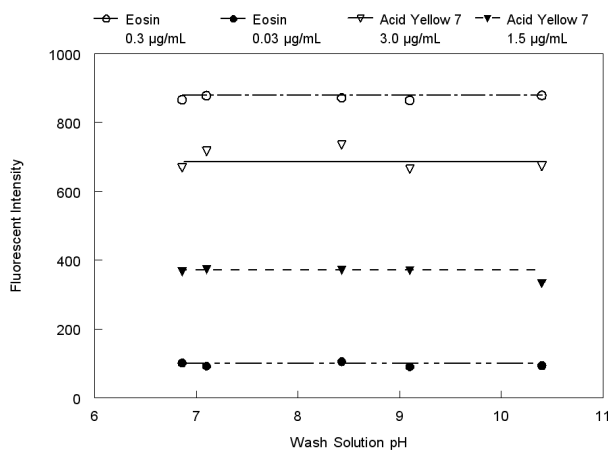


Figure 3. Effect of wash solution pH conditions on fluorescent intensities of solutions containing 0.03- and 0.3-µg/mL Eosin and 1.5- and 3.0-µg/mL Acid Yellow 7, respectively.

Table 2. Ratio of fluorescent intensities between two concentrations at different pH values for tracer solutions containing Acid Yellow 7, Eosin, and Fluorescein.

Wash Solution pH Value	Acid Yellow 7 Rc ^[a] = 2	Eosin Rc = 10	Fluorescein	
			Rc = 2	Rc = 4
6.86	1.8	9.1	1.8	4.3
7.41	1.9	8.8	N/A	4.2
8.43	2.0	7.8	1.9	4.3
9.10	1.8	10.9	1.9	3.9
10.40	2.1	8.9	1.9	3.9

^[a] Rc represents the ratio of two concentrations, which is 3.0 µg/mL to 1.5 µg/mL for Acid Yellow 7, 0.3 to 0.03 µg/mL for Eosin, and 0.03 to 0.015 µg/mL and 0.03 to 0.0075 µg/mL for Fluorescein, respectively.

water or tap water, respectively. The initial tracer solution was 0.05% of the final wash solution. Under the same pH conditions, there was no significant difference ($p < 0.05$) in measured fluorescent intensity for Tinopal solutions using either tap water or distilled water in the initial tracer solution. However, when the pH of the wash solution was higher than 7.41, the Pyranine solution made with tap water had slightly greater fluorescent intensity than the solution containing distilled water.

Thus, the pH providing peak fluorescent intensity varied depending on the tracer. Acid Yellow 7 and Eosin displayed stable fluorescent readings over the pH range from 6.86 to 10.40. Pyranine was the most sensitive to pH changes among the five tested fluorescent substances, while Fluorescein and Tinopal were somewhat affected by the pH of the wash solution. However, Fluorescein, Pyranine, and Tinopal were more fluorescent sensitive than Acid Yellow 7 and Eosin. Based on the results of this research, it required 0.024-µg/mL Fluorescein, 0.055-µg/mL Pyranine, 0.026-µg/mL Tinopal, 0.24-µg/mL Eosin, or 3.04-µg/mL Acid Yellow 7 for the fluorescent intensity to reach 700 with the stable solution pH range (8.43 or higher). This result indicated that at a solution pH of 8.43 or higher, the fluorescent sensitivity of Fluorescein was 127 times that of Acid Yellow 7 and 10 times that of Eosin, Tinopal was 116 times that of Acid Yellow 7 and 9 times that of Eosin, and Pyranine was 55 times that of Acid Yellow 7 and 4.4 times that of Eosin. It was necessary that the solution pH must be adjusted to be greater than 8.43 to reduce the analytical errors if Fluorescein, Pyranine, or Tinopal was used as the tracer to measure spray deposition and drift.

CONCLUSIONS

- For the water soluble tracers evaluated in this study, fluorescent intensity of Pyranine, Fluorescein, and Tinopal

Table 3. Effect of distilled water and tap water existing in the initial tracer solutions on the fluorescent intensity of final solutions containing 0.0315-µg/mL Tinopal or 0.05-µg/mL Pyranine.^[a]

	Fluorescent Intensity			
	Tinopal (0.0315 µg/mL)		Pyranine (0.05 µg/mL)	
	Distilled Water	Tap Water	Distilled Water	Tap Water
6.86	725.1 (26.8)	740.9 (4.7)	210.3 (23.2)	210.9 (19.6)
7.41	802.2 (36.6)	779.6 (50.1)	295.6 (19.6)	335.3 (5.6)
8.43	804.6 (25.7)	804.1 (31.4)	625.3 (15.2)	679.8 (10.6)
9.10	846.4 (11.7)	824.0 (27.7)	626.5 (21.0)	684.9 (19.1)
10.40	843.2 (4.4)	840.3 (11.6)	648.7 (2.5)	672.6 (18.0)

^[a] Standard deviations were given in parentheses.

was influenced by the solution pH conditions; however, the effect was not the same for all tracers evaluated. The fluorescent intensity of Pyranine, Fluorescein, and Tinopal tracers decreased as the solution became more acidic. The fluorescence of Acid Yellow 7 and Eosin remained nearly constant over the solution pH range from 6.86 to 10.40. The fluorescent intensity tended to become constant for all tracers at a rather alkaline pH greater than 8.43.

