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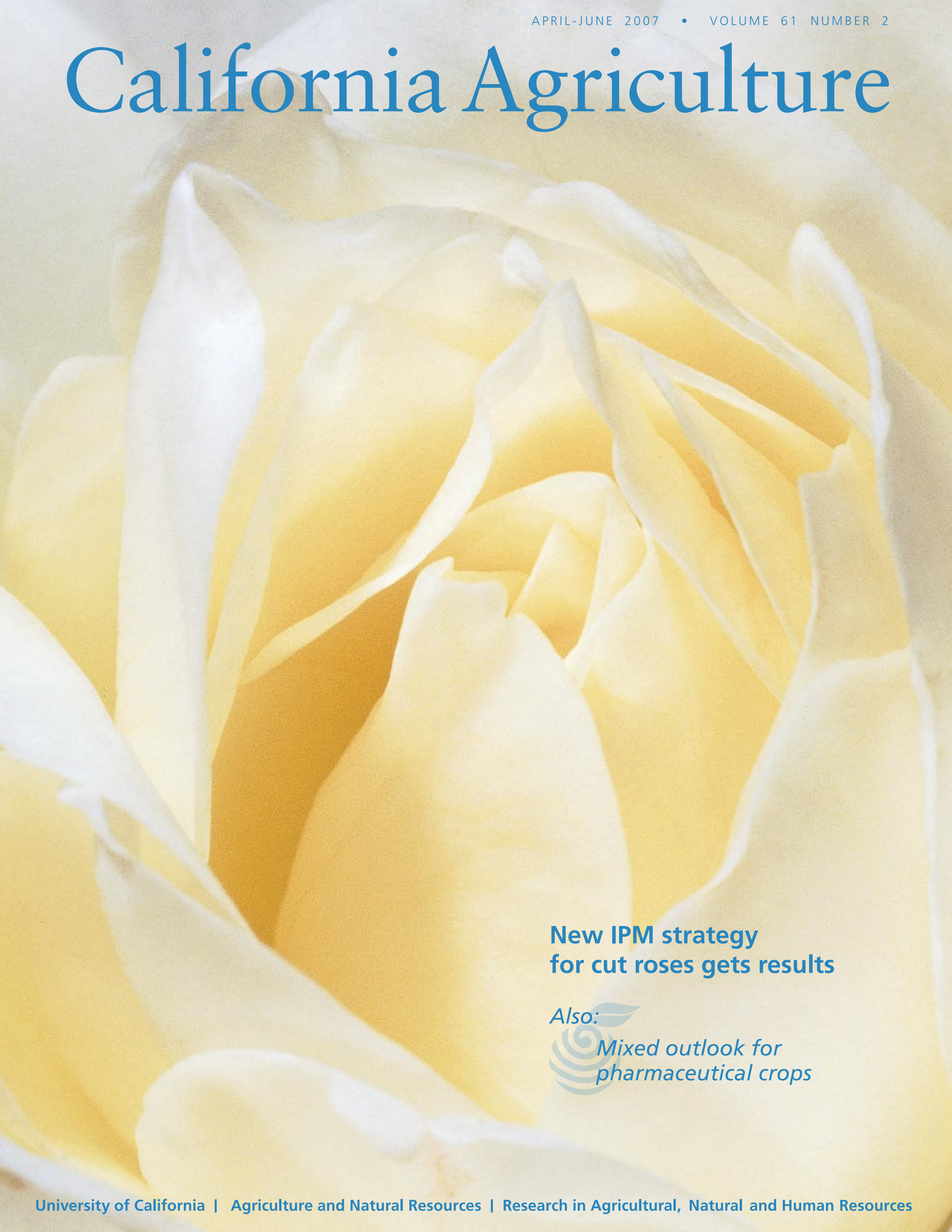
California Agriculture



New IPM strategy for cut roses gets results

Also:

Mixed outlook for pharmaceutical crops

Jerry Gillespie
Director,
Western Institute
for Food Safety
and Security, UC Davis

Lessons from the E. coli *outbreak — understanding the complexity of foodborne disease*

We are in the "recovery period" of the spinach-related, foodborne-disease outbreak of August 2006, caused by *Escherichia coli* O157:H7. This outbreak resulted in at least 204 illnesses in 26 states, three deaths and more than 100 hospitalizations; 29 had hemolytic uremic syndrome, and it is suspected that many more became ill. As of March 2007, reports from official investigations have not been released. Nevertheless, we have learned enough to draw some important conclusions.

Perhaps the most significant lesson is that foodborne disease outbreaks are complex and multidimensional; they require a multidisciplinary, multi-industry approach if preventative measures are to be found. In recent years, regulatory agencies have focused their epidemiological investigations on production units (farms) identified as sources of contaminated produce that caused previous foodborne disease outbreaks linked to fresh produce. For example, researchers examined water (irrigation, flood, runoff), soil and fertilizers, production practices, wildlife, labor, equipment, and other potential routes of introduction of foodborne pathogens (such as *E. coli* O157:H7) on the farm (Sargeant et al. 1999). Microbiologists with diverse backgrounds in genomics, diagnostics, pathogen biology, and bacterial culture and isolation technologies, to name a few, have been central to this research. Much of the initial research involved collecting large numbers of samples from the farm environment in search of *E. coli* O157:H7 (*California Agriculture* 61[5]).

These investigations suggest that *E. coli* O157:H7 might have been introduced to the spinach by water, wildlife fecal matter or other materials brought to the farm from off-site sources. To explore these sources, we must understand livestock management practices, environmental biology, waterway dynamics and farming practices. The fundamental question is: What is the ecology of *E. coli* O157:H7 in spinach production, processing, shipping and retail marketing?

We know that we can isolate this pathogen from fecal matter from animals (including humans), occasionally from waterways, and soil and plant surfaces. We do not know specifically how long *E. coli* O157:H7 can survive in each of these environments nor precisely how it moves from one to the next (such as from fecal matter to spinach leaves). Using current technology, *E. coli* O157:H7 is difficult to isolate, and its concentration in any environment varies from time to time. It is also known that as few as 10 *E. coli* O157:H7 organisms can cause disease in people. Those few organisms would be easy to miss because of how difficult it is to isolate this strain of *E. coli* from environmental or food samples.

Even with the causal associations made in the current investigations, there is no definitive epidemiological pathway identified for the spinach contamination. In fact, it is possible there were several simultaneous routes of contamination, some not recognized in any investigation to date. For example, scientists have found the specific pathogenic strain of *E. coli* O157:H7 (EXHX01.0124) in samples from cattle manure near spinach fields, in wild pig manure from spinach fields, in recovered bags of spinach, and from stool samples from those affected, suggesting a path from cattle to pigs to spinach field to humans. However, investigators cannot rule out that it reached the spinach from fecal discharges by birds flying over, or contaminated workers' shoes or tractor tires.

Each of these modes of introduction would require somewhat different preventative practices to forestall contamination of product. The investigations are not complete or comprehensive enough to provide producers with definitive actions to prevent future contamination. Many believe other segments of the spinach continuum need to be rigorously investigated. For example, are the trucks that carry the product contaminated? Are there sites within packaging plants that are adding pathogens to the product? Are there practices in the storage or cooling plants that are allowing the introduction of pathogens? Are retail outlets (grocery stores or restaurants) potential sites for contamination? Finally, we cannot rule out deliberate contamination by a disgruntled individual acting alone or as part of a terrorist cell.

Our task of enhancing food safety is made all the more daunting by the rapid globalization of the food supply and the rapid changes in the technologies used at each step in production, from farm to consumer (*California Agriculture* 54[5]). Investigators must keep track of changes in production and identify new potential risks. Our risk analysis must also look at intentional contamination; for example, how vulnerable are seaports to agroterrorism?

To seriously address leafy-green contamination, we need more comprehensive research, including more academic disciplines and more segments within the leafy-green continuum. We need research expertise on the livestock industry, wildlife biology and water. This is precisely why UC and its partners formed the Western Institute for Food Safety and Security (WIFSS) in 2002, to build needed expertise and foster partnerships between university scientists and industry.

To make the most out of WIFSS partnerships, the WIFSS laboratory must provide a consistent and focused long-term research program that persists between foodborne disease outbreaks and food-system disasters. Partnerships are important to expanding research capacity and, in particular, for filling research niches that cannot be covered by the WIFSS laboratory. These partnerships will involve scientists from a wide spectrum of disciplines and experts from every segment of the global food system.

Sargeant JM, Hafer DJ, Gillespie JR, et al. 1999. The prevalence of *Escherichia coli* O157:H7 in white-tailed deer sharing rangeland with cattle. JAVMA 215:792–4.

TABLE OF CONTENTS

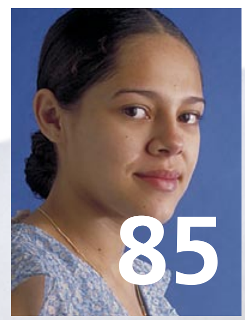
COVER: A new IPM program for cut roses succeeded in controlling twospotted spider mites and reducing pesticide use (see page 71). *Photo: Jack Kelly Clark*

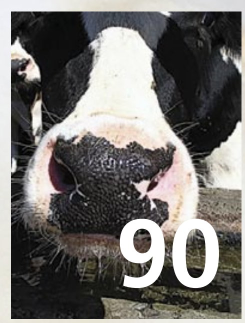
News departments

Research and reviews

Biotech risks & benefits

In the July-September 2006 issue, *California Agriculture* initiated a series of articles on the risks and benefits of agricultural biotechnology (see http://californiaagriculture.ucop.edu/0603JAS/toc.html). In this issue, the series continues.





Letters

WHAT DO YOU THINK?

The editorial staff of *California Agriculture* welcomes your letters, comments and suggestions. Please write to us at 1111 Franklin St., 6th floor, Oakland, CA 94607 or calag@ucop.edu. Include your full name and address. Letters may be edited for space and clarity.

Classic *Hilgardia* on figs posted

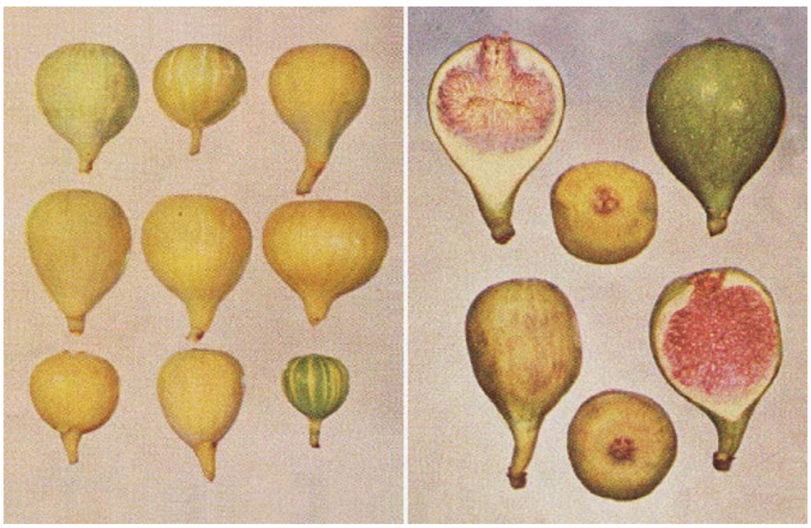
In February 1955 when Ira Condit's "Fig Varieties: A Monograph" was published in *Hilgardia*, it was the definitive work on figs. It remains so in 2007. I am pleased that Dr. Condit's publication is now available to anyone wishing a copy as a downloadable PDF at: http://californiaagriculture.ucop.edu/0702AMJ/letters.html.

This PDF is the result of more than 135 hours of patience, scanning, optical character recognition (OCR) conversion, retyping unrecognizable text and most importantly, editing for accuracy. Despite the effort, there are still punctuation errors and no doubt typographic errors resulting from the OCR conversion. Although there have been some minor changes including eliminating hyphenation and placing some text on adjacent pages for easier reading, it is my hope that none of the original publication's intent has been lost in the PDF.

Since most fruit production is totally dependent on microclimate conditions and finding suitable varieties, I initiated the Puget Sound Regional Fig Variety Test in 2000 to determine which fig varieties will produce best for home gardeners in western Washington state. The best available information was necessary, hence the need for Dr. Condit's publication.

Bob "Kiwibob" Glanzman, Proprietor
Puget Sound Kiwi Co.
Seattle, Washington

Editor's note: Hilgardia, *a monograph series published by ANR, ceased publication in 1995. Copies can be found in university libraries. We cannot confirm the detailed accuracy of this 247-page document, but we gratefully acknowledge Glanzman's heroic effort.*



Plates from Ira Condit's "Fig Varieties: A Monograph," published in 1955. The classic 247-page work has been posted online.

Radiofrequency promising for herbs

Re: Lagunas-Solar et al. "Radiofrequency power disinfects and disinfests food, soils and wastewater" (October-December 2006).

Golden State Herbs in Indio farms 600 acres of conventional and organic herbs, and operates an air-drying facility. We ship our herbs globally, and are the largest grower/processor of air-dried herbs in the United States. The herb and spice industry has relied on various gases, steam or even irradiation for sterilization. This article fascinated me, and I would like to know if the work is applicable to air-dried herbs and spices.

Jack Vince, Vice President
Golden State Herbs, Indio

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Manuel Lagunas-Solar, research chemist with the UC Davis Crocker Nuclear Laboratory, responds: Indeed, radiofrequency power is an excellent optional procedure for simultaneously disinfecting and drying herbs, dried food and food-additive commodities. We have studied the dielectric properties of various herbs and spices with excellent results. The chemical composition of dried products makes them very efficient in absorbing radiofrequency power to convert it to thermal energy. In this process, we achieved about 90% overall energy-use efficiencies at selected frequencies, making the economics of the process attractive as well. The radiofrequency process is an efficient and reliable alternative to replace chemical-based, conventional heating and irradiation.

At our laboratory, we have several prototype systems available for demonstration projects. One such system is being prepared for technical demonstrations in Antalya, Turkey, and we are considering projects with private companies in China, Brazil and Canada.

Keep on publishing

I read every issue of *California Agriculture* and forward or route it to people who should read the information that I feel is in their line of work. As a teenager I attended Picnic Day at UC Davis (1955) and learned about agriculture, not college. After working for 44 years — 20 years at Crocker Bank (computers) and 24 years for the city and county of San Francisco (PUC computers) — I have used the information in the magazine for databases in portfolios, engineering, water samples and more important, my daily life and raising a family of five. Please continue to publish your magazine (as well as post it on the Web) because online publications get lost, whereas magazines can be reread, routed and filed easier for future reference, and used as references in business, work, schools and home.

Bill Flaherty
San Francisco

Appreciates e-mail notification

Editor's note: California Agriculture *provides an e-mail notification listing highlights of each new journal edition as it is posted on the Web. If you wish to sign up, please write to CalAg@ucop.edu.*

Thank you for the "mind-tickling" content messages for *California Agriculture*. I can pick and choose what I want to delve into as I have time. Eventually I get through all the subjects, but it's great for me and my burgeoning schedule to be able to prioritize.

Pamela Cornelison
UCCE Master Gardener
Mariposa

Science briefs

California Dept. of Water Resources

The Delta's 1,100 miles of levees are increasingly vulnerable to earthquakes, floods, subsidence and other factors. The cost to repair the Jones Tract Levee, which failed in June 2004, was $90 million.

Report: Delta failure costs could top $40 billion

The costs for a single episode of unexpected levee failure in the Sacramento–San Joaquin Delta could reach $40 billion, according to a report released in February by the nonprofit Public Policy Institute of California and written in collaboration with five UC Davis professors.

The 300-page report, *Envisioning Futures for the Sacramento–San Joaquin Delta,* found that such an episode would affect drinking water for millions of people and agricultural animals, such as the state's huge dairy herd, as well as irrigation water for food crops and water supplies for industry.

"After Hurricane Katrina, people realized that catastrophic collapse of these levee and water systems is a very real possibility," says co-author Richard Howitt, UC Davis professor of agricultural and resource economics. "There's a 64% probability of something like this happening in the next 50 years. That's too high for public infrastructure."

According to the report, the Delta is increasingly threatened by floods, earthquakes, sinking land, rising sea levels, regional climate change, invasive species and urbanization. CALFED, the government consortium charged with solving the Delta's problems, is itself challenged by underfunding and internal dissent.

The report considers nine alternatives for Delta management and evaluates the performance of each in three key areas: water supply, environmental effects and economic costs.

The authors recommend that scientific work in the Delta be refocused on a new problem-solving framework that includes levee replacement, ecosystem adaptation, flood control and island land management.

In addition to PPIC research fellow Ellen Hanak, the interdisciplinary team of UC Davis professors included Howitt; Jeffrey Mount, UC Davis Center for Watershed Sciences director; Peter Moyle, Department of Wildlife, Fish and Conservation Biology; and William Fleenor and Jay Lund, Department of Civil and Environmental Engineering.

The full report is available at: www.ppic.org.

Photos: Jeannette Warnert

USDA plant physiologist David Obenland demonstrates the use of a blacklight to identify freeze-damaged oranges, which fluoresce with dots.

2007 freeze: *UV could cast new light on citrus damage*

The toll of the January freeze on California agriculture is still being tallied by state officials, with the latest estimates hovering at $1.2 billion and rising. But simple ultraviolet (UV) technology could make it possible for orange growers to estimate damage while fruit is still on the tree or in the packinghouse.

This year's freeze, which began in the wee hours of Jan. 12 and lasted anywhere from 5 to 15 days depending on the location, assaulted the state's agricultural crops with temperatures ranging from the teens to the high 20s.

A wide variety of California's fruit and vegetable crops were affected, including citrus, avocados, strawberries, artichokes, broccoli, celery, blueberries and cut flowers. Gov. Arnold Schwarzenegger has requested disaster relief for 24 of the state's 59 counties.

The biggest financial loss, about $700 million, was shouldered by the state's citrus industry, followed by avocado growers with an estimated loss of $108 million.

"Our most recent estimate is a 27% loss for avocadoes," says Tom Bellamore, senior vice president of the California Avocado Commission. "The hardest hit areas were San Luis Obispo, the San Joaquin Valley, Fillmore and the Ojai area of Ventura County."

Citrus farmers expect damage when temperatures drop below 28°F for more than 5 or 6 hours, so the extended period of cold nights hit especially hard.

Farmers made efforts to save their crops by blowing warmer air through 30-foot wind machines and irrigating trees, but in most cases fruit still sustained some damage.

Ted A. Batkin, president of the Citrus Research Board, estimated losses of about 45% for fresh-market citrus products and 35% overall, which accounts for juice recovery. Citrus growers began harvesting the navel orange crop in late October, more than 2 months before the freeze set in, so about 30% of the crop had already been harvested. That left about 70% still on the trees during the cold snap. "It looks like we are recovering 30% to 40% of what was left on the trees," Batkin says.

New technology to estimate damage

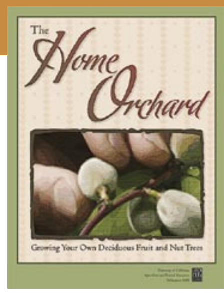
Loss estimates for the 2007 freeze are still somewhat fluid because it is difficult for citrus growers to determine exactly how much freeze damage fruit has incurred.

Currently, the industry needs to cut open fruit to see if it has been damaged. But this approach is far from foolproof, says Mary Lu Arpaia, UC Cooperative Extension subtropical horticulture specialist and member of the UC Riverside botany and plant sciences department. "Fruit-cutting is a laborious process," Arpaia explains. For several weeks after a freeze, fruit are evaluated using the "segment cut," in which the middle third of the fruit is opened up to examine the segment walls.

But that may be changing.

One upside of the freeze is that the scientists have been able to go out into orange groves to test a new method that utilizes ultraviolet (UV) light to assess freeze damage while fruit is still on the trees, without cutting the fruit.

Funded by the Citrus Research Board and headed by Jim Thompson, UC Cooperative Extension agricultural engineering specialist at



A new ANR Communication Services book, *Home Orchard: Growing Your Own Deciduous Fruit and Nut Trees,* was developed for backyard orchardists, rare fruit growers and small-scale growers; go to http://anrcatalog.ucdavis.edu for more information.

UC Davis, a group of UC and U.S. Department of Agriculture (USDA) scientists appear to have identified a nondestructive method.

Researchers in the lab of USDA plant physiologist David Obenland and USDA plant pathologist Dennis Margosan noticed that the outside peel of freeze-damaged oranges fluoresces with yellow pinpoint dots when put under UV fluorescent light (UVA at 365 nanometers wavelength). “Freezing causes tiny amounts of orange oil to become visible on the rind’s surface,” Thompson says. “The specks of oil glow like stars on a moonless night when exposed to blacklight.”

The group started testing the method last spring in the lab with artificially frozen fruit, but with this year’s freeze they had an opportunity to test it with a simple UV flashlight in orange groves at night and in blacklight rooms inside packinghouses. Already they’ve found differences between the lab and the field.

“The fluorescence was not as dramatic as when you put fruit in the freezers,” says Arpaia, who joined the team for the validation phase of the research. “We have had to recalibrate.” They are also investigating how long fluorescence lasts on the fruit.

In addition, the team is doing preliminary testing to see if different kinds of navel oranges fluoresce differently. The material that fluoresces in some citrus fruit is called “tangeritin,” which is found in the peel of oranges, grapefruit and some mandarins; it is not found in lemons and therefore, they do not fluoresce. Likewise, the method does not appear to work in other, noncitrus crops.

The researchers also tested another potential method of evaluating frost damage in oranges using a Breathalyzer, a tool more commonly used by law enforcement to identify drunk drivers. Frost-damaged oranges emit ethanol, so researchers placed samples of damaged fruit into closed plastic bags for 20 to 30 minutes, then used a Breathalyzer fitted with a hypodermic needle (instead of a mouthpiece) to measure the amount of ethanol produced. The initial field tests showed the technique was not as reliable as UV light.

“We’re hoping we have some of these variables worked out,” Arpaia says. “We have more work to do before the next freeze.”

— Celeste Durant and Editors

Photos: ANR Communication Services

Freeze-damaged tree and fruit.

Frost-damaged plants may need pruning, but wait until spring

Freezing temperatures in many parts of California injured some citrus trees and other frost-sensitive subtropical plants in January, but since the full extent of injury can not be known for several months, UC Cooperative Extension (UCCE) horticulture advisor Ed Perry recommends that gardeners wait until spring before pruning and removing damaged trees and plants.

“While you may be tempted to prune out damaged branches right away, it’s best to wait until spring when new growth will show you the extent of the injury,” says Perry of Stanislaus County. “Earlier pruning often results in leaving some limbs which might continue to die back, and the removal of limbs which might recover.”

Frost injury to plants depends upon a number of factors, including species, age, health, soil moisture and location. Frost injures plants by causing ice crystals to form in plant cells, making water unavailable to plant tissues and disrupting the movement of fluids. Frost-damaged leaves appear water-soaked, wither, and turn dark brown or black. Unprotected, sensitive young trees may be killed, but frost rarely kills mature trees in California.

The only treatments that should be applied rapidly after a freeze are whitewashing to prevent “sunburn” and picking frost-damaged fruit to remove stress from the tree, says UCCE citrus farm advisor Ben Faber of Ventura County.

The UC advisors also recommend withholding nitrogen fertilizer applications to severely damaged citrus trees and irrigating carefully. Over-irrigation may induce root damage and encourage the growth of root-rotting organisms. Irrigation should be less frequent and in smaller amounts until trees have regained their normal foliage. *— Jeannette Warnert*

UC Malaria Research and Control Group vows to defeat malaria

Twenty-one scientists from five UC campuses are partnering with the Mosquito and Vector Control Association of California to defeat one of the world's oldest and deadliest diseases: malaria.

Malaria infects some 350 to 500 million people a year, killing between 1 million and 2.5 million, according to the World Health Organization. Ninety percent of the global incidence of malaria occurs in Africa, where a child dies from the disease every 30 seconds.

The UC Malaria Research and Control Group (MRCG) vows to change that. The group, formed in February 2006, is a branch of the UC Mosquito Research Program, a statewide program of the UC Division of Agriculture and Natural Resources.

"We're firmly committed to defeating the most formidable and challenging mosquito-borne disease," says medical entomologist and MRCG director Gregory Lanzaro, who also directs the UC Mosquito Research Program and the UC Davis Center for Vectorborne Diseases.

"This is all about saving lives," Lanzaro says. "It's the right thing to do. We are combining compassion, technology and science to defeat a killer."

Malaria, first recognized 4,000 years ago and eradicated in the United States in the early 1950s, has been eliminated in many parts of Asia, Europe and the Americas, but is raging uncontrolled in many parts of Africa, Lanzaro says. "The spike can be attributed to more efficient mosquito vectors, increased pesticide and drug resistance, and socioeconomic factors, including struggling health systems."

Malaria threatens more than 100 countries and territories, with more than 40% of the world's population at risk, according to the U.S. Centers for Disease Control and Prevention. Children under age 5 and pregnant women are most susceptible.

UC Davis scientist recounts battle with neuroinvasive West Nile virus

Keira Simmons knew something was wrong the minute she woke up that fateful morning in mid-June 2005.

A strange red rash splotched her inner arms and torso. Within 4 days, the rash covered her entire body, even between her fingers and toes. A knife-splitting headache, coupled with crushing muscle pain, vomiting, neck stiffness, fatigue, dizziness and nausea ensued.

Her normal 98.5°F degree temperature spiked to 102°F and then raged to 106.5°F. She dropped 20 pounds in 10 days.

The UC Davis scientist remembers four trips to the hospital emergency room, a 3-day hospitalization to treat the raging fever, worsening pain, dizziness and dehydration, and a 3-week recovery period at home. The illness incapacitated her for 7 weeks.

It nearly killed her.

"The doctors thought I had the flu," recalls Simmons, then 27 and a postgraduate researcher in a UC Davis School of Medicine lab.

It was not the flu.

Only after she had accepted a research position in November 2005 at the UC Davis Center for Vectorborne Diseases (known as CVEC), did a routine blood test confirm her worst suspicions: neuroinvasive West Nile virus , the most severe virus spread by *Culex* mosquitoes.

For more information:

Symptoms of West Nile virus
www.cdc.gov/ncidod/dvbid/westnile/qa/symptoms.htm

UC Center for Vectorborne Diseases
http://www.vetmed.ucdavis.edu/cvec

Kathy Keatley Garvey

Keira Simmons, now a researcher at the UC Davis Center for Vectorborne Diseases, still suffers side effects from West Nile virus.

"Keira's blood test proved strongly positive for the West Nile virus," said CVEC research entomologist William Reisen, who researches *Culex* mosquitoes.

Looking back, Simmons speculates that the infected mosquito bit her when she and her fiancé were hiking along Putah Creek on the UC Davis campus.

Hospital technicians drew her blood, but Simmons learned later that it was never tested for West Nile virus, even though she had repeatedly asked for the test. "They ran a few noninvasive tests and some cultures. When none of them was conclusive, they simply gave me IV fluids and sent me home. They said I had the flu."

The illness alarmed her family and friends. "No one around me had been sick and no one was getting sick from exposure to me. It was quite frightening for my

Lanzaro, who researches *Anopheles gambiae*, the principal vector of malaria in Africa, says the most deadly parasite is *Plasmodium falciparum*. It can kill within hours of noticeable symptoms, which include high fever, severe headache, drowsiness, delirium and confusion. The malaria mosquitoes bite at night, usually between 10 p.m. and 4 a.m.

Shirley Luckhart, a UC Davis medical entomologist, traveled to Mali in summer 2006 as part of the UC Mosquito Research and Control Group's efforts to wipe out malaria, a devastating mosquito-borne disease.

Focus on research and education

At its organizational meeting in May 2006, MRCG agreed to focus on academic research, education and public service. Its mission is threefold: facilitate collaborative activities, including organized research and training; mitigate the malaria burden in Africa; and provide technical advice to public health agencies on malaria control programs, based on mosquito abatement in Africa.

Individual members of MRCG are involved in eight African partnerships and two research-training grants.

Basic researchers study mosquito molecular genetics, population genomics and the ecology of malaria vectors, mosquito mating biology, and the genetics of immunology and biochemistry of *A.gambiae/P. falciparum* interactions.

Applied research involves the evaluation of existing insecticide-based control strategies, the development of novel mosquito attractants, new assays for the detection of metabolic insecticide resistance in mosquitoes, the role of agricultural insecticide use in the development of resistance in mosquitoes, the mass-rearing of *A. gambiae,* and models for malaria associated with rice agriculture.

Lanzaro and UC Davis medical entomologist Anthony Cornel of the UC Mosquito Research Laboratory, located at the Kearney Agricultural Center in Parlier, have conducted fieldwork in Africa for more than 15 years, zeroing in on insecticide and drug resistance and population genetics. Last summer, medical entomologist Shirley Luckhart, a UC Davis School of Medicine faculty member, and entomology graduate students Tara Thiemann and Lisa Reimer joined them in Mali. Cornel, a native of South Africa, and Thiemann also worked in Cameroon last summer.

Funded by a National Institutes of Health grant, Lanzaro is researching the complex genetic structure of *A. gambiae.* "Using DNA markers we have been able to demonstrate that subpopulations of this mosquito exist in nature that do not interbreed and therefore do not exchange genes," Lanzaro says. "These subpopulations often exist even within a single village. This has important

mom, dad, sister and fiancé to stand by, powerless, and watch me deteriorate."

When her personal physician admitted her to the hospital, "I wasn't real aware of my surroundings or what was going on at that time. I had literally started to mentally check out," she says. "I was quite fortunate that I had my fiancé, family and my physician advocating on my behalf."

Today side effects still persist. "I feel generally healthy," Simmons says, "but I still have about three or four headaches of migraine-intensity a week. I also still have some weakness in my arms and hands, affecting my manual dexterity. I have transient spells of vertigo that leave me unable to work in my capacity as a researcher."

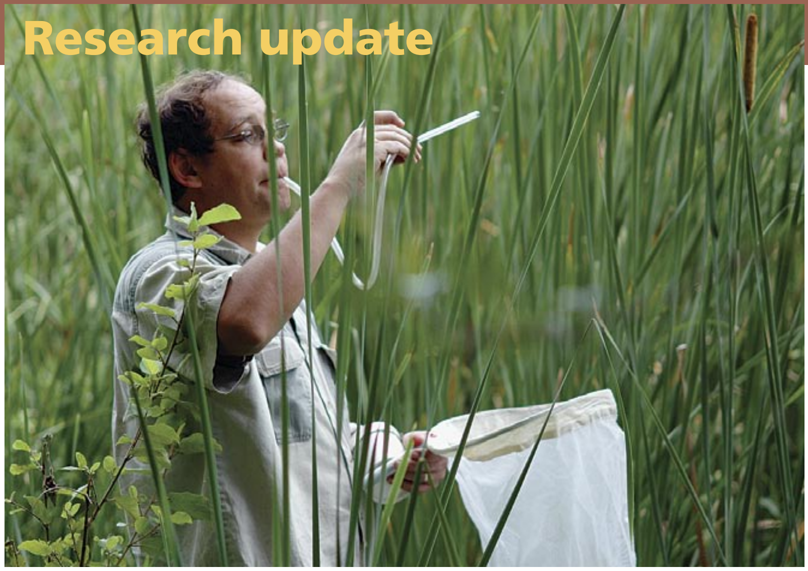
"I have no idea when these symptoms will resolve. West Nile virus is so new to our population that they really don't have any idea how it may affect people or their quality of life."

Since her encounter with the infected mosquito, Simmons works with a renewed interest as a researcher and the lead molecular technician at CVEC, testing mosquito pools and dead bird tissue submitted from throughout the state.

Had she not changed jobs and taken the mandatory blood test, Simmons believes the disease may have gone undiagnosed. Her reaction to the positive blood test? "Vindicated. Validated. I knew there was something seriously wrong with me."

— *Kathy Keatley Garvey*

Rory McAbee



Anthony "Anton" Cornell, UC Davis medical entomologist, collects mosquitoes; one focus of his work is insecticide resistance in malaria vectors.

consequences to understand patterns of resistance to insecticides that form the basis of malaria control campaigns."

Cornel's work focuses on understanding environmental exposures to insecticides and the various mechanisms responsible for mosquito resistance to insecticides. This includes developing field assays to monitor resistance, an important factor in malaria control programs.

Luckhart's research is aimed at understanding the relationship between malaria parasites and their mosquito vectors. "Her work is improving our understanding of why some mosquitoes are capable of transmitting this deadly parasite, while others do not," Lanzaro says.

Delegations to Tanzania, the White House

A four-member MRCG delegation, led by Lanzaro and Cornel, journeyed to Tanzania in mid-October to develop collaborations and build partnerships for malaria control and research. "Malaria is the leading cause of death in both children and adults in Tanzania," Lanzaro says. "In 2003, the most recent year for which information is available, there were more than 10 million cases of malaria in Tanzania."

The delegation included two representatives from the Mosquito Vector and Control Association of California: Major Dhillon, manager of the Northwest Mosquito and Vector Control District, Corona; and Steve Mulligan, who manages the Consolidated Mosquito Abatement District, Selma.

Lanzaro also represented MRCG at the Dec. 14, 2006, White House Summit on Malaria, which brought together international experts; corporations and foundations; African civic leaders; and voluntary, faith-based and nonprofit organizations. The goal is to raise awareness of malaria and to mobilize a grassroots effort to save millions of lives in Africa. President Bush declared April 25 as Malaria Day.

In response, Lanzaro has organized the first-ever Malaria Awareness Day symposium on the UC Davis campus for April 25, gathering members of the scientific community to discuss malaria and the UC Davis commitment to global health. Topics will range from the history of malaria in California to current novel malaria-control strategies in Africa.

Speaking on the history of malaria in California will be Robert K. Washino, professor and chair emeritus of the UC Davis entomology department, and co-author of *Mosquitoes of California.*

"Malaria," Washino says, "will continue to be of concern to residents of California due to continued travel outside the United States by civilian and military personnel, immigration policies and most recently, the potential effect of global warming on mosquito-parasite interactions involved in malaria transmission." — *Kathy Keatley Garvey*

Anthony Cornel

In Africa, the ***Anopheles gambiae*** mosquito transmits ***Plasmodium falciparum***, the parasite that causes malaria.

For more information:

UC Malaria Research and Control Group
www.mrcg.ucdavis.edu

UC Davis Malaria Awareness Day
www.mrcg.ucdavis.edu/news/malariasymposium.html

UC Mosquito Research Program
www.ucmrp.ucdavis.edu

Lanzaro Vector Genetics Lab
http://entomology.ucdavis.edu/faculty/lanzaro/index.htm

Centers for Disease Control and Prevention
www.cdc.gov/malaria

Malaria No More
www.malarianomore.org

World Health Organization
www.who.int/topics/malaria/en

REVIEW ARTICLE

Pharmaceutical crops have a mixed outlook in California

by Michelle Marvier

Crops are being genetically engineered to produce a wide variety of drugs, vaccines and other pharmaceutical proteins. Although these crops may open the door to less expensive and more-readily available drugs, there is concern regarding the potential for contamination of human food and livestock feed, as well as environmental harm. The outlook for the production of pharmaceutical crops in California currently appears mixed. To date, 18 federal permits for field trials involving pharmaceutical or industrial proteins have been approved in California. However, the state's farming community and general public have thus far rejected pharmaceutical crop production, and a handful of local governments have recently banned the cultivation of genetically modified crops, including pharmaceutical crops. In light of the many pros and cons, three major approaches — the precautionary approach, risk analysis and cost-benefit analysis — could be used to move the debate about pharmaceutical crops forward.

USDA-ARS/Doug Wilson

Proponents of crops genetically engineered to express pharmaceutical proteins believe that these crops could increase the availability of medicines and vaccines, and lower costs. To date, about two-thirds of pharmaceutical field-trials in the United States have involved maize, a wind-pollinated species (conventional corn is shown).

Even science fiction writers did not dream that we would someday use maize fields to produce insulin, or rice paddies to grow anticoagulants. Today, however, crops are being turned into factories producing not just food, but drugs, vaccines, enzymes and antibodies. The first step in using crops to produce pharmaceutically active proteins is the synthesis or isolation of genes that code pharmaceutical proteins, followed by the transfer of those genes into the DNA of crop plants. These transferred genes, or "transgenes," can potentially come from a different plant species, an animal (often a human) or a bacterium. The genetically modified crops are then cultivated and harvested.

In most cases, the crop-produced pharmaceutical protein is extracted, purified and possibly modified further before it is administered to humans or livestock. In some instances, however, crops are being engineered so that a vaccine can be delivered through the direct consumption of leaves, fruits or other plant parts, without the cost and inconvenience of extracting the proteins and delivering them via pills or injections (Sala et al. 2003).

Benefits of pharmaceutical crops

Scientists are drawn to the genetic engineering of crops as a means of quickly producing large quantities of drugs and vaccines, with the hope that this technology can reduce costs and increase the availability of much-needed pharmaceuticals (Fischer et al. 2004; Giddings et al. 2000; Horn et al. 2004; Ma et al. 2003; Ma, Barros et al. 2005). The potential products of transgenic plants include blood thinners, hemoglobin, insulin, growth hormones, cancer treatments and contraceptives. Products already in the pipeline include plant-produced vaccines for hepatitis-B, cholera, rabies, HIV, malaria and influenza. One company is developing genetically modified maize (corn) to produce lipase, a digestive enzyme used to treat patients with cystic fibrosis. Arthritis and other autoimmune diseases are also targets for plant-produced vaccines (Sala et al. 2003). Researchers have focused on maize, bananas, tomatoes, carrots and lettuce as possible oral-delivery mechanisms for such vaccines because these foods can be eaten raw, thereby avoiding the protein denaturing that typically occurs during cooking (Sala et al. 2003). Producing vaccines in food plants would eliminate the need for refrigeration, which limits the usefulness

Human error occurs and, frankly, is unavoidable.

of certain vaccines in many parts of the world (Walmsley and Arntzen 2000).