- For the same concentration, fluorescent intensity varied considerably with tracers. Pyranine, Fluorescein, and Tinopal produced greater fluorescent intensity than Acid Yellow 7 and Eosin at the same concentration. However, the tracer concentration did not significantly influence the tracer recovery rate under different wash solution pH conditions.
- Wash solution pH should be adjusted to above 8.43 instead of using purified distilled water only to increase fluorescence and minimize analytical errors for Pyranine, Fluorescein, and Tinopal tracers. While this study centered on the pH of wash solution used for tracer recovery, further studies are necessary to evaluate the effect of pH on the photo-degradation of fluorescent tracers as the assessment of spray movement.

ACKNOWLEDGEMENTS

The authors greatly acknowledge Eva Lu, Leslie A. Morris, and L. E. Horst for their technical assistance.

REFERENCES

- Anderson, M., and R. Magleby. 1997. Pesticides. In *Agricultural Resources and Environmental Indicators, 1996-97*, Agricultural Handbook Number 712, U.S. Department of Agriculture. Washington, D.C.: U.S. Government Printing Office.
- Anonymous. 2002. Acros Organics 2002/03 Catalog of Organics and Fine Chemicals. Morris, N.J.: Fisher Scientific International L.L.C.
- Cai, S. S., and J. D. Stark. 1997. Evaluation of five fluorescent dyes and triethyl phosphate as atmospheric tracers. *J. Environ. Science and Health B32(6)*: 969-983.
- Carlton, J. B., L. F. Bouse, H. P. O'Neal, and W. J. Walla. 1981. Measuring spray coverage on soybean leaves. *Transactions of the ASAE 24(5)*: 1108-1110.
- Cross, J. V., R. A. Murray, M. S. Ridout, and P. J. Walklate. 1997. Quantification of spray deposits and their variability on apple trees. *Aspects of Applied Biology 48*: 217-224.
- Derksen, R. C., and C. Jiang. 1995. Automated detection of fluorescent spray deposits with a computer vision system. *Transactions of the ASAE 38(6)*: 1647-1653.
- Derksen, R. C., R. D. Fox, R. D. Brazee, and C. R. Krause. 2000. Coverage and drift produced by air induction and conventional hydraulic nozzles used for orchard applications. ASAE Paper No. 001137. St. Joseph, Mich.: ASAE.
- Farooq, M., and M. Salyani. 2002. Spray penetration into the citrus tree canopy from two air-carrier sprayers. *Transactions of the ASAE 45(5)*: 1287-1293.
- Fox, R. D., D. L. Reichard, R. D. Brazee, and C. R. Krause. 1993. Downwind residues from spraying a semi-dwarf apple orchard. *Transactions of the ASAE 36(2)*: 333-340.
- Gan-Mor, S., A. Grinstein, H. Beres, Y. Riven, and I. Zur. 1996. Improved uniformity of spray deposition in a dense plant canopy: methods and equipment. *Phytoparasitica 24(1)*: 57-67.
- Pergher, G. 2001. Recovery rate of tracer dyes used for spray deposit assessment. *Transactions of the ASAE 44(4)*: 787-794.

- Pergher, G., and R. Gubiani. 1995. The effect of spray application rate and airflow rate on foliar deposition in a hedgerow vineyard. *J. of Agricultural Engineering Research* 61: 205-216.
- Pringsheim, P. 1949. *Fluorescence and Phosphorescence*. New York: Interscience Publishers, Inc.
- Salyani, M. 1993. Degradation of fluorescent tracer dyes used in spray applications. *Pesticide Formulations and Application Systems*, Vol. 13, eds. P. D. Bergwer, B. N. Devisetty, and F. R. Hall. Philadelphia, Pa: American Society for Testing and Materials.
- Walker, J. T., and G. Huitink. 1989. Penetration of tilt into a rice canopy. ASAE Paper No. 891007. St. Joseph, Mich.: ASAE.
- Zhu, H., D. L. Rowland, J. W. Dorner, R. C. Derksen, and R. B. Sorensen. 2002. Influence of plant structure, orifice size and nozzle inclination on spray penetration into peanut canopy. *Transactions of the ASAE* 45(5): 1295-1301.
- Zhu, H., J. W. Dorner, D. L. Rowland, R. C. Derksen, and H. E. Ozkan. 2004. Spray penetration into peanut canopies with hydraulic nozzle tips. *Biosystems Engineering* 87(3): 9-17.