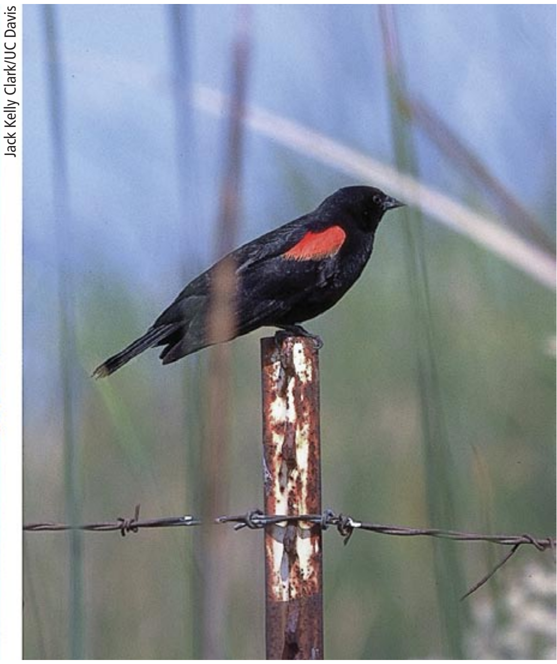
In some cases, the pharmaceuticals targeted for production in transgenic plants are currently produced by cultures of transgenic animal, bacterial or yeast cells in large vats. Plants are an attractive alternative because they could potentially produce greater yields. This is especially important for monoclonal antibodies (such as etanercept, which is used to treat rheumatoid arthritis) because current production methods cannot keep up with increasing demand (Elbehri 2005). Moreover, faster and less expensive production could reduce prices for consumers. Another major benefit of utilizing plants is the reduced risk of disease transmission; there is concern that producing drugs via mammalian cell cultures or animal milk could facilitate the movement of certain viruses to humans.

Despite these potential advantages, drugs produced by pharmaceutical crops have not yet appeared on the U.S. market. Several are currently making their way through field and clinical trials, and the first drugs derived from pharmaceutical crops could be on the market within a few years (Ma, Chikwamba et al. 2005).

Containment risks

There is a long history of cultivating plants to produce pharmaceutical compounds, and at least one-fourth of modern medicines contain plant-derived ingredients (Raskin et al. 2002). Some plants that are used for pharmaceutical production (such as opium poppies) are also food crops (such as poppy seeds). Despite such precedents from nature, genetically modifying major commodity grains such as maize and rice to produce pharmaceutical proteins has raised new levels of concern and public anxiety (Stewart and McLean 2004). Although earlier methods of pharmaceutical production often involved cultures of genetically modified cells, these cells were kept under strict confinement in laboratories. The production of pharmaceutical proteins in maize or rice, on the other hand, will typically be done in the field where it will be impossible to completely contain the crops, transgenes and pharmaceutical proteins (Ellstrand 2006). Production of these crops in contained greenhouses or underground caves has been proposed as a potential, albeit far less cost-effective, solution.

Suzanne Paisley/UC Davis



Jack Kelly Clark/UC Davis

Whenever pharmaceutical-producing crops are grown outside, it is virtually inevitable that birds, insects and other wildlife will eat them, resulting in unknown risks to the animals, and that the pollen and seeds will be transported offsite. *Left,* bees on a corn stalk. *Right,* a red-winged blackbird.

Contamination of food and feed. In 2002, 130 acres of pharmaceutical maize were cultivated in the United States in field trials. Two-thirds of all pharmaceutical plantings in the United States are maize, but soybeans, rice, potatoes, alfalfa, wheat, tobacco and other crops are also being used. The primary concern is that the public might someday find unwanted medicines in their food or in livestock feed (Elbehri 2005; Kirk et al. 2005; Mascia and Flavell 2004; Peterson and Arntzen 2004).

Food can be contaminated when transgenes are not contained, or if plant products intended only for medicinal uses accidentally comingle with those headed for our dinner tables. Transgenes can escape when pollen from pharmaceutical crops drifts into and fertilizes fields of nonpharmaceutical crops. Due to the energetic costs that producing pharmaceutical proteins likely entails, it is unlikely that transgenes coding for pharmaceutical products would persist for very long within the recipient gene pool. However, even transient transgene flow could cause problems. For example, if transgenic pollen fertilizes seed kernels on a nontransgenic maize plant, the kernels could produce and contain the pharmaceutical protein. Alternatively, if seeds are left behind in the soil following harvest, "volunteer" pharmaceutical plants could establish themselves in subsequent growing seasons, possibly in mixture with nonpharmaceutical crops. Because some pharmaceutical compounds are effective in very low concentrations, even low-level contamination may pose risks.

Transgene escape from food crops. Although pharmaceutical crops are still rarely produced and only under tightly regulated conditions, there already has been one revealing case of transgene escape involving field trials of pharmaceutical maize in Nebraska and Iowa. In November 2002, the U.S. Department of Agriculture (USDA) discovered that ProdiGene had failed to comply with federal regulations in two of its field trials, which involved maize genetically modified to produce a vaccine that pre-

Greenpeace/Laura Lombardi

In 2002, field trials of pharmaceutical maize went awry when the producer failed to destroy volunteer maize during the subsequent growing season. As a result, 500,000 bushels of harvested soybeans were destroyed in Aurora, Nebraska. Greenpeace activists hung a banner on the silo.

vents diarrhea in pigs. In both locations, ProdiGene failed to destroy volunteer maize plants in the subsequent growing season. In Nebraska, the mistake was not discovered until after the volunteer maize had been shredded and mixed among soybeans that had been subsequently planted at the site. This meant that 500,000 bushels of soybeans had to be destroyed. In Iowa, there was no mixing with soybeans, but 155 acres of maize surrounding the pharmaceutical-crop test site were destroyed because of possible contamination via pollen from volunteer plants. ProdiGene was fined $300,000 for these violations, and also paid $2.7 million in damages and cleanup costs.

A half-dozen more examples of human error involving other, nonpharmaceutical-producing types of genetically modified crops were reviewed by Marvier and Van Acker (2005). Since the publication of that paper, Syngenta admitted to accidentally distributing the seeds of an unapproved variety of genetically modified insect resistant Bt10 maize over a 4-year period (Macilwain 2005), and traces of Bayer's Liberty Link 601 herbicide-resistant rice have been detected in both U.S. and European long-grain food rice, even though the variety was never approved or marketed (Vogel 2006). The lesson from these events is that human error occurs and, frankly, is unavoidable.

Food versus nonfood crops

The possible escape of pharmaceutical products from engineered crops into the food supply is of concern to the promoters of genetic engineering, as well as environmentalists. For example, an editorial in the journal *Nature Biotechnology* offered two suggestions that could help industry to avoid foreseeable problems (Editors of Nature Biotechnology 2004). First, engineered crops could be geographically isolated to reduce the chances of contamination in the general food supply. For example, pharmaceutical crops might be cultivated on islands where the food crop is otherwise absent. Second, the editors recommended that food crops should not be used to produce pharmaceutical proteins, and that nonfood crop alternatives such as tobacco might be a wiser choice. The National Research Council (2004) concurred, stating, "Alternative nonfood host organisms should be sought for genes that code for transgenic products that need to be kept out of the food supply."

Despite the National Research Council's recommendations, many biotechnology firms are nonetheless using food grains as platforms for pharmaceutical production. As of 2003, over three-quarters of field trials conducted to produce pharmaceutical or industrial proteins (including fibers, plastics and enzymes) had involved maize, a wind-pollinated species (Union of Concerned Scientists 2003). Grain crops are favored because protein yields from the large seeds of maize, rice and barley are typically much higher than those obtained from leaves and other vegetative parts. In addition, pharmaceutical proteins can remain stable in dried grain for several years, compared to the much-reduced stability of these same proteins in leaf tissue. Moreover, maize is generally recognized as safe for ingestion by the U.S. Food and Drug Administration (FDA) and therefore can be used as an inactive carrier, suitable for drug delivery.

Despite these advantages, warnings from critics may be having an effect. A growing number of companies are focusing on tobacco, or even mosses, algae and duckweed, as platforms for pharmaceutical production (Fischer et al. 2004). These plants, however, pose risks of their own that must be considered. Algae and duckweed, if cultivated, would have greater potential than highly domesticated crop species to escape from cultivation.

Additional routes of exposure

Even if the production of pharmaceutical proteins was limited to nonfood crops, potential risks would remain. Pollen, fine particles of leaves that are crushed during harvest, and possibly even runoff water contaminated with proteins from decomposing plants, could expose people and wildlife that live on or near pharmaceutical-producing fields to the transgenic material. Whenever production occurs outside, birds, insects and other wildlife can consume pharmaceutical crops, regardless of where they are grown or what species they are. Pharmaceutical crops may also affect soils and the community of soil-dwelling organisms.

Such impacts on wildlife and soils have received scarce attention from scientists and regulators, but surely will vary greatly by variety depending on the nature of the protein being produced. One possible strategy to avoid these problems would be to engineer proteins so that they do not become biologically active until after they are extracted and further processed in a laboratory.

Regulatory responses

Pharmaceutical crop varieties are not expected to be deregulated; rather, it is likely that they will only be produced in field trials as permitted under USDA regulatory oversight. Initially, field-trial applications for pharmaceutical crops were treated like those for any other regulated, genetically modified crop. However, the USDA recently published stricter requirements specifically designed for plants genetically engineered to produce pharmaceutical and industrial proteins (Federal Register 2003). These new requirements aim to reduce the risk of gene flow and the contamination of food and feed. Confinement measures now required for pharmaceutical crops include greater geographic isolation from other fields of the same species, buffer zones of bare soil around the field edge, scouting for and destroying volunteer plants in subsequent field seasons, and the dedication of equipment for use only on the trial fields.

There is a precedent for the successful segregation of crop varieties intended for use in food from those intended for industry. Rapeseed varieties containing high levels of erucic acid are segregated from those used to produce canola oil, which must contain less than 2% erucic acid (Ma, Chikwamba et al. 2005). Erucic acid is used to create lubricants, coatings and surfactants, but the regular consumption of large amounts of erucic acid has been linked to heart disease in animal studies. Producers of high-erucic-acid rapeseed must maintain a minimum 16.4-foot buffer zone around their fields and clearly label harvested products. In addition, erucic acid levels in canola oil are regularly monitored by various food inspection agencies.

Although this example demonstrates the potential for successful segregation, more-stringent protocols would be required to produce pharmaceutical proteins in food crops. In the case of erucic acid, a low level of cross contamination is acceptable (Bilsborrow et al. 1998), but for pharmaceutical compounds there is generally zero tolerance. Studies examining the potential for the coexistence of other types of genetically modified crops with nongenetically modified varieties demonstrate that contamination can be limited (for example, less than 0.9%) but not entirely prevented (EuropaBio 2006). Moreover, in the rapeseed example, only one or two compounds must be monitored. In contrast, if maize is eventually used to produce some 50 or 100 different pharmaceutical compounds, the costs for systematic monitoring to ensure that none of these compounds contaminates maize intended for food or feed could be prohibitive.

In addition to rules governing how pharmaceutical crops are grown, USDA inspectors have publicly announced that field-test sites of such crops will each be inspected five times during the growing season and twice postharvest (Stewart and Knight 2005). However, based on an audit that included site visits to 91 field-test locations in 22 states, the USDA Office of the Inspector General found that this level of inspection was not consistently maintained. The audit report concluded that weaknesses in the regulatory oversight of genetically modified crop field-trials increase the chance that these crops will inadvertently persist in the environment (USDA 2005). Of additional concern, the audit found that, "At the conclusion of the field test, APHIS does not require permit holders to report on the final disposition of genetically modified pharmaceutical and industrial harvests. . . . As a result, [the inspectors] found two large harvests of genetically modified pharmaceutical crops remaining in storage at the field-test sites for over a year without APHIS's knowledge or approval of the storage facility" (USDA 2005). Clearly, better adherence to monitoring requirements is needed to minimize the risk of a loss of containment.

Although the 2003 regulations set forth by USDA are an important step, the proposed rules make no attempt

ANR Communication Services

In California, rice farmers strongly opposed efforts to grow 120 acres of rice genetically engineered to produce proteins for two pediatric medicines, fearing that their exports to Asia would be jeopardized. *Above*, a California rice farm (not genetically engineered).

to protect wildlife (fencing or netting are not required), assess how pollen or fine particulate matter from the crop might affect humans, or test soils and groundwater for pharmaceutical residues. Also missing is any requirement that the pharmaceutical variety be readily identifiable. For example, several authors have suggested that pharmaceuticals could be produced in "identity-preserved varieties, such as white tomatoes or maize, which are easily identified by their pigmentation" (Ma et al. 2003).

No specific requirements were proposed for molecular solutions to contamination, presumably because these are not sufficiently developed yet. However, molecular strategies hold great promise for the improved containment of transgenes. Examples include the genetic modification of chloroplast DNA rather than nuclear DNA (for crop species in which pollen does not contain chloroplasts, transgenes would not move with pollen) (Daniell et al. 2002) and the inducible production of pharmaceuticals (for example, the pharmaceutical protein is activated by exposure to ethanol vapor) (Mascia and Flavell 2004). The tissue-specific expression of pharmaceutical proteins may also reduce or eliminate certain avenues of exposure (such as the possibility of exposure via pollen inhalation), and gene deletion technologies could potentially be used to remove transgenes from certain tissues (such as pollen) to reduce the possibility of transgene spread (Keenan and Stemmer 2002).

If transgenes could be contained, then regulations could be much more permissive about which traits are allowed in crop plants. On the other hand, if transgenes will inevitably escape and spread — despite our best intentions for containment — then we must be much more cautious about which traits are allowed to be developed in crop plants. Alternatively, the cultivation of crops engineered to produce particularly hazardous pharmaceutical proteins might be restricted to greenhouses or other enclosed facilities, such as caves. Although production in such facilities is feasible, it would likely be far more expensive than field production.

Field-testing in California

The USDA database of field-trial permits for plants expressing pharmaceutical and industrial proteins includes many entries for which the petitioning organization has used a claim of Confidential Business Information to withhold from the public any information regarding the transgene, its source or the traits that have been altered (USDA APHIS 2007). It is therefore difficult to know exactly how many field trials of pharmaceutical crops have been approved in California. However, the Union of Concerned Scientists (2007) estimates that 18 permits for field trials involving pharmaceutical or industrial proteins were approved in California, the earliest in 1996 and one as recently as 2006 (table 1). According to this analysis, California is tied with Kentucky for seventh among U.S. states and territories, after Nebraska with 41 approved permits, Hawaii with 40, Puerto Rico

TABLE 1. USDA-approved field-trial permits allowing the growth of crops genetically engineered to produce pharmaceutical or industrial proteins in California, 1996–2006

Engineered crop	Applicant	Issued/ effective	Source of gene*	Pharmaceutical or industrial protein
Maize	Dow	6/2002	CBI†	CBI: Unidentified pharmaceutical protein
	Monsanto	3/2001	CBI	CBI: Unidentified transcriptional activator (pharmaceutical)
		3/2001	CBI	CBI: Unidentified transcriptional activator (pharmaceutical)
	Pioneer	3/2000	Unclear‡	CBI: Unidentified novel protein that may have pharmaceutical or industrial uses
		4/2001	Unclear	CBI: Unidentified novel protein that may have pharmaceutical or industrial uses
		4/2002	Unclear	CBI: Unidentified industrial enzyme and unidentified novel protein that may have pharmaceutical or industrial uses
		4/2004	Unclear	CBI: Unidentified novel protein that may have pharmaceutical or industrial uses
Leaf mustard	USDA Agricultural Research Service	3/2004	Unclear	CBI: Unidentified industrial enzyme
CBI		3/2004	CBI	CBI: Unidentified industrial enzyme
Rapeseed	Pioneer	9/1996	CBI	CBI: Unidentified pharmaceutical protein
Rice	Ventria Bioscience (formerly Applied Phytologics)	3/1997	Humans	Pharmaceutical proteins: Antithrombin and serum albumin
		2/1998	Humans	Pharmaceutical proteins: Antitrypsin, antithrombin and serum albumin
		2/1998	CBI	CBI: Unidentified pharmaceutical protein
		5/2000	CBI	CBI: Unidentified pharmaceutical protein and unidentified novel protein that may have pharmaceutical or industrial uses
		4/2001	Humans	Pharmaceutical proteins: Antitrypsin, lactoferrin and lysozyme
		4/2003	Humans	Pharmaceutical proteins: Lactoferrin and lysozyme
		5/2004	Humans	Pharmaceutical proteins: Lactoferrin and lysozyme
Tobacco	Planet Biotechnology	6/2006	Mice, rabbits, CBI	Antibodies to tooth decay and common cold

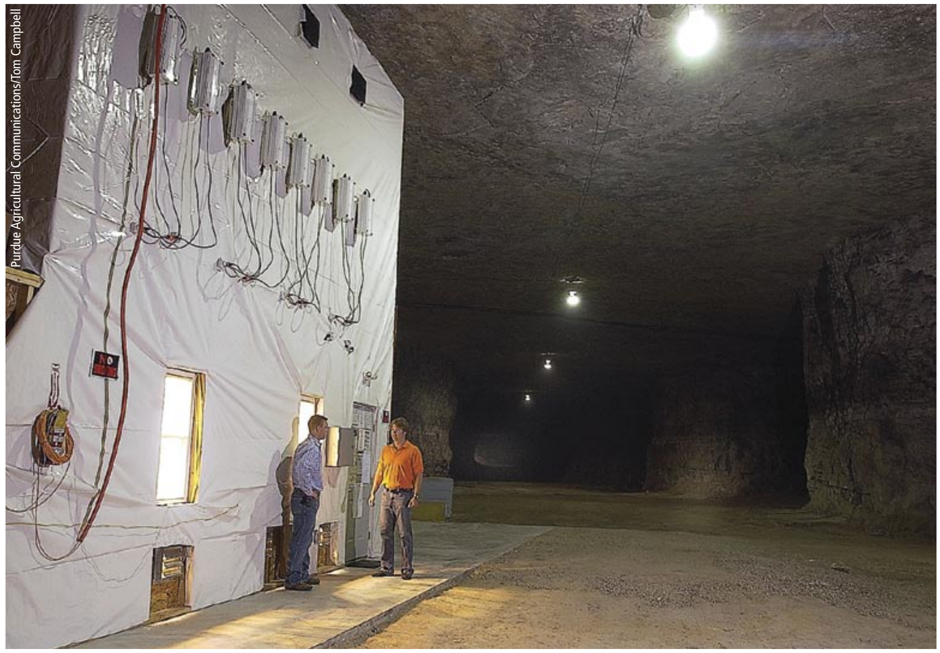
* Refers specifically to the gene coding for the industrial or pharmaceutical protein.
† CBI = Confidential Business Information.
‡ Source of gene coding for industrial and/or pharmaceutical protein(s) cannot be determined from publicly available information.
Source: Union of Concerned Scientists 2007.

with 39, Wisconsin with 29, Iowa with 27 and Illinois with 19.

Pharmaceutical rice. The production of pharmaceutical proteins in transgenic crops is meeting with some resistance in California, as Ventria Bioscience recently discovered. Ventria had received federal permits to grow approximately 100 acres of pharmaceutical rice in California almost annually since 1997 (see table 1). However, the company's plans to expand its 2004 field trials to 120 acres of rice engineered with synthetic human genes were met with strong opposition from California rice farmers and environmentalists. Ventria's rice has been genetically engineered to produce lactoferrin and lysozyme, compounds used to treat severe diarrhea in infants. However, farmers were concerned that even low levels of contamination of their rice crops could threaten exports to Asia.

The California Rice Certification Act of 2000 gave the California Rice Commission the authority to devise protocols governing the cultivation of any new rice variety that requires segregation. Despite farmers' concerns, on March 29, 2004, the commission approved planting guidelines for Ventria's expanded plantings in a 6 to 5 vote, on the condition that the field trials be conducted in counties such as Orange and San Diego, remote from the state's rice-growing regions. Due to the late timing of the commission's decision and the need to plant immediately, Ventria then asked the California Department of Food and Agriculture (CDFA) to issue an emergency permit for the proposed field trials. On April 9, 2004, CDFA decided not to approve Ventria's proposal because federal regulators at USDA had not yet completed their review of Ventria's permit application. California regulators essentially deferred to federal regulation, reasoning that federal oversight of the field-trial application is both necessary and sufficient. In 2005, Ventria attempted to move its field trials to Missouri, where it met similar resistance from major rice purchasers.

Local bans. Although California regulators may be happy to defer to USDA judgment when it comes to genetically modified crops, the public and local communities are not always so accommodating. Several counties have considered banning genetically modified crops outright, and in some cases bans have indeed been implemented. Bans on all genetically modified plants are in effect in four counties: Mendocino (Measure H, passed by voters in March 2004), Trinity (passed by the county board of supervisors in August 2004), Marin (Measure B, passed by voters in November 2004) and Santa Cruz (unanimously passed by the county board of supervisors in June 2006). In contrast, voters rejected initiatives to ban genetically modified crops in four counties: Humboldt, San Luis Obispo and Butte in 2004, and Sonoma in 2005. Supervisors in several other California counties, including Fresno, Kern and Kings, have passed resolutions supporting the use of genetically modified crops.


Purdue Agricultural Communications/Tom Campbell

In an abandoned Indiana mine, Controlled Pharming Ventures is working with Purdue University researchers to develop techniques for growing pharmaceutical crops underground, in order to limit risks.

The political future of local measures, either for or against genetically engineered crops, was recently challenged by Senate Bill 1056, which would have prohibited California counties, towns and cities from passing any local regulation of seeds and nursery plants. However, in September 2006, this bill failed to make it out of committee and died with the close of the legislative year. The failure of this bill leaves open the possibility of additional local restrictions on genetically modified crops in the future.

Economic considerations. In the end, economic concerns regarding the containment of food crops may outweigh concerns for the environment or even food safety. The contamination of U.S.-produced rice with the unapproved Liberty Link 601 (herbicide-resistant) variety has had an enormous economic impact on U.S. rice growers. U.S. exporters of long-grain rice lost about $150 million because genetically modified rice is banned throughout most of the European Union, a major importer of U.S. long-grain rice. Even greater economic losses would likely occur if a crop were found to be contaminated with a pharmaceutical protein. Whether pharmaceutical-producing crops will be accepted in California will likely depend on the economic value of other markets that might be placed at risk. A proposal to produce pharmaceutical rice within a major rice-producing area such as the Sacramento Valley is unlikely to be welcomed. However, a proposal to grow that same pharmaceutical rice in an area with very little other rice production may be acceptable.

Evaluating risks and benefits

All forms of agriculture entail some risks to the environment. Whenever food is grown, some species lose their habitat and some may be poisoned, trapped or shot; species extinctions are also possible. Pharmaceutical crops entail all of these same risks plus additional ones — the contamination of food and feed being the most serious. There

USDA/Ken Hammond

In a 2004 report, an expert panel of the National Research Council recommended that food crops should not be used to produce pharmaceutical crops, suggesting instead that nonfood crops such as tobacco (shown in Virginia) would be a wiser choice.

are three major approaches to evaluating the potential benefits and risks.

(1) Precautionary approach. A precautionary approach typically shifts the burden of proof onto the producer, so that a practice or product is not approved until there is sufficient scientific understanding of the potential risks. This approach has been adopted in many legal and policy arenas, including the transnational movement of living, genetically modified organisms under the Cartagena Protocol on Biosafety. Since all nations with commercial transgenic production must undertake safety testing (of some sort) prior to the commercial production of transgenic crops, a precautionary approach is already being applied to a certain degree (Conko 2003).

However, interpretations of the precautionary approach vary. A strong interpretation mandates that the producer demonstrate the absence of harmful effects prior to the release of the product. Given that harmful effects could be exceedingly rare, this represents an impossible standard from a scientific perspective. In contrast, a weak interpretation mandates that regulators should only consider delaying the approval of a practice or product when sufficient evidence of risk exists (Conko 2003). California counties with moratoria on all transgenic crops are adopting a strong interpretation of the precautionary approach, similar to European countries that require the labeling of any foods with genetically modified plant ingredients.

(2) Formal risk-assessment framework. The U.S. Environmental Protection Agency embraces a risk-assessment approach in all of its regulatory capacities, including the regulation of chemical pesticides and “biopesticides,” such as plants genetically modified to express insecticidal proteins. Risk is defined as a function of both hazard and exposure, such that either a low hazard or low probability of exposure will reduce the assessed level of risk. Hazard is a measure of the harmful effects of the pharmaceutical proteins on people and the environment; as such, not all are equally hazardous. For example, lactoferrin is naturally produced in human tears and breast milk. Assuming that plant-produced lactoferrin is very similar to human-produced lactoferrin, this compound would present little if any hazard to humans.

With regard to exposure, the potential routes and amounts of exposure to pharmaceutical compounds are expected to be highly variable. Exposure will depend upon which crop species is used as the production platform, where it is grown, and where the protein is and is not expressed within the plant (pollen, for example, is highly mobile). The amount of land needed to produce sufficient quantities of particular pharmaceuticals must also be considered; this will depend both upon demand for the product and the protein yields obtained per plant. Incorporating transgenes into chloroplast DNA rather than nuclear DNA could reduce exposure both by limiting the expression of the protein in pollen and by boosting the production of target proteins to a level where sufficient quantities could be produced in very small fields (Daniell et al. 2002).

(3) Cost-benefit analysis. An important component of the cost-benefit analysis approach is “fairness” — who benefits and who pays the costs. Fairness is a core value of many Americans, and environmental policy discussions increasingly focus on equitability and fairness. The precautionary approach and risk-assessment framework do not require the consideration of costs and benefits to stakeholder groups. But one explanation for the public’s reluctance regarding the production of pharmaceutical proteins in crop plants could be that the distribution of benefits (primarily to corporations) does not match the distribution of risks (primarily falling upon the general public).

Because biotech and pharmaceutical companies are the primary economic beneficiaries, the key questions for a cost-benefit approach applied to pharmaceutical crop production are whether the economic rewards outweigh the potential risks of unwanted pharmaceutical exposure, and whether the distribution of the costs and benefits is equitable and fair (Elbehri 2005). If economic profits are reinvested into the research and development of new drugs, then additional benefits for human and animal health may be achieved. In addition, drug prices might be reduced if it becomes inexpensive to manufacture drugs in large quantities. However, because most pharmaceutical crops are designed to produce patented pharmaceutical compounds, there would typically be little competition to drive prices lower. Furthermore, the research and development of pharmaceutical crops will likely remain very expensive.

Other potential benefits are possibly increased income for farmers and higher tax revenues (Wisner 2005). There is much hope that pharmaceutical crops will improve farmer incomes, but these benefits are unlikely in a global market where the production of pharmaceutical proteins in genetically modified crops could be undertaken in whichever nation has the lowest production costs and weakest regulatory restrictions (Wisner 2005). Another important issue for farmers concerns liability for contamination incidents. In the only precedent to date, ProdiGene was held accountable for its mistakes. Communities or regulatory agencies considering allowing the production of pharmaceutical crops will want assurances regarding who pays for any damages.

A promising new technology?

Like many new technologies, the genetic engineering of crops to produce pharmaceutical products has great promise. Bananas that could cheaply and easily deliver vaccines to children throughout the tropics could be a wonderful invention. But there are downsides; it will be difficult to avoid food contamination and potential harmful effects to wildlife if pharmaceuticals are widely produced in food crops grown out of doors.

Finally, the pros and cons of alternative strategies to achieve the same goals should be assessed (O'Brien 2000). For example, could certain pharmaceutical crops reasonably be confined to greenhouses, caves or other enclosed facilities? Are there other possible routes to the inexpensive and efficient production of drugs that perhaps do not involve the transgenic manipulation of crop plants? The future course of this technology will require thoughtful input from ecologists, public health experts and medical researchers — as well as those who genetically engineer these crops in the first place.

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RESEARCH ARTICLE

Growth stage influences level of resistance in glyphosate-resistant horseweed

by Anil Shrestha, Kurt J. Hembree *and* Neil Va

While glyphosate-resistant horseweed has not previously been reported in California, we suspected that it might exist, especially in noncrop areas. We collected horseweed seeds from two locations in the San Joaquin Valley and treated greenhouse-grown plants at different stages with different amounts of glyphosate. This study showed that a glyphosate-resistant biotype of horseweed exists in the noncrop areas of Dinuba, in Tulare County, and that the level of resistance may be influenced by the plant's growth stage at the time of glyphosate application.

A glyphosate-resistant horseweed plant shows the regrowth of new tissue 3 to 4 weeks after glyphosate was applied.

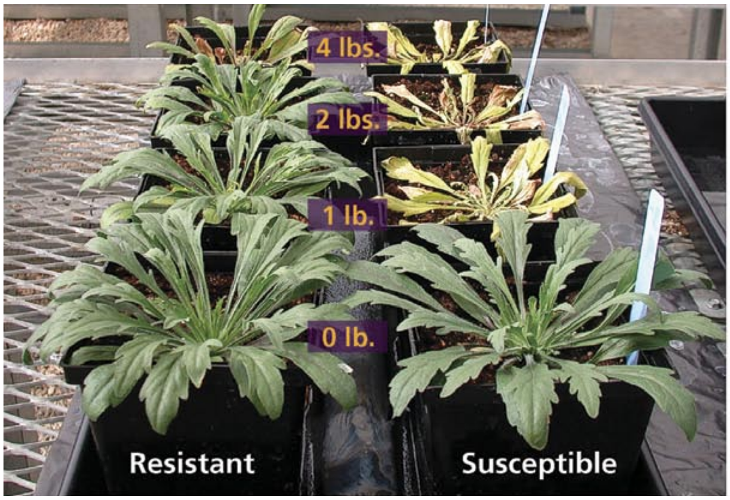
Horseweed, or marestail (*Conyza canadensis* L. Cronq.; Asteraceae family), is an annual, native, North American plant, which often colonizes roadsides, fallow fields, fencerows and the nontilled rows of perennial crops in the San Joaquin Valley. Although horseweed has been in California for a long time, increased invasions of this weed have been observed in orchards and vineyards in the southern San Joaquin Valley over the past 5 years.

In the past, horseweed was adequately controlled by pre- and postemergence herbicide treatments, fall and/or spring cultivation, or hand-pulling in perennial tree and vine crops. However, in recent years this weed has become more difficult to control. This may be because of emerging air-quality regulations that have led to restrictions on agricultural cultivation near urban areas, and water-quality concerns that have led to restrictions on the use of certain preemergence herbicides in the San Joaquin Valley. Furthermore, in years when commodity prices for crops such as raisins and stone fruits were low, weed control efforts were reduced in vineyards and orchards, and horseweed densities increased.

Growers who choose chemical weed control face challenges in the San Joaquin Valley. Many vineyards, orchards and noncrop areas (such as canal banks) in this region are located in Ground Water Protection Areas, state-designated areas that are vulnerable to pesticide leaching or runoff into groundwater (DPR 2004). Several preemergence herbicides, including simazine, that are effective on horseweed are now severely restricted in Ground Water Protection Areas. Instead, relatively inexpensive, broad-spectrum, postemergence herbicides — primarily glyphosate (Roundup) — are often used in these areas. (Postemergence herbicides are applied on the weeds after they emerge and thus they do not leach into the groundwater, as opposed to preemergence herbicides that are applied to the soil and incorporated.)

However, in various parts of the world horseweed has developed resistance to broad-spectrum herbicides such as the triazines (Gressel et al. 1982; Heap 2007), paraquat (Smisek et al. 1998), the acetolactate synthase inhibitors (Heap 2007) and glyphosate (VanGessel 2001). The first case of a glyphosate-resistant horseweed in North America was reported in Delaware in 2000 (VanGessel 2001). Since then, 13 other states have reported glyphosate-resistant horseweed (Heap 2007). All of these reports were from annual row-crop systems such as cotton (*Gossypium* sp.) and soybean (*Glycine max*). Repeated use of the same herbicide is the main reason that weeds develop herbicide resistance worldwide (Holt 1992). Researchers believe that the intensive use of glyphosate in crops that are genetically engineered to be resistant to this herbicide has resulted in the selection of weed populations that are also naturally resistant (Nandula et al. 2005). Although there are reports of glyphosate-resistant horseweed in pe-

Relevance to biotech risks and benefits: This article reports data of value to those dealing with weeds resistant to glyphosate herbicides (e.g. Roundup). The development of herbicide-resistant weeds is a risk in places where genetically modified, glyphosate-resistant crops are grown. However, this article did not specifically examine that possibility.



Glyphosate was applied at increasing rates (0, 1, 2 and 4 pounds active ingredient per acre) to resistant horseweed plants from Dinuba, Tulare County (left) and susceptible plants from Fresno County (right).

Seeds for this study were collected from horseweed plants that had survived glyphosate applications along a canal bank in Dinuba, Tulare County.

rennial cropping systems in Brazil and China (Heap 2007), there have been no reports of glyphosate-resistant horseweed in perennial cropping systems or noncrop areas in North America.

A field manager in Dinuba, Tulare County, recently reported poor control of horseweed with glyphosate on an irrigation canal bank. Glyphosate had been used repeatedly at this site during the previous several years, and we suspected glyphosate resistance after observing plants that escaped treatment and those that were controlled by glyphosate at this site. Preliminary studies at this site and in the greenhouse showed that while horseweed seedlings were effectively controlled by glyphosate at a rate of 4 pounds active ingredient per acre (ai/ac), about 40% of the horseweed at the 18- to 21-leaf rosette stage did survive. We decided to test the Dinuba horseweed for glyphosate resistance and evaluate how glyphosate rate and plant growth stage affect resistance.

Testing for glyphosate resistance

We collected horseweed seeds from the suspected glyphosate-resistant (GR) population in Dinuba (36°29′15″ N; 119°24′10″ W) and from a population in western Fresno County believed to be glyphosate-susceptible (GS), where effective control had been obtained with a labeled rate of glyphosate (control). Seeds were collected in fall 2004 and stored at room temperature (70°F). The experiment was done twice, first with seeds planted in early spring and second with seeds planted in late summer. The first experiment was conducted from April 6 to Sept. 4, 2005, and the second from Aug. 10, 2005, to Jan. 3, 2006.

Horseweed seeds were planted in plastic germination trays in the lab and moved to a greenhouse following emergence. The greenhouse temperatures were set at 75°F to 80°F during the day and 60°F to 65°F at night. No supplemental lighting was used. When the seedlings developed two to three leaves, they were transplanted into plastic pots (6 inches deep and 4 inches wide) containing a commercial potting mix. For the purpose of this study, seeds from the Dinuba and west Fresno County sites were designated as GR and GS horseweed biotypes, respectively.

The experimental design was a two-factor, completely randomized block with five replications. The two factors included five glyphosate application timings based on horseweed growth stage (5 to 8 true leaves, 11 to 15 true leaves, 18 to 21 true leaves, bolting to 6 inches tall, and 6.1 to 12 inches tall) and four rates of glyphosate (0, 1, 2 and 4 pounds ai/ac). A fully expanded leaf was considered a true leaf. Four extra plants (two GR and two GS) were included in each replication at 18 to 21 true leaves (rosette stage) for glyphosate treatments of 8 and 16 pounds ai/ac. These extra plants were included because growers and land managers generally treat horseweed at the rosette stage in early spring in the San Joaquin Valley. There were 44 pots containing either the GR or GS biotype for each of the five growth stages, for a total of 220 pots. The plants were watered regularly and fertilized twice during the growing season with a commercial fertilizer (MiracleGro).

Glyphosate, formulated as Roundup Weathermax (5.5 pounds ai/gallon), was used in the study. No additional surfactants were added to the spray solution. Treatments were applied at the designated growth stage with a carbon dioxide backpack-sprayer. The spray was discharged 18 inches above the target plants through a 40-inch boom with a single flat-fan nozzle (TeeJet XR8002EVS) in the center and a blank at each end. The system was pressurized to 30 pounds per square inch (psi) to deliver the herbicide solution at 35 gallons per acre (gpa; broadcast acre basis) in a 20-inch band. The plants were moved outside the greenhouse, sprayed and moved back into the greenhouse. The mortality of each plant was evaluated weekly, and classified as "alive" or "dead." Plants were designated as dead when the aboveground plant parts disintegrated and showed no traces of green tissue.

In the first experiment, survivors were allowed to grow until anthesis (the period during which a flower is fully open) of the first flower, and then the aboveground biomass was collected. In the second experiment, biomass was collected prior to flowering due to a severe aphid infestation. In both experiments, plants were clipped at the surface of the soil, placed in separate paper bags, dried

Problematic horseweed infestations have become more common among perennial crops in the San Joaquin Valley, such as near this Fresno County raisin vineyard. Treatments should be applied soon after horseweed emerges, before plants develop more than eight true leaves.

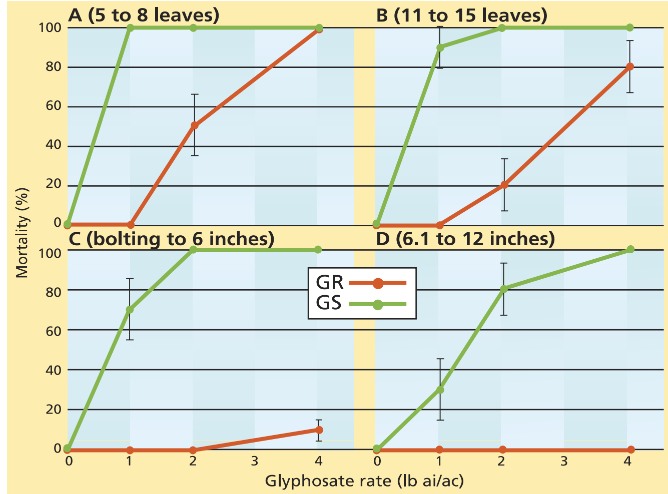


Fig. 1. Percentage mortality of glyphosate-resistant (GR) and glyphosate-susceptible (GS) horseweed plants under different glyphosate rates sprayed at the (A) 5- to 8-leaf, (B) 11- to 15-leaf, (C) bolting to 6-inch and (D) 6.1- to 12-inch stages.

to constant weight in a forced-air oven at 140°F, and their dry weights recorded.

Mortality and shoot biomass data were subjected to analysis of variance using GLM procedures in SAS with an alpha level of 0.05. Mortality data for both experiments were combined because there were no interactions ($P > 0.05$) between experiment and biotype or between experiment and glyphosate rate for plant mortality. Shoot biomass data was analyzed separately for the two experiments because of differences in the development stage of the plants at the time of harvest.

Mortality of the GS and GR horseweed plants at the rosette stage was regressed against glyphosate rate using a nonlinear sigmoidal dose-response model in SigmaPlot:

$$Y = \text{min.} + \frac{\text{max.} - \text{min.}}{1 + 10^{(LD_{50} - x)}} \quad [1],$$

where Y is plant mortality, min. is the minimum response limit (the minimum dose required for plant mortality among a group of plants), max. is the maximum response limit, LD_{50} is the herbicide rate to achieve 50% mortality, and x is the concentration of glyphosate rate.

Growth stage and mortality

While Koger et al. (2004) found that the growth stage of horseweed had little effect on the level of glyphosate resistance, our data indicated that the level of resistance is influenced by the growth stage at the time of glyphosate application. At the 5- to 8-leaf stage, all of the GR horseweed plants survived the 1-pound glyphosate treatment (fig. 1A). However, only half survived the 2-pound treatment, while none survived the 4-pound treatment. At this stage, none of the GS plants survived any of the glyphosate treatments (fig. 1A).

At the 11- to 15-leaf stage, all of the GR horseweed plants survived the 1-pound glyphosate treatment while only 20% survived the 4-pound treatment (fig. 1B). In contrast to the 5- to 8-leaf stage, 10% of the GS plants at the 11- to 15-leaf stage survived the 1-pound glyphosate treatment (fig. 1B). After the plants bolted, most of the GR plants survived the 4-pound glyphosate treatment (figs. 1C, 1D). Similarly, delaying glyphosate application until bolting increased the chances of survival for GS plants, as 30% of them survived the 1-pound treatment (fig. 1C). Tolerance of GS plants to glyphosate further increased when the herbicide was applied at the 6.1- to 12-inch stage, as 70% and 20% of the plants survived the 1- and 2-pound treatments, respectively (fig. 1D).

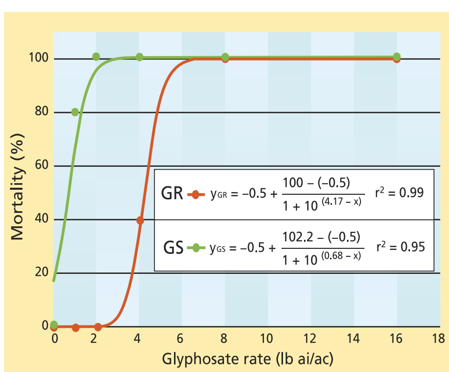


Fig. 2. Nonlinear regression of percentage mortality of glyphosate-resistant (GR) and glyphosate-susceptible (GS) horseweed plants as a function of different glyphosate rates sprayed at the 18- to 21-leaf (rosette) stage.

Glyphosate resistance in horseweed is believed to be due to the limited translocation of glyphosate to the roots and growing points of the plant (Feng et al. 2004). This could also be the reason for the increased glyphosate resistance that we found at later growth stages in the GR plants.

Based on a nonlinear regression model predicting percentage mortality as a function of herbicide rate, the glyphosate treatments required to kill 50% of the GR and GS plants at the rosette stage were 4.17 and 0.68 pounds, respectively (fig. 2). We found that at the rosette stage, 20% of the GS plants survived the 1-pound treatment and none survived the higher treatments, while none of the GR plants survived the 8- or 16-pound treatments. Therefore, based on the model, the GR plants were approximately six times more resistant to glyphosate than the GS plants at the rosette stage. Similarly, VanGessel (2001) reported an 8- to 13-fold increase in glyphosate resistance in GR horseweed biotypes from Delaware, and Koger et al. (2004) reported an 8- to 12-fold increase in glyphosate resistance in GR horseweed biotypes from Mississippi.

Plant biomass effects

The GR plants from Dinuba generally grew bigger than the GS plants

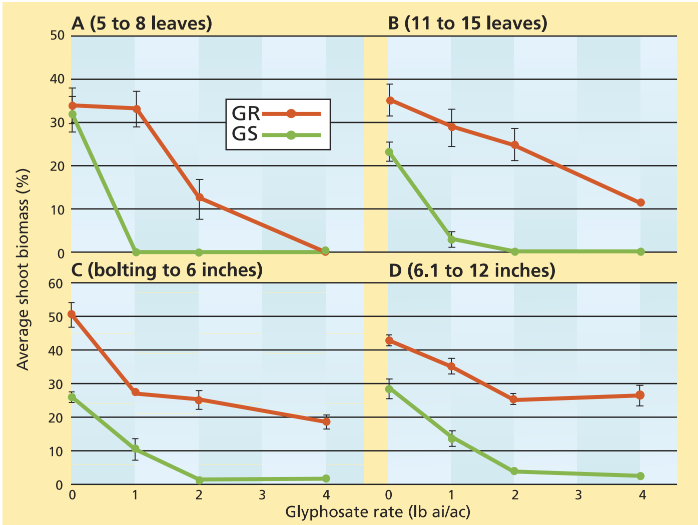


Fig. 3. Average shoot biomass (± SE) of glyphosate-resistant (GR) and glyphosate-susceptible (GS) horseweed plants in experiment 1 as a function of different glyphosate rates sprayed at the (A) 5- to 8-leaf, (B) 11- to 15-leaf, (C) bolting to 6-inch and (D) 6.1- to 12-inch stages.

from Fresno County. A *t*-test for the control treatment (no glyphosate) showed that at the time of flowering, the GR plants had 31% more aboveground biomass than the GS plants in the first experiment, and 27% more in the second experiment.

Higher glyphosate levels generally reduced the shoot biomass of the GR plants that survived the herbicide application (figs. 3A–D). For example, shoot biomass was reduced 18%, 30% and 67% by the 1-, 2- and 4-pound glyphosate treatments, respectively, when the herbicide was applied at the 11- to 15-leaf stage (fig. 3B). When glyphosate was applied at the 5- to 8-leaf stage, some GR plants survived the 2-pound treatment, but they were stunted and accumulated very little biomass compared to the untreated control plants (fig. 3A). In addition, the shoot biomass of the GR horseweed was greater than that of the GS horseweed even under nonsprayed conditions (figs. 3B–D). Although a few GS plants escaped the 1- and 2-pound treatments when glyphosate was applied postbolting, their shoot biomass was reduced up to 92% (fig. 3D). Similar reductions in shoot biomass of GR plants were also observed in the second experiment as glyphosate rates increased (data not shown).

Resistance and horseweed control

In these experiments, most of the GR horseweed plants that initially appeared to be dead began growing again approximately 3 to 4 weeks after glyphosate application, producing green leaves from the center of the rosette and starting to reaccumulate shoot biomass. Although the biomass of the horseweed was reduced in our study, all the surviving plants still produced flowers in the first experiment. We found that the GR plants from Dinuba amassed more aboveground biomass than those from Fresno County and appeared more vigorous. However, it is difficult to associate glyphosate resistance with plant vigor. A comparative study of progeny of GR and GS plants derived from the same parents may be needed to verify if there are any fitness or plant vigor costs associated with glyphosate resistance in horseweed.

Our results showed that the horseweed from Dinuba was resistant to glyphosate, but that the level of resistance varied with growth stage. GR plants from Dinuba could likely be controlled at the 5- to 8-leaf stage with 2- and 4-pound glyphosate treatments. At later stages, even some of the GS horseweed from Fresno County escaped the lower rates of glyphosate. These results highlight the importance of controlling weeds at an early growth stage.

When a postemergence herbicide such as glyphosate is used for horseweed control, it is important to apply the treatment soon after the horseweed emerges, preferably before plants develop more than eight true leaves. This may result in complete control of GS and partial control of GR plants. The application of glyphosate to horseweed during or after the bolting stage can result in some escapes of GS plants and no control of GR plants. If the horseweed population is to be reduced, several successive postemergence herbicide applications are needed to control plants that may emerge in multiple flushes over the growing season. This could result in further increase in herbicide use in Ground Water Protection Areas and perennial cropping systems that rely solely on postemergence weed control.

In order to prevent or delay the onset of herbicide resistance in horseweed, an integrated program should be developed to manage this plant, particularly in noncrop areas, orchards and vineyards located in Ground Water Protection Areas of the San Joaquin Valley. Similarly, a resistance management strategy must also be adopted for this weed in glyphosate-resistant crops. Although the difference in the intra- and interspecific competitive ability of the two biotypes is unknown, it may be beneficial to prevent the spread of glyphosate-resistant Dinuba-type horseweed because this biotype appears to be more vigorous than the glyphosate-susceptible Fresno County biotype.

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RESEARCH ARTICLE

IPM program successful in California greenhouse cut roses

by Christine Casey, Julie Newman, Karen Robb, Steven A. Tjosvold, James D. MacDonald *and* Michael P. Parrella

We developed and tested an integrated pest management (IPM) program for the key pests of cut roses, which was based on fixed precision sampling plans, thresholds, biological control, directed sprays of reduced-risk pesticides, and cultural control. This program represented the largest effort to date to implement an IPM program in U.S. floriculture. The biological control of mites was successful at all locations, and pesticide use was generally lower in the IPM greenhouses. Future work will concentrate on reducing scouting time, improving natural-enemy release methods, and developing IPM techniques for secondary pests and powdery mildew.

Jack Kelly Clark/UC Davis

California nurseries produce two-thirds of the cut roses grown in the United States, with a wholesale value of $45 million. Pest control options have been limited in the past, resulting in the heavy use of pesticides and increasing resistance in important pests such as western flower thrips and twospotted spider mites.

Rose production is currently the largest component of California's $300 million cut-flower industry. In 2001, California growers produced 66% of the U.S. rose crop, with a wholesale value of $45 million (USDA 2002). The key pests of cut roses are twospotted spider mites *(Tetranychus urticae),* western flower thrips *(Frankliniella occidentalis)* and rose powdery mildew *(Sphaerotheca pannosa rosae).*

The twospotted spider mite is a foliage feeder that extracts the cell contents from leaves. This feeding causes foliar stippling and can disrupt the plant's photosynthetic and water balance mechanisms (Tomczyk and Kropczynska 1985). The western flower thrips is both a foliage and flower feeder, although it feeds primarily on flowers in the cut-rose system (Robb 1989). Powdery mildew is probably the most widespread and best-known disease of roses. The fungus produces a white, powdery-appearing growth of mycelium and conidia on leaves, which can cause distortion, discoloration and premature senescence. Although it causes some disruption of photosynthesis and transpiration control, the key impact of powdery mildew is reduced aesthetic value caused by the white, powdery spots and leaf distortion.

Fresh cut roses are often harvested twice daily, so revised reentry intervals imposed by the U.S. Environmental Protection Agency (EPA) after pesticide application limit the number of pesticides that are useful in this production system (EPA 1995). In addition, the typical number of pesticide sprays applied to roses grown for cut flowers has impeded the implementation of integrated pest management (IPM) procedures, particularly the use of biological controls. The IPM approach to pest management incorporates all cost-effective control tactics appropriate for the crop, including biological, cultural and chemical controls.

Pesticides that target hard-to-kill floriculture pests frequently kill natural enemies as well, which favors continued reliance on conventional pesticides while discouraging the adoption of biological control. Heavy pesticide use against key pests in the greenhouse has resulted in the widespread development of pesticide resistance in western flower thrips (Immaraju et al. 1992; Jensen 2000), mites (Ramdev et al. 1988; Fergusson-Kolmes et al. 1991), whiteflies (Prabhaker et al. 1985), aphids (Kerns and Gaylor 1992) and leafminers (Sanderson et al. 1989). The heavy use of pesticides in cut roses is also a worker safety concern in global (Tenenbaum 2002) and local (Warrick 2000) production. California rose growers reached a crisis point about 8 years ago, when pesticide resistance, costs and limited pesticide availability threatened the growers' ability to effectively manage twospotted spider mites.

At the same time, a new cut-rose production system that favors the success of IPM was gaining widespread acceptance. Roses were traditionally grown in soil with a hedgerow training system, where flowers are cut in a manner that gradually creates a 7-foot

Traditionally, greenhouse cut roses were grown in hedges, *right.* In the late 1990s, a new bent-shoot system, *left,* was developed that cultivates plants in raised containers with modified hydroponics. The new bent-shoot system helped lay the groundwork of an integrated pest management program for rose growers.

(2.13-meter) or taller hedge. The hedges are pruned back annually to about a 3-foot height and the process is begun again. With the new bent-shoot method, plants are grown in raised containers in a modified hydroponics system. Most of the shoots are bent downward at the crown to intercept more light, creating a perennial lower canopy that exists for the 5 to 8 years of crop production. The upper canopy contains only stems that produce flowers, which take 45 to 52 days to develop. The bent-shoot method creates a spatial separation between the harvested flowers and perennial foliage that does not exist in standard roses. Pesticides to control western flower thrips and powdery mildew that are more compatible with mite predators have also recently become available. These developments, coupled with the difficulty that rose growers were facing in controlling spider mites, made us confident that we could develop a successful IPM program that rose growers would adopt.

This project was initiated in 2000 with major funding from the Pest Management Alliance Program of the California Department of Pesticide Regulation and was later supplemented with additional funding. The goal of the Alliance project was to foster a team approach to the development and implementation of IPM programs in a given commodity and to document a reduction in traditional pesticide use. Our Alliance team included researchers, county-based advisors, growers, chemical and biological-control industry representatives, commodity associations and government officials. Our objective was to develop a cost-effective IPM program for the key pests of cut roses that included sampling, thresholds, biological control and directed sprays of reduced-risk pesticides.

Implementing the IPM program

Eight growers spanning the major rose-producing areas of California (San Diego, Santa Barbara and Santa Cruz counties) participated in the program. Each grower contributed an IPM and a conventional-practice greenhouse; all greenhouses were between 5,000 and 10,000 square feet (465 to 929 square meters) in size. All pest management decisions in the IPM greenhouses were based on the IPM program that we developed, while the grower made all pest management decisions in the conventional greenhouses. Data was collected and compared on a weekly basis by trained scouts using a comprehensive sampling plan that provided information about the density of insects, mites and diseases. The project included growers with several different rose varieties and both the bent-cane and hedgerow training techniques, but we kept these two variables standardized within a location. Implementation began in March 2000 and continued until January 2001.

Fixed precision sampling plans that had been previously developed for twospotted spider mites (Casey 2002) and western flower thrips (Casey and Parrella 2000) were used in our scouting program. This type of sampling plan was developed through intensive surveys of a crop to determine a pest's spatial distribution. The degree of acceptable error (the "precision" of the plan) was decided upon (or "fixed") in advance, and the number of samples needed to obtain that precision was calculated using knowledge of the pest's spatial distribution in the crop. We used a precision of 0.25, which is acceptable for pest management sampling (Southwood 1978). Generally, as spatial distribution becomes more aggregated (clumped), more samples are required to determine pest density with the desired precision. Although they take some effort to develop, these types of sampling plans are often more accurate and efficient than other sampling approaches. This study represents the first use of such plans in a floriculture IPM program. Sampling for all other pests was done during sampling and inspection for twospotted spider mites. Data was collated and summarized by the scouts and then discussed by members of the Alliance team. The scouts then met with the growers to discuss control strategies. Based on thresholds developed for each of the pests, no action was taken; cultural controls were used; biological control agents were released; or a pesticide application was made. Each greenhouse was a replicate, and ANOVA was used to determine whether there were differences between the conventional and IPM treatments.

Photos: Jack Kelly Clark/UC Davis

Left, **the adult western flower thrips feeds primarily on rose flowers, leaving scabby, brown scars, *right,* that can indicate feeding in unopened buds. The IPM program developed weekly threshold limits for treating thrips, and tested targeted lower-volume sprays to just the upper canopy of flowers.**

Twospotted spider mites

The first leaf above the bend on 38 randomly selected plants was sampled per 10,000 square feet (929 square meters) of greenhouse area to estimate mite density at the desired precision. Plants were classified as infested if the scout found more than five mobile mites (eggs were not counted) on the sampled leaf, or not infested if there were five or fewer. These samples were also used to determine co-occurrence of twospotted spider mites with the predatory mite *Phytoseiulus persimilis,* and they were inspected for secondary pests and diseases. In addition to the fixed samples, the scouts took directed samples as they walked down each row and noticed damage by insects, mites or pathogens. These plants were flagged for potential spot treatments.

In the IPM greenhouses, mite treatments were initiated according to the percentage of infested plants (table 1). Chemical controls included azadirachtin (Azatin), bifenazate (Floramite) and insecticidal soap (M-Pede), all of which provide some level of compatibility with *P. persimilis*. Releases of predatory mites were based on the co-occurrence of twospotted spider mites and predators on the sampled leaf. Co-occurrence is the percentage of plants with twospotted spider mites on which *P. persimilis* also occurs. This idea has been discussed in the literature as a theoretical basis for natural enemy releases, but has never been tested in practice (Nachman 1981; Ryoo 1996; Greco et al. 1999). We chose to include this method in our program because our natural enemy supplier recommended it to growers. Additional predatory mites were released when co-occurrence was less than 10%. All predator releases were made to leaves just below those on which twospotted spider mites were present. Predators were kept refrigerated and were released as soon as possible after arrival at the greenhouse, as per the supplier's instructions.

Targeting western flower thrips

A fixed precision sampling plan for western flower thrips was also developed (Casey and Parrella 2000). This sampling plan used yellow sticky traps and a general threshold of 25 to 50 thrips per trap per week (Parrella et al. 2003). Three 4-by-6-inch (10-by-15-centimeter) yellow sticky traps (Seabright Laboratories) with both sides exposed were placed per 10,000 square feet (929 square meters). The traps were placed at flower level and were evenly distributed in the greenhouse (for example, at the ends and center of the middle row). The lower threshold of 25 thrips per trap per week was used in more-susceptible varieties (generally white or yellow

TABLE 1. Control actions for twospotted spider mite based on percentage of infested plants

Mite density	Action
% samples infested	
0–10	Do nothing
> 10–25*	Biological control *(Phytoseiulus persimilis)*, with release rate based on proportion of co-occurrence of mites and predators
> 25	Chemical controls

* 25% infested = 4.5 *T. urticae*/leaf.

Photos: Jack Kelly Clark/UC Davis

The twospotted spider mite feeds on foliage, disrupting photosynthesis and water usage.

An infestation of twospotted spider mites causes stippled, bleached rose foliage.

***Phytoseiulus persimilis* eats twospotted spider mite eggs, acting as a biological control.**

flowers) and in areas of heavy thrips pressure. The higher threshold of 50 thrips per trap per week was used in less-susceptible varieties (generally red flowers).

There is currently no cost-effective biological control agent for western flower thrips in cut roses, so control of this pest in the IPM greenhouses included both cultural and chemical methods. Although the female thrips lays eggs in the flower or in foliage directly below the flower, the development time for eggs and larvae is longer than the 5 to 6 days between sepal split (when eggs are first laid) and flower harvest (Robb 1989). Routine flower harvest removes immature thrips from the greenhouse and subsequently there is little thrips reproduction in the rose greenhouse unless open flowers (those that are too mature for harvest) are left on the rose plant. Teerling (2000) has measured significantly higher thrips populations in Canadian rose greenhouses when these flowers are not removed.

Cultural control was the removal of open flowers, and chemical control was applications of spinosad (Conserve) or azadirachtin (Azatin) directed to the flowers when the thrips-per-trap-per-week threshold was reached. Research on the distribution of thrips in the rose range has revealed that most thrips are found near the developing flower (Parrella et al. 2003). Based on these findings, we then conducted a trial to determine whether sprays directed toward the flowers would provide control equivalent to full-volume wet sprays.

Such a study is critical to the implementation of IPM in the rose range, because a typical full-volume spray in roses may reach hundreds of gallons of water per acre. Such high volume thoroughly wets the foliage, but creates problems with runoff and affects biological control agents regardless of where they are on the plant. In separate rose greenhouses, we initiated a replicated study where rose beds were divided into 20-foot sections and applications of registered pesticides were made using full-volume wet sprays at 275 gallons per acre (2,555 liters per hectare) versus the same material applied just to the upper canopy (the flowers) at 70 gallons per acre (662.5 liters per hectare). Registered materials — acephate (Orthene), methiocarb (Mesurol) and spinosad (Conserve) — at label-recommended rates were used in the study. There were four replicated, 20-foot (6-meter) rows for each material per volume combination, and applications were made for 4 weeks.

At the end of this time, 10 flowers were removed from each section and examined for the presence of thrips.

Predatory mites were successfully used in all of the IPM greenhouses and almost eliminated the need for miticides.

Analysis of the mean number of thrips per flower (t-test, $P > 0.05$) revealed no difference in the performance of any material, despite the reduction in spray volume (fig. 1). In all subsequent control efforts against thrips in our IPM program, lower-volume directed applications were made. This reduced the amount of runoff and active ingredient used and helped conserve *P. persimilis* that had been released into the lower canopy.

Powdery mildew control

Our effort to introduce IPM principles in the management of powdery mildew

Jack Kelly Clark/UC Davis

Powdery mildew is a fungus that grows on rose leaves, but usually not flowers or petals. It is generally treated with fungicides to improve the plant's appearance. A better model is needed to predict when and if they should be sprayed.

centered on an attempt to use a predictive model for powdery mildew of grapevines (Gubler et al. 1999). The UC Davis powdery mildew risk-assessment model for grapevines is based on the effect of temperature on the reproductive rate of the pathogen following initial plant infection. As temperatures are recorded in vineyards, risk points are accumulated if temperatures are favorable (between 70°F and 85°F for 6 hours or longer) or subtracted if temperatures are not favorable. When risk points (on a scale of 0 to 100) reach a predetermined threshold, fungicide application is recommended. This model has been effective in determining if and when fungicide treatments need to be applied to grapevines, and has resulted in effective disease management with significantly reduced fungicide usage in California.

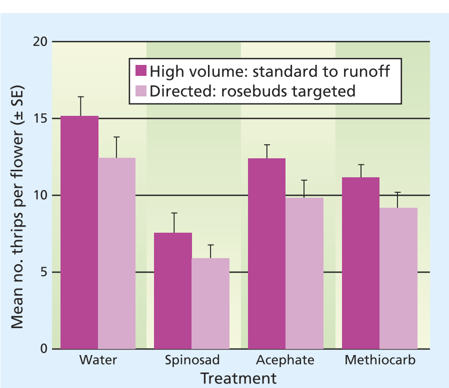

Fig. 1. Thrips control using selected pesticides and different volumes of water. No significant differences were detected when different volumes of water were used, regardless of the insecticide (*t*-test, $P > 0.05$).

In commercial rose greenhouses, growers spray regularly weekly during mildew season in Central California (April to October) and all year long in Southern California. It is not unusual for half of all yearly pesticide sprays in a rose crop to be for mildew control, presenting a strong argument for matching applications to actual risks. Although the powdery mildew fungus attacking roses is a different species, its response to environmental conditions (Horst 1989) is similar to that of the species attacking grapevines. For this reason, we sought to determine whether the grapevine mildew model (GMM) could be easily adapted to greenhouse-grown roses.

The greenhouses used in this effort were instrumented so that temperature, relative humidity and leaf wetness were measured at 30-minute intervals throughout the day and night. Temperature data was fed into the GMM to add or subtract risk points. In order to correlate actual disease development with the GMM risk points, a trained scout evaluated plants in the greenhouses weekly. This was accomplished by walking through the greenhouses in a predetermined pattern, stopping at regular intervals and evaluating one plant at each stop-point to assess disease incidence and severity. Disease incidence was determined by the presence or absence of mildew lesions on the leaves of harvestable stems. Disease severity was determined by counting the actual number of lesions on leaves attached to the harvestable stems. This data was used to calculate an overall disease rating for the crop that could be compared to risk predictions based on the GMM.

Along with the disease incidence and severity data, we recorded the timing of all chemical fungicide and insecticide applications made by the growers in the IPM houses so that we could evaluate these effects on disease ratings. As a resistance management practice, growers typically varied the fungicide materials used throughout

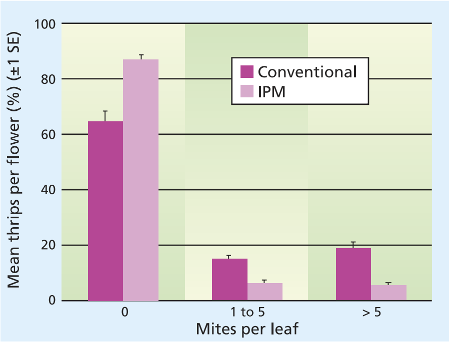

Fig. 2. Twospotted spider mite densities under conventional and IPM programs across all nurseries. There were significantly more plants with no mites ($P < 0.0001$; F = 33.84) and significantly fewer plants with mites at the other levels measured (1 to 5/leaf, $P < 0.0001$, F = 22.88; > 5/leaf, $P < 0.0001$, F = 23.33).

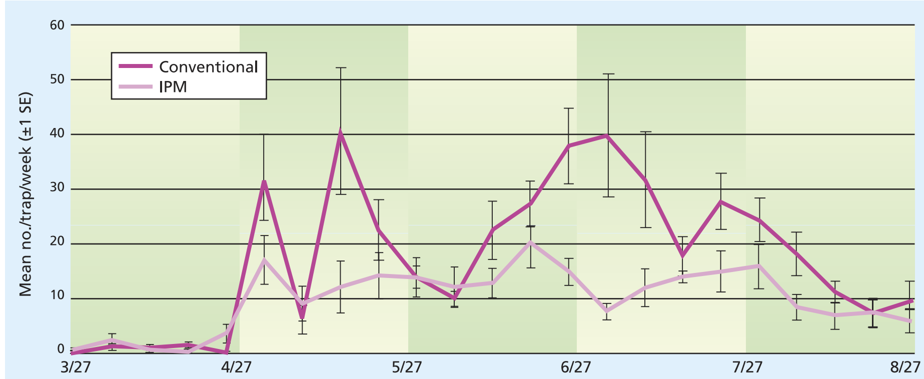

Fig. 3. Western flower thrips populations in conventional versus IPM greenhouses by date. The largest differences in thrips levels between the conventional and IPM treatments were observed from mid-June to mid-August 2000, the period of peak thrips pressure. There was a significant difference between thrips populations under the two control techniques ($P < 0.0001$, F = 34.13)

the season. A few fungicides were common across all locations, but growers did differ in some of the materials applied. For example, if powdery mildew became severe in a greenhouse, growers at all locations would typically apply piperalin (Pipron) because of its eradicative properties. Other materials used at the various locations included myclobutanil (Systhane), chlorothalonil (Daconil), benzeneacetic acid (Compass), azoxystrobin (Heritage), insecticidal soap (M-Pede) and potassium bicarbonate (Kaligreen).

Monitoring for secondary pests

Plants in both the IPM and conventional greenhouses were inspected for whiteflies, aphids, mealybugs, Botrytis, downy mildew and rust as part of the inspections for twospotted spider mites. The same traps that were used to monitor western flower thrips were also used to monitor whiteflies and winged aphids. We emphasized the use of materials that were compatible with the *P. persimilis* predator for control of these pests when necessary.

Was the IPM program successful?

Twospotted spider mites. Predatory mites were successfully used in all of the IPM greenhouses and almost eliminated the need for miticide applications in those houses. A comparison of twospotted spider mite levels under IPM and conventional control across all nurseries revealed that there were significantly more plants with no mites (0 mites/leaf) and significantly fewer plants with mites at the two levels measured in the IPM greenhouses (1 to 5 mites/leaf and > 5 mites/leaf) (fig. 2). Similar results were observed at the individual nurseries.

The cost of IPM during the first 8 weeks was higher than the cost of conventional control (table 2). Higher release rates were needed during this startup period for several reasons, including increased predator mortality as growers learned proper release techniques and the desire of some growers to begin biological control when twospotted spider mite densities were greater than the 25% infested threshold. After several releases had been made and predators became established, the release rate dropped and costs for the two control programs were comparable.

Western flower thrips. The monitoring program and the use of reduced-risk pesticides to control western flower thrips worked very effectively in the IPM greenhouses. This was a critical component of the entire program, because thrips are considered the key pest of roses. The need to control thrips with pesticides often limits the use of biological control in floriculture crops. Significantly fewer western flower thrips were caught in the IPM houses than in the conventional houses across all nurseries. The largest differences in thrips levels between the two treatments occurred during the summer months, when western flower thrips pressure is generally highest (fig. 3). There were also greater fluctuations in the overall densities of western flower thrips in the conventional houses, as well as more variation between individual conventional houses during the time of peak thrips pressure, compared to the IPM greenhouses. We attribute both of these observations to the regular removal of open flowers in the lower canopy that occurred under IPM but not in the conventional houses.

Powdery mildew. Our attempt to use the grape mildew model without modification to predict powdery mildew infection in greenhouse-grown roses was not satisfactory. The GMM is based on a sustained (6 hours or longer) temperature threshold of 70°F to 85°F, which is a little higher than optimum for mycelial growth of the rose mildew pathogen (Horst 1989). For this reason, we attempted to improve the performance of the model by running it with a temperature range of either 65°F to 85°F or 65°F to 80°F.

Generally, we found the model to be of limited value in Southern California;

TABLE 2. Miticide costs under conventional control, IPM startup (first 4 to 8 weeks) and IPM maintenance

Treatment	Cost/ft^2/ application	Amount used per application*
Conventional	$0.006 to $0.01	100 to 150 gallons
IPM startup	$0.02 to $0.03	1 to 50 vials
IPM maintenance	$0.005 to $0.008	2 to 5 vials

* Per 10,000 square feet; one vial contains 2,000 *Phytoseiulus persimilis.*

Jack Kelly Clark/UC Davis

A scout uses a hand-magnifier to count insects on a yellow sticky trap, in order to help growers make better pest-management decisions.

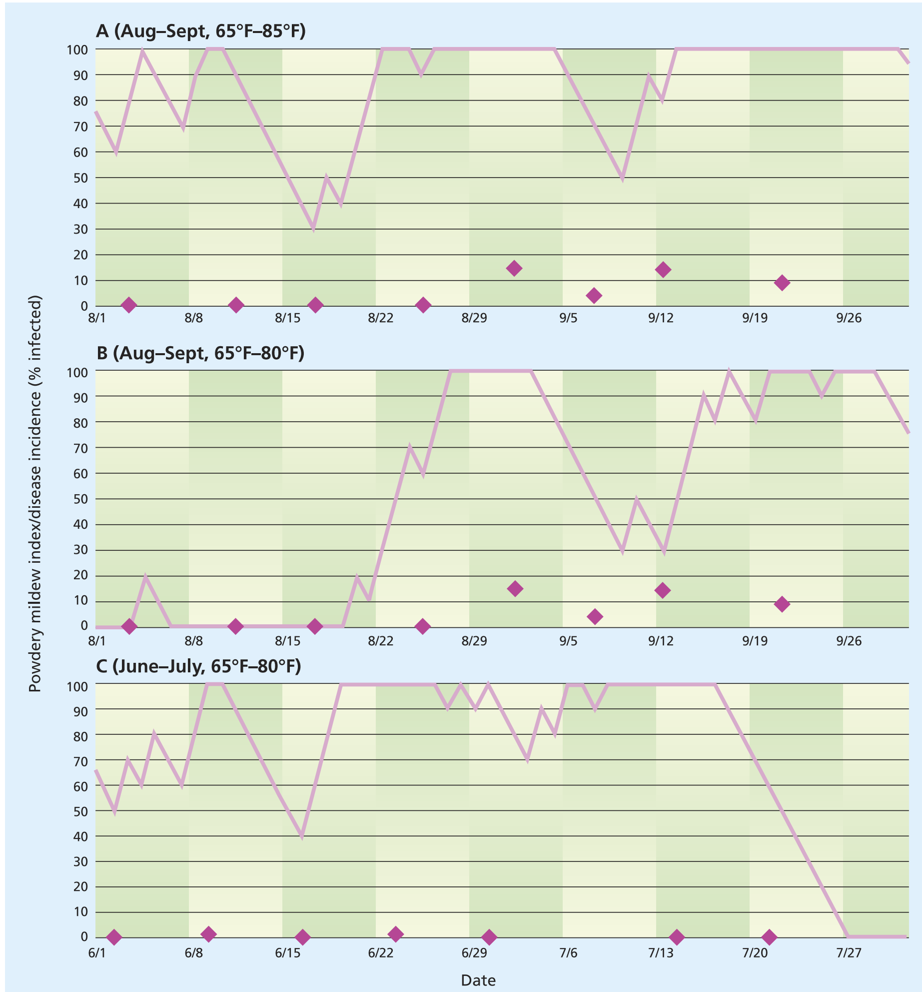

Fig. 4. Comparison of the powdery mildew index (PMI) computed by the grapevine mildew model (solid lines) relative to observations of actual disease incidence (diamonds) in a Monterey rose greenhouse for (A and B) August to September and (C) June to July 2000.

it showed a high level of disease risk most times of the year, and disease was a chronic problem. There was no clear start to a mildew season, and there was little success in identifying environmental changes associated with changes in disease pressure. On the other hand, Central California greenhouses did appear to have a seasonal component to disease, with powdery mildew on greenhouse roses starting in early spring (coincident with mildew on roses outside the greenhouse) and tapering off by early fall.

However, even under these conditions, the model was not successful in identifying triggering events. For example, there was a poor relationship between the powdery mildew index (PMI) in a greenhouse near Monterey when the model was run with a temperature range of 65°F to 85°F (fig. 4A). This relationship was improved somewhat by running the model for the same data using a temperature range of 65°F to 80°F (fig. 4B). However, there were many times in the spring and early summer when the PMI indicated high disease risk but no disease was evident on the crop (fig. 4C). We have no explanation for these persistent failures. Perhaps there was no inoculum in the greenhouse; perhaps we were not fully aware of all fungicide treatments; or perhaps greenhouse humidity is interacting in a way that confounds the model.

Clearly a model that could predict the most opportune times for applying fungicide treatments to control powdery mildew on roses would be beneficial. We were encouraged by the fact that the model never indicated low risk when there was in fact significant disease (data not shown), and that we sometimes saw a rise in mildew incidence after a rise in the index with an appropriate latent period lag (figs. 4A, 4B). However, our research showed that the UC Davis powdery mildew risk assessment model for grapevines is not easily adapted to the challenge of powdery mildew on greenhouse roses. Additional research is needed to develop a more suitable modeling platform before it will be possible to effectively advise growers regarding risk periods.

Secondary pests. Effective IPM implementation was hindered at two sites by the citrus mealybug *(Planococcus citri).* This pest is generally not a problem for rose growers until IPM is implemented, when the cessation of broad-spectrum pesticide applications can allow this pest to develop. It is generally a problem only at sites where roses are or were grown adjacent to other flower crops such as *Stephanotis,* an important citrus mealybug host plant. Unfortunately, natural enemies of the citrus mealybug are not regularly available at the commercial level, and the most effective mealybug pesticides are detrimental to spider mite predators. We are working with the natural enemy suppliers to try to change this situation, and we continue to evaluate reduced-risk pesticides for efficacy against the citrus mealybug.

◀ **Citrus mealybug became a pest at two study sites after broad-spectrum pesticide spraying ceased. This is generally a problem only where roses are grown adjacent to other flowers that serve as mealybug hosts.**

Past success, future work

Overall, we believe that the rose IPM program was successful. For example, most of the growers participating in the study wanted to abandon their conventional treatments in favor of using a biological control, predatory mites, to control twospotted spider mites; we allowed them to do so after we felt that enough data had been collected for a good comparison of the IPM and conventional treatments. Future work should concentrate on reducing the sampling effort while still collecting sufficient information to support good pest management decisions. In addition, more work is needed on refining the predictive powdery mildew model as well as on developing effective IPM techniques for secondary pests.

This program represents the first and largest effort to demonstrate and implement an IPM strategy on floriculture crops in the United States. Drawing on the partnerships that are central to the Pest Management Alliance concept, we have shown that high-quality roses can be produced with substantially fewer pesticides and with the incorporation of biological control into mainstream floriculture. Effective partnering with the biological control industry has also been a hallmark of this program. This has led to the widespread use of predatory mites in commercial rose production in California, representing the largest use of biological control by the floriculture industry in the United States.

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Native roadside perennial grasses persist a decade after planting in the Sacramento Valley

by Ryan E. O'Dell, Stephen L. Young
and Victor P. Claassen

Restoring native grassland along roadsides can provide a relatively low-maintenance, drought-tolerant and stable perennial vegetative cover with reduced weed growth, as opposed to the high-maintenance invasive annual cover (requiring intensive mowing and herbicide treatments) that dominates most Sacramento Valley roadsides. A survey of long-established roadside native-grass plantings in Yolo County showed that once established and protected from disturbance, such plantings can persist with minimal maintenance for more than a decade, retaining a high proportion of native species. The survey also showed that each species of native perennial grass displays a microhabitat preference for particular roadside topographic positions, and that native perennial grass cover is negatively affected by disturbance.

Grasslands cover approximately 17% (almost 20 million acres) of California's landscape (Huenneke and Mooney 1989). Although the range of California's grassland communities has changed little since European settlement more than 200 years ago, their species composition has been altered dramatically. Heavy livestock grazing, cultivation, wildfire suppression and the introduction of annual species from the Mediterranean have transformed California's once-pristine and diverse grasslands, which were dominated by perennial bunchgrasses, to invasive, annual-dominated grasslands with lower species diversity (Dyer and Rice 1997; Heady et al. 1992; Huenneke and Mooney 1989). Less than 10% of California native perennial grassland is estimated to remain (Huenneke and Mooney 1989).

The remaining perennial grasslands in California's interior are dominated by the native species purple needlegrass (*Nassella pulchra* [A. Hitchc.] Barkworth), blue wildrye (*Elymus glaucus* Buckley), bluegrass (*Poa secunda* J.S. Presl.), California melic (*Melica californica* Scribner), creeping wildrye (*Leymus triticoides* [Buckley] Pilger) and meadow barley (*Hordeum brachyantherum* Nevski) (Hickman 1993). Purple needlegrass, blue wildrye, bluegrass and California melic are drought-tolerant species that typically occupy well-drained upland sites. In contrast, creeping wildrye and meadow barley are less drought-tolerant and typically grow in the moist soils of seeps, streams and wetland margins (Walker 1992; Hickman 1993). Creeping wildrye and meadow barley are also flood-tolerant.

California annual exotic grasslands are largely composed of the species Italian ryegrass (*Lolium multiflorum* Lam.), soft chess (*Bromus hordeaceus* L.), ripgut grass (*Bromus diandrus* Roth), wild oat (*Avena fatua* L.), medusahead (*Taeniatherum caput-medusae* [L.] Nevski) and foxtail barley (*Hordeum murinum* L.). Yellow starthistle (*Centaurea solstitialis* L.) and broadleaf filaree (*Erodium botrys* [Cav.] Bertol.) form a large component of the associated invasive annual broadleaf biomass (Heady et al. 1992; Lulow 2004; Pitcairn et al. 2006). Except for yellow starthistle, all of these invasive species complete their life cycles by the time soils become dry in the summer

At relatively undisturbed site 1 (looking west), vegetation from the road edge (left) to swale (bottom right to center) is dominated by the native perennial purple needlegrass. The swale is periodically inundated in winter and contains a few individuals of the native perennial meadow barley distributed among a dense cover of common vetch (*Vicia sativa*), an invasive annual.

The road edge of heavily traveled site 4 (looking east) is bare (bottom right to center). A dense strip of stunted, invasive annual grasses (Italian ryegrass and foxtail barley) occurs to the left of the road edge on the shoulder (bottom center to center). A strip of the native perennial purple needlegrass occurs on the much-less-disturbed backslope (bottom left to center).

(Huenneke and Mooney 1989). In contrast, yellow starthistle is deeply rooted, drought-tolerant and continues active growth throughout most of the growing season, until it completes its life cycle in late summer or early fall (Morghan and Rice 2005).

Restoring native perennial grass

The regeneration of native perennial grasslands is desirable to improve the quality of grazing forage; establish stable vegetative cover for soil conservation; provide habitat for wildlife; reduce fire hazards associated with thick, matted, invasive annual thatch; and suppress resident invasive annual species (Brown and Rice 2000; Bugg et al. 1997; Kemper et al. 1992). Although the establishment of native grass stands is initially labor-intensive, the long-term management time and costs required are substantially lower than that for controlling the growth and spread of noxious invasive species along roadsides, generally with intensive herbicide applications and repeated mowing (Westbrooks 1998). The cost of installing and maintaining native grassland can vary considerably from site to site. Robins et al. (2001) estimated installation costs (earthwork, tillage, herbicide, seeding) at $522 to $1,433 per acre of roadside, using current costs for seed; maintenance costs for each of the first 3 years of establishment were an estimated $52 to $153 per acre, with similar costs occurring periodically (2 to 3 years) in following years.

Early attempts to restore native perennial grasslands in annual grass–dominated pasturelands were largely unsuccessful due to inadequate preparation of the soil prior to planting and suppression by competitive, invasive species after planting, as well as heavy grazing (Kay et al. 1981). Recently, the successful establishment of native perennial grasses has been attributed to preplanting site preparation, including burning to reduce invasive-species seed and thatch loads, ripping and disking to improve the seedbed, and pre-emergent and postemergent herbicide treatments to reduce residual invasive annuals (Anderson 2001; Bugg et al. 1997; Lulow 2004; Stromberg and Kephart 1996).

In their roadside perennial-grassland restoration studies, Bugg et al. (1997) described environmental gradients, particularly with respect to soil moisture across roadside topographic zones. The gradient across topographic zones creates the potential for variations in species distribution within the planting. The authors examined the distribution of native perennial grass species with respect to roadside topographic zones. Although they predicted that the optimal environmental and tolerance features of each native California perennial grass species should result in the selective establishment of certain species within particular roadside topographic zones, their results did not reveal any such trend for 2 years after seeding.

TABLE 1. Management practices used by landowners on roadside planting sites established by Yolo County Resource Conservation District

Site	Established	Mowing regime	Spraying regime*	Burning regime
1	1993	Once postplanting	Yearly spot application chlorsulfuron, 2,4-D and clopyralid	Alternate years
2	1993	Once postplanting	Yearly spot application chlorsulfuron, 2,4-D and clopyralid	Alternate years
3	1996	Yearly	Yearly entire site application clopyralid	Twice postplanting
4	2001	Twice yearly	None	None
5	1998	Twice postplanting	Alternate years entire site application clopyralid, 2,4-D or bromoxynil	Once postplanting
6	1999	Twice yearly	Yearly spot application clopyralid	None
7	2001	Yearly	Yearly entire site application clopyralid or 2,4-D	Alternate years
8	2001	Twice yearly	Yearly entire site application triclopyr or 2,4-D	Alternate years
9	2001	Yearly	One to three times yearly entire site application triclopyr or 2,4-D	Alternate years

* Chlorsulfuron (Telar) is a preemergent herbicide that targets and inhibits seed germination and seedling establishment. Clopyralid (Transline), bromoxynil (Buctril), triclopyr (Garlon 4) and 2,4-D are postemergent broadleaf herbicides. Herbicide application varied by site and degree of invasive species cover.

Yolo County planting history

In 1993, the Yolo County Resource Conservation District (YCRCD) began to establish permanent, native perennial grass plantings as an alternative to managing invasive annual grass–dominated roadside rights-of-way (Rose 1998). YCRCD established 30 such plantings between 1993 and 2001 throughout Yolo County in the Sacramento Valley. Roadside and fieldside areas were required to be at least 11.5 feet (3.5 meters) wide for proper seeding and maintenance-equipment access, and to accommodate agricultural-implement turns. Sites were also required to have slopes of less than four-to-one (horizontal-to-vertical) for safety and ease of equipment operation during establishment and maintenance. The quantity and timing of runoff to roadside ditches were considered in the selection of plant species seeded at each site. The soil texture class at all sites was a silty clay loam (Andrews 1972).

All sites were lightly disked in the fall to prepare the seedbed. Before seeding each site, a single application of glyphosate (Roundup) herbicide was applied to reduce competition by invasive species with newly emerged native-grass seedlings. Each site was then seeded with purple needlegrass, blue wildrye, creeping wildrye and meadow barley at approximately 30 pounds per acre (34 kilograms per hectare) of pure live seed (Rose 1998) using precision broadcast seeders or hand-held belly grinders. An ATV pulling a straight-toothed and flexible harrow was used to incorporate the seed into the soil. Following fall planting, a selective broadleaf herbicide was applied in late winter to control broadleaf invasive species.

In the second year, landowners assumed invasive species management responsibilities, which included occasional mowing, spot treatments with herbicides to control invasive species and burning (table 1). Some owners

Relatively undisturbed site 9 (looking west) is bordered by a bike path (left) and road (right). Dense strips of the native perennial purple needlegrass (straw-colored inflorescences) are on the backslope (left) and shoulder (right of the phone poles), and a dense strip of the surrounding native perennial creeping wildrye (dark green) is in the swale (surrounding the phone poles).

Vegetation cover on the road edge and shoulder (bottom right to center) of site 3 (looking north) is low. Low mowing on the shoulder has resulted in a monoculture stand of Bermuda grass (*Cynodon dactylon*), an invasive perennial. The narrow swale (bottom center to center) is dominated by Italian ryegrass, an invasive annual. Vegetation on the backslope (bottom left to center) is dominated by the native perennial purple needlegrass, with some invasive annual common vetch.

chose to seed native broadleaf species into their native plantings. Those species included yarrow (*Achillea millefolium*), California poppy (*Eschscholzia californica*), gumplant (*Grindelia camporum*) and lupine (*Lupinus* sp.), which were seeded at unknown rates.

Planting survey

Although 30 YCRCD sites were established, complete records on post-establishment treatments (including herbicide treatment and burning) had been kept for only nine long-established (5 to 13 years) sites (table 2). We surveyed these sites in late spring 2006. We sought to determine: (1) the restoration success of matured roadside perennial grass plantings compared to adjacent unrestored roadsides, as represented by plant cover and density; (2) whether certain restoration species dominated particular roadside topographic zones (microhabitats); and (3) whether disturbance affects the native-versus-invasive composition of the planting.

Data collection. Point-transect plant cover and species identification were collected at each of the nine sites. Topographic zones — including edge, shoulder, swale and backslope — were delineated at each site, similar to those delineated by Bugg et al. (1997) (fig. 1; table 3).

The distance of the topographic transects from the road pavement edge was dictated by each site's unique topogra-

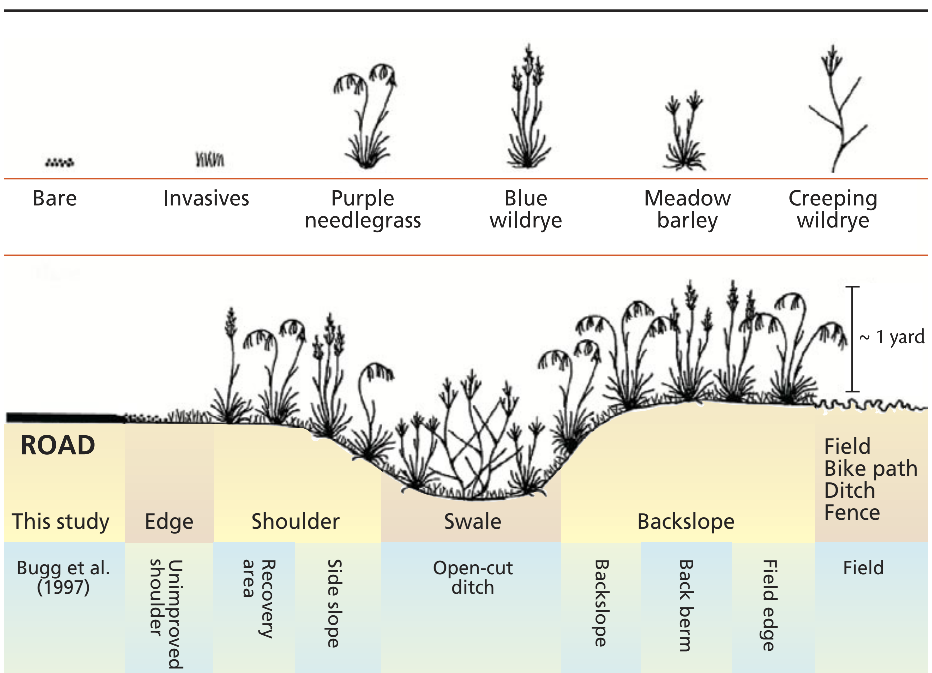

Fig. 1. Roadside topographic zones delineated for this study as compared to Bugg et al. (1997). The grass species distribution depicted reflects the general trends in species distribution observed at various sites.

TABLE 2. Yolo County Resource Conservation District survey site locations

Site	Location
1	0.5 miles west of intersection of roads 89 and 27 (north side of road 27)
2	0.5 miles west of intersection of roads 89 and 27 (south side of road 27)
3	1 mile north of intersection of roads 89 and 23 (west side of road 89)
4	0.5 miles west of intersection of roads 89 and 23 (north side of road 23)
5	1 mile north of intersection of roads 102 and 16 (east side of road 102)
6	1 mile north of intersection of roads 89 and 31 (west side of road 89)
7	Intersection of I-505 and road 13 (southwest side of intersection)
8	1 mile west of intersection of Russell Blvd. (Davis) and road 87 (north side of Russell Blvd.)
9	0.5 miles west of intersection of Russell Blvd. (Davis) and road 96 (south side of Russell Blvd.)

TABLE 3. Distribution of topographic zone-impact combinations across survey sites

Topograhic zone	Observed condition	Site 1	2	3	4	5	6	7	8	9	No. sites
Edge	Heavily disturbed			X	X						2
Edge	Lightly disturbed		X			X	X	X		X	5
Edge	Undisturbed	X									1
Shoulder	Heavily disturbed			X			X	X			3
Shoulder	Undisturbed	X	X		X	X			X	X	6
Swale	Heavily disturbed			X	X	X	X		X		5
Swale	Undisturbed	X						X		X	3
Backslope	Heavily disturbed					X			X		2
Backslope	Undisturbed	X		X			X	X		X	5

TABLE 4. Criteria used to categorize vegetation cover in topographic zones and impacts that may have caused the condition

Topographic zone	Condition	Appearance	Impact
Edge	Heavily disturbed	Plants nonexistent (bare ground)	Heavy travel, soil disturbance, scalping, herbicide
Edge	Lightly disturbed	Plants flattened to ground	Light travel, soil disturbance, scalping, herbicide
Edge	Undisturbed	Plants in dense stands and upright	None
Shoulder	Heavily disturbed	Plants flattened to ground; plants sparse or bare soil patches	Light travel, soil disturbance, scalping, herbicide
Shoulder	Undisturbed	Plants in dense stands and upright	None
Swale	Heavily disturbed	Plants flattened to ground; plants sparse or bare soil patches	Inundation, travel, soil disturbance, scalping, herbicide
Swale	Undisturbed	Plants in dense stands and upright	None
Backslope	Heavily disturbed	Plants flattened to ground; plants sparse or bare soil patches	Light travel, soil disturbance, scalping, herbicide
Backslope	Undisturbed	Plants in dense stands and upright	None

TABLE 5. Effect of topographic zone-impact interactions on cover types*

Topographic zone	Condition	Cover † Bare	Invasives	PN/BW	CW/MB	Other natives
		. . . .	. . . .	%	. . . .	. . . .
Edge	Heavily disturbed	90.7 ± 2.9	9.3 ± 2.9	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Edge	Lightly disturbed	0.4 ± 0.3	91.4 ± 1.5	5.1 ± 1.1	0.0 ± 0.0	3.1 ± 1.0
Edge	Undisturbed	4.5§	39.5§	46.0§	0.0§	10.0§
Shoulder	Heavily disturbed	8.3 ± 1.7	91.7 ± 1.7	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Shoulder	Undisturbed	0.3 ± 0.1	22.0 ± 4.7	76.0 ± 4.6	1.1 ± 1.1	0.6 ± 0.5
Swale	Heavily disturbed	7.5 ± 1.9	76.4 ± 6.0	3.9 ± 2.3	12.2 ± 2.7	0.0 ± 0.0
Swale	Undisturbed	0.5 ± 0.5	44.1 ± 5.2	8.2 ± 2.8	47.0 ± 5.3	0.2 ± 0.2
Backslope	Heavily disturbed	15.4 ± 3.3	76.0 ± 4.0	8.3 ± 1.5	0.0 ± 0.0	0.3 ± 0.3
Backslope	Undisturbed	1.3 ± 0.8	22.5 ± 3.3	75.9 ± 3.3	0.3 ± 0.2	0.0 ± 0.0

* n = 1–6, mean ± standard error of the mean (SEM). Dominant cover types for each topographic zone-impact combination are shown in red.

† Bare, invasives, purple needlegrass/blue wildrye (PN/BW) species assemblage, creeping wildrye/meadow barley (CW/MB) species assemblage, natives assemblage.

§ No SEM due to only one replicate.

phy and was variable between sites. Contiguous 6.56-foot (2 meter) point-transect surveys (n = 10) were conducted per topographic zone (edge, shoulder, swale, backslope) per site. This linear transect layout was necessary to accommodate the geometry of the roadside sites. The transect starting points were randomly selected. Such systematic sampling is regarded as analogous to simple random sampling when the population sampled is in random order (Williams 1978). Point-transect cover (bare or plant) and species identification (when plants were present) were collected at 0.33-foot (0.1-meter) intervals (20 intervals total per 0.33-foot transect).

The same point-transect data collection method was used to collect data from the shoulder topographic zones of an unplanted roadside area adjacent to each roadside planting site (control). The disturbance condition of each topographic zone (heavily disturbed, lightly disturbed, undisturbed) was determined based on vegetation appearance and evidence of adverse impacts, including prolonged inundation, vehicle travel impact, disking, scalping/low mowing and herbicide application (table 4).

In order to examine species microhabitat preferences at each site, cover data for the drought-tolerant species, purple needlegrass (PN) and blue wildrye (BW), were combined (PN/BW) within individual topographic zones (edge, shoulder, swale, backslope), as was cover data for the drought-susceptible species, creeping wildrye (CW) and meadow barley (MB). Invasive and native species cover (excluding PN, BW, CW and MB) were combined into separate groups according to individual topographic zones as well; these species groups are termed assemblages.

Statistics. AR1 structure (autoregressive of order 1) analyses of errors across the contiguous transects confirmed that autocorrelation was insignificant (maximum autocorrelation estimate = 0). Zone differences within a given assemblage were evaluated by two-way ANOVA. Mean separation between factors was established by Fisher's LSD. The significance level was set at $P = 0.05$. All statistical analyses were conducted using Statistica 6.1. To examine the trend effects of disturbance on species assemblage cover, data be-

A dense strip of invasive species dominates the road edge (bottom left to center) of site 5 (looking north). The shoulder (bottom center to center) contains the native perennial purple needlegrass intermixed with Italian ryegrass and soft chess, invasive annual species. The swale and backslope (bottom right to center) have been disked (far right). The swale is periodically inundated by irrigation runoff in summer. Heavy disturbance in the swale and backslope has resulted in dominance by the invasives field bindweed, summer mustard (*Hirschfeldia incanna*) and wild radish (*Raphanus sativus*).

tween sites was grouped by condition according to topographic zone (table 5). Due to critically low replicates in some groups (n < 3; see table 3), no statistical tests could be run using this data set, but strong trends were evident through comparison of the means.

Persistence, microhabitat

The native perennial grass species originally planted, including purple needlegrass, blue wildrye, creeping wildrye and meadow barley, continued to dominate most of the plantings more than a decade after establishment. All of the native grasses had flowered and produced a profusion of seed, much of which had fallen to the ground below the parent plants. In addition to native grasses, herbaceous broadleaf species such as yarrow, gumplant, California poppy and lupine were common among the bunchgrasses. Invasive annual and perennial species common in portions of some of the sites included Italian ryegrass, soft chess, foxtail barley, yellow starthistle and field bindweed *(Convolvulus arvensis).* In highly disturbed areas of the sites, a thick thatch of these invasive species had accumulated. None of the adjacent, unplanted, control roadside sites contained a single native plant.

In general, the recruitment of native perennial grasses in annual-dominated grasslands is strongly suppressed due to competition from fast-growing resident invasive annual species (Brown and Rice 2000; Dyer and Rice 1997; Dyer and Rice 1999; Hamilton et al. 1999; Lulow 2004; Seabloom et al. 2003; Stromberg and Kephart 1996). Although native California perennial grass species are effective competitors once established, they are relatively poor competitors with invasive annual species in the early stages of seedling establishment. Invasive annual grass species complete their life cycles early in the growing season (spring and early summer) by virtue of rapid growth rates, high shoot-to-root biomass allocation and the efficient production of very-fine-diameter roots to acquire water resources from the upper soil profile (Holmes and Rice 1996).

Native perennial bunchgrass species, in contrast, allocate a much greater proportion of their biomass to the production of a deep root system, in order to access deep soil moisture during the dry season. Evidence strongly suggests that competition for water between invasive annuals and native perennial grass seedlings, which are shallow-rooted in their early stages of growth, limits the establishment of perennial grass seedlings on invasive, annual-dominated roadsides and other annual grasslands (Dyer and Rice 1997; Dyer and Rice 1999; Hamilton et al. 1999; Holmes and Rice 1996).

Water availability is one of the most important resources that controls plant cover, composition and distribution across the landscape. Roadsides may have sharp gradients in soil-water availability associated with the sloped topography of the site. Road edge, shoulder and backslope topographic zones are typically well drained and have low soil-water availability during the summer. In contrast, swales tend to have greater soil-water availability that persists throughout the summer. Additionally, swales may be inundated for extended periods due to excessive winter precipitation or periodic summer irrigation runoff.

Site surveys in this study demonstrated that assemblages of native perennial grass species dominated particular roadside topographic positions. Cover by the drought-tolerant PN/BW species assemblage was significantly greater in shoulders and backslopes than in the road edges and swales ($P < 0.001$) (table 5). In contrast, cover by the drought-susceptible, flood-tolerant CW/MB species assemblage was significantly greater in the swales than in the road edge, shoulder and

None of the adjacent, unplanted, control roadside sites contained a single native plant.

backslope zones ($P < 0.001$). Invasive species cover was significantly greater in road edges and swales than in either the shoulders or backslopes ($P < 0.001$), coincident with areas that experience the greatest disturbance.

Disturbance effects

Increasing levels of disturbance tended to favor dominance by invasive species in the planting. Undisturbed road edges were dominated by native perennial grass species (table 5).

The light disturbance of road edges resulted in dominance by invasive species, while heavy disturbance was so detrimental to plant growth that the ground was essentially bare. Disturbance had the same detrimental impact in the shoulder, swale and backslope zones as in the road edges.

Disturbance has a strong negative impact on the persistence of native perennial grasses. For example, plowing for agriculture and heavy grazing were major factors responsible for the degradation and loss of native perennial grasslands in California following European settlement (Bartolome 1981; Burcham 1957; Huenneke and Mooney 1989; Mack 1989). Disturbances detrimental to the persistence of native perennial grasses in the YCRCD roadside plantings included (impact followed by cause): prolonged inundation (winter precipitation and summer irrigation runoff, flooded more than 2 weeks); travel (vehicle drift off pavement, farm equipment); soil disturbance (roadside grading, disking); scalping (improper mowing height, less than 6 inches); and nonselective herbicide application (inadvertent or intentional, to reduce weed biomass).

Managing roadway environments

Planting and management plans should recognize the potential environmental and human impacts that may adversely affect the persistence of native grassland communities at the site. Additionally, plans should consider that each native perennial grass species has an optimal microhabitat within the roadside topography. Soil moisture availability in roadside topographic zones can vary greatly between the shoulder and backslope (drier) and swale (wetter). For example, purple needlegrass and blue wildrye are more suitable for shoulder and backslope topographic zones, due to their drought-tolerant characteristics; creeping wildrye and meadow barley are less drought- and more flood-tolerant, so they are more suitable for swales. If a roadside site's local soil-moisture conditions are not known or are highly variable across the site, planting a mix of all four species in all topographic zones allows each species to establish itself in its optimal micro-environment.

Management activities that integrate multiple invasive-species control methods and reduce disturbance should be carefully considered with respect to site conditions, season, spatial application and frequency of application. Broadleaf herbicides and herbicide spot treatments shortly after planting can be beneficial to reduce competition by invasive species, but the broad use of nonselective herbicides is detrimental to native perennial grasses and should be avoided. Physical disturbances should also be avoided, including excessive travel, roadside grading, disking, and scalping due to low mowing height.

Once established, native grasslands can provide an attractive and lower-maintenance alternative to invasive annual grasslands. Native grasslands remain green well into the dry season (reducing fire hazards) and provide higher-quality forage and habitat for native animals. Although the efforts needed to regenerate native perennial grass communities can be intensive, this study confirms that they can persist for many years in right-of-way environments and can reduce the density of invasive annual species.

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Low-income women in California may be at risk of inadequate folate intake

by Emily R. Cena, Amy Block Joy,
Karrie Heneman *and* Sheri Zidenberg-Cherr

Folate plays a major role in preventing neural tube defects in the developing fetus, as well as in reducing the risks of cardiovascular disease, certain types of cancer and some mental health problems. We assessed the folate intakes of socioeconomically disadvantaged women of childbearing age participating in California's Food Stamp Nutrition Education program. Of 195 women studied, 59% failed to meet the Institute of Medicine's folate intake recommendations for women capable of becoming pregnant. We found significant differences among the ethnic groups studied: 45% of Hispanic, 65% of white and 77% of black women did not meet the recommendation for synthetic folic acid intake. This study supports the need for developing targeted nutrition-education lessons focusing on the importance of adequate folate consumption.

USDA/Ken Hammond

Daily folic acid intake is important for all women who could become pregnant, because it reduces the risk of neural tube defects in the developing fetus. A daily folic acid–containing supplement is an effective way for women to meet the folic acid recommendation.

Folate is a B vitamin required for critical bodily functions such as DNA synthesis and repair, and amino acid metabolism. This vitamin is found in foods such as liver, lentils and other legumes, oranges and orange juice, and dark-green leafy vegetables. It is well established that folate deficiency causes macrocytic, hyperchromic anemia, a red-blood-cell condition that causes weakness, fatigue, loss of appetite and confusion. More recently, suboptimal folate levels have been associated with cardiovascular disease (Boushey et al. 1995), certain types of cancer (such as colon and pancreatic) (Jennings 1995) and some mental health disorders, including depression and dementia (Bodnar and Wisner 2005; Campbell et al. 2005).

Low folate levels are also associated with birth defects. Women deficient in this vitamin are more likely to have babies with neural tube defects such as spina bifida or anencephaly (MRC 1991). These defects occur during the first 4 weeks of embryonic development due to incomplete closure of the neural tube, which ultimately becomes the brain and spinal cord. Neural tube defects can cause physical abnormalities, developmental problems, partial or complete paralysis, and may even cause death (before or after birth).

Adequate folate intake can reduce the risk of neural tube defects by as much as 72%, according to a randomized, double-blind vitamin supplementation trial involving 1,817 women of childbearing age from seven countries (MRC 1991). In an effort to reduce the incidence of neural tube defects, the United States has required the mandatory fortification of enriched grains with synthetic folic acid (SFA) since 1998; some other countries also mandate folate fortification, including Canada and Chile.

Folate recommendations

There are currently two types of folate in the U.S. food supply: (1) SFA and (2) folate that occurs naturally in a limited number of foods, such as spinach and beans. SFA is added to enriched grain products, including ready-to-eat breads and breakfast cereals, and is also found in folic acid–containing vitamin supplements. The primary difference between these two types of folate is that SFA is more easily digested and

The Food Stamp Nutrition Education program teaches low-income people how to purchase and prepare healthy foods. In this study, researchers evaluated the folate intakes of female program participants. Yolanda Lopez, FSNE program nutrition educator in Fresno County (right), and a volunteer conduct a cooking demonstration.

absorbed, making it more readily available to the body's tissues (bioavailable) (IOM 1998).

To account for the difference in bioavailability between types of folate, the intake of this vitamin is typically quantified with a standard unit called the dietary folate equivalent (DFE). In 1998, the Food and Nutrition Board of the Institute of Medicine (an independent arm of the National Academy of Sciences) published the Dietary Reference Intakes for folate (IOM 1998). They include a Recommended Dietary Allowance (RDA), a Tolerable Upper Intake Level (UL), and a special recommendation for women capable of becoming pregnant to consume at least 400 μg SFA/day, in addition to the natural food folate found in a varied diet (see glossary).

Impact of fortification uneven

Research suggests that the U.S. folate fortification program has been only partially successful. On the positive side, the general population has significantly higher mean folate levels in red blood cells and serum, according to a comparison of National Health

Glossary

Block DFE screener: An instrument that quickly estimates the usual folate intake from 21 food and supplement sources. The screener identifies individuals who may be at risk of low folate status, especially in low-income populations.

Dietary folate equivalent (DFE): The standardized unit for measuring folate intake: 1 microgram (μg) DFE = 1 μg natural food folate = 0.5 μg SFA from a supplement that is taken on an empty stomach = 0.6 μg SFA taken with food or from a fortified food source.

Dietary Reference Intakes (DRIs): Recommendations by the Institute of Medicine for nutrient intakes, which can be used for planning and assessing diets. The DRIs for folate include a Recommended Dietary Allowance, Tolerable Upper Intake Level, and a special recommendation for women capable of becoming pregnant.

Folate: B vitamin required by the body for a variety of functions, including DNA synthesis and repair; includes naturally occurring food folate and SFA.

Recommended Dietary Allowance (RDA): The intake level for a nutrient that the Institute of Medicine considers sufficient to meet the needs of almost all healthy people of a given age and gender. For folate, the RDA for adults is 400 μg DFE per day, based on the amount of dietary folate required to maintain normal blood concentrations of certain folate status indicators.

Special recommendation for women of childbearing age: A recommendation for women capable of becoming pregnant set forth by the Institute of Medicine, in addition to the RDA. In order to reduce the risk of giving birth to a child with a neural tube defect, women of childbearing age are recommended to consume 400 μg SFA per day, in addition to the natural food folate supplied by a varied diet.

Synthetic folic acid (SFA): A human-made form of folate found in fortified grain products and vitamin supplements. The bioavailability of SFA is greater than that of natural food folate.

Tolerable Upper Intake Level (UL): The highest daily intake level for a nutrient that is likely to be safe for almost all healthy people of a particular age and gender. For folate, the UL for adults is 1,000 μg SFA per day, regardless of the natural food folate consumed. This value is based on the possibility that very high intakes of SFA from supplements and fortified foods might mask a vitamin B-12 deficiency.

USDA-ARS/Scott Bauer

Fortified breakfast cereals, broccoli and beans are good dietary sources of folate. More than half of the women studied were not getting enough folate from their diets or vitamin supplements.

USDA-ARS/Keith Weller

and Nutrition Examination Surveys (NHANES) before and after the fortification program began (1988-1994 and 1999-2000, respectively) (CDC 2002). In addition, there were 26% fewer neural tube defects 2 years after the program began than 3 years before (2000 and 1995, respectively) (CDC 2004). While this is a noteworthy improvement, it is still far less than the 72% reduction in neural tube defects expected if all women of childbearing age consumed adequate amounts of folate.

Moreover, other studies suggest that certain population subgroups may still be at risk. For example, the NHANES data indicates that non-Hispanic black women have 23% lower red-blood-cell folate levels and 26% lower serum folate levels than non-Hispanic white women (CDC 2002). In addition, socioeconomically disadvantaged women had 16% lower red-blood-cell folate levels and 24% lower serum folate levels than their socioeconomically advantaged counterparts, according to a study in Southern California (Caudill et al. 2001).

Assessing folate intake

The purpose of our study was to assess folate intake among low-income, food stamp–eligible (≤ 130% of federal poverty level) women of childbearing age in California. In California, the Food Stamp Nutrition Education (FSNE) program serves approximately 138,000 food stamp–eligible families each year by providing nutrition education and skills training about selecting, purchasing and preparing healthy foods. Funding is provided via an interagency agreement among the U.S. Department of Agriculture, the California Department of Social Services and UC Davis.

Participants and instruments. During spring 2005, 211 women participating in California's FSNE program were recruited for a cross-sectional survey of folate intake, from 12 counties: Alameda (n = 59), Amador (n = 3), Calaveras (n = 14), Fresno (n = 10), Los Angeles (n = 11), Nevada (n = 12), Placer (n = 8), Riverside (n = 7), San Diego (n = 33), Shasta (n = 29), Trinity (n = 20) and Tuolumne (n = 5). Study participants were between 18 and 45 years old, nonpregnant and able to read and understand English or Spanish. The Institutional Review Board at UC Davis approved the study.

Study participants were asked to complete two forms: (1) a demographic survey, which was adapted from the FSNE program's Adult Family Record, and (2) the Block Dietary Folate Equivalents (DFE) Screener, a one-page, rapid screener developed to measure the usual intake of dietary folate in low-income populations. The DFE Screener has previously been demonstrated to reflect red-blood-cell folate status in this population (Clifford et al. 2005).

The Block DFE screener is used to estimate folate intake in order to quickly and easily determine the risk of low folate status. However, it does not account for all sources of dietary folate. Rather, it measures the frequency of intake for the most common food sources of folate in the United States. A comprehensive, traditional, food-frequency questionnaire could be used for more quantitative folate-intake assessment, but we did not choose this option because of the substantial participant burden it entails, particularly in a low-income population. In addition, as with all studies of self-reported food intake, participants in our study may have under- or over-reported their consumption of certain food groups. Finally, the food items included in the screener may not fully represent the dietary choices of different ethnic groups or individual variations.

The completed folate screeners were scanned and scored by Block Dietary Data Systems in Berkeley, California. Data from the demographic survey and folate screener were analyzed by independent *t*-tests, one-way analysis of variance and Tukey post-hoc multiple comparisons, using SPSS version 13.0.

Demographics. Of the 211 study participants, six were excluded due to incomplete surveys and 10 were excluded as outliers. As a result, 195 women were included in the final analysis. The average age (± SE) of study participants was 33.5 ± 0.5 years old, with 45.1% (n = 88) being white, 30.8% (n = 60) Hispanic and 13.3% (n = 26) black. Due to small sample sizes of Asians, Pacific Islanders and Native Americans, these women as well as those who wrote in a different response were combined into an "other" category, which comprised

TABLE 1. Estimated folate intake from food and supplement sources (n = 195)*			
Type of folate	Mean intake ± SE for each folate type†	Bioavailability factor	Mean DFE intake ± SE for each folate type‡
	μg		*μg DFE*
Naturally occurring folate in foods	198.0 ± 14.6	1.0	198.0 ± 14.6
SFA from fortified foods	270.5 ± 10.0	1.7	459.9 ± 17.1
SFA from supplements	148.8 ± 16.6	1.7	253.0 ± 28.2

* For each type of folate, mean intake × bioavailability factor = mean DFE intake.
† Mean total SFA intake = 419 ± 18 μg.
‡ Mean total DFE intake = 911 ± 33 μg DFE.

TABLE 2. Study participants not meeting Institute of Medicine recommendations for folate intake (n = 195)	
Recommendation	Women not meeting recommendation
	n (%)
RDA for adults: ≥ 400 μg DFE/day	17 (8.7)
Special recommendation for women of childbearing age: ≥ 400 μg SFA/day	114 (58.5)
UL for adults: No. with intake > 1,000 μg SFA/day	3 (1.5)

6.7% (n = 13) of respondents. The remaining 4.1% (n = 8) did not report their ethnicity. Approximately two-thirds (71.3%) of the respondents completed the surveys in English.

Folate consumption evaluated

The Block DFE screener provided information about participants' intake levels of naturally occurring food folate, SFA from fortified foods and SFA from folic acid–containing vitamin supplements. Of the 195 participants, 41% (n = 80) reported taking a supplement containing folic acid at least twice per month. The mean estimated total intake of folate from all sources was 911 ± 33 (mean ± SE) micrograms (μg) DFE per day (table 1). Of this, 78% (713 μg DFE) was in the form of SFA from fortified foods and supplements (table 1). Although the mean intake for total DFE exceeded the RDA, and mean total SFA intake exceeded the special recommendation for women of childbearing age to consume at least 400 μg SFA per day, nearly 59% of the participants did not meet the latter recommendation (table 2).

After comparing the mean folate intakes to dietary recommendations, we tested for differences in intake according to demographic characteristics. One-way analysis of variance revealed significant differences in folate intake between ethnic groups (fig. 1). On average, Hispanic women consumed 48% more SFA than black women ($P < 0.05$). Additionally, Hispanic women consumed, on average, 28% more total DFE than white women, and 46% more total DFE than black women ($P < 0.01$). Even so, 45% of the Hispanic women in this study had usual SFA intakes below the recommendation. Suboptimal SFA intakes were more prevalent in the other two largest ethnic categories, with 65% of white women and 77% of black women in this study failing to meet the SFA recommendation.

The mean intakes for women classified as "other" were not significantly different from those of the other three groups for either total DFE or SFA, possibly due to the small sample size. In addition, there were no significant differences in total dietary folate intake or SFA intake according to county of residence, language or age.

Role of vitamin supplements

More than half of the women participating in our study had suboptimal SFA intakes, suggesting that despite the national fortification program, low-income women of reproductive age in California may be at risk of suboptimal folate status. Furthermore, supplement use and the regular consumption of cereals and bread products were the dominant factors in how well women met their folate needs. We found that 90% of the women with SFA intakes below the recommended level reported taking folic acid–containing supplements once per month at most. In contrast, 81% of women with adequate SFA intakes reported taking supplements with folic acid at least twice a month, and most took them more frequently. Of the 13 women who reported taking a folic acid–containing supplement more than once per month but still failed to meet the SFA recommendation, most only took the supplement two to three times per month. They also tended to consume low amounts of foods that are typically fortified with SFA, such as breakfast cereals and bread products. Folate intake from most vegetables appeared to be inadequate to overcome low SFA intake levels.

Similar conclusions can be drawn regarding the 17 women who had total DFE intakes below the RDA. All of the women in this group reported taking supplements once per month at most. In general, women in this group also reported the infrequent consumption of cold breakfast cereal and relatively low intakes of bread products. While some of these women did report the regular consumption of salads and other vegetables, their folate intake from fortified foods was low.

The UL for folate is based on the hypothesis that excessive SFA intake may mask the symptoms of a vitamin B-12 deficiency. Of the 195 study participants, only three had SFA intakes that exceeded the UL. All three of them reported taking a folic acid–containing

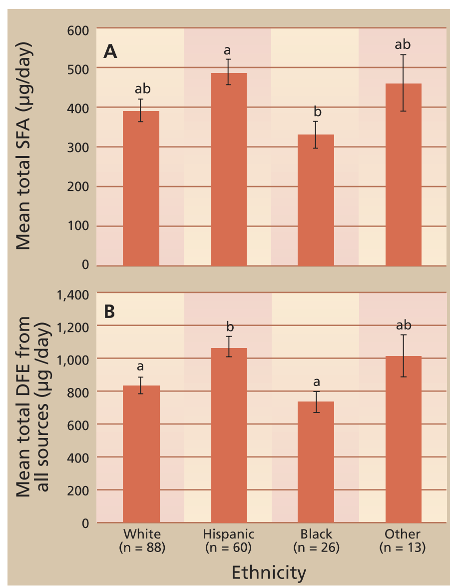

Fig. 1. Mean total (A) synthetic folic acid (SFA) and (B) dietary folate equivalents (DFEs) by ethnicity (n = 187). Groups with different letters are significantly different from one another in A (*P* < 0.05) and B (*P* < 0.01). Error bars represent ± SE.

Despite the national fortification program, low-income women of reproductive age in California may be at risk of suboptimal folate status.

multiple vitamin supplement every day, a folic acid or B-complex supplement and/or consuming ready-to-eat breakfast cereal every day, and consuming dark, leafy greens every day. These findings suggest that the risk of folic acid toxicity in this population is limited to a small minority of individuals who consume excessive amounts of SFA daily.

The results demonstrate that folic acid–containing supplements have a considerable impact on total SFA intake in this population. Likewise, in a study of low-income women in the Sacramento area, Clifford et al. (2005) found that the mean (± SE) total SFA intake for nonpregnant women of childbearing age was 950 ± 64 μg per day, including a daily supplement of 600 μg SFA per day. Before the supplementation period began, the same group of women had a mean SFA intake of 321 ± 34 μg per day, which is below the special recommendation for women of childbearing age.

Ethnicity and folate status

Our finding that Hispanic women consumed more SFA and total folate than black women is consistent with results from NHANES 1999-2000. However, the finding that Hispanic women also consumed more total folate than white women was surprising. Researchers from the U.S. Centers for Disease Control and Prevention analyzed the NHANES dataset to compare serum and red-blood-cell folate status among women of childbearing age from three ethnic groups (non-Hispanic white, non-Hispanic black and Mexican American). They found that non-Hispanic white women had the highest blood folate values, followed by Hispanic women, and then non-Hispanic black women (CDC 2002).

There are a few possible explanations for the high folate intakes of Hispanic women in California. One is the possibly higher consumption of certain folate-rich foods, such as beans, fortified tortillas and fortified rice. Although we did not compare the intake of specific food items among ethnic groups, the traditional diet of Hispanic women of Mexican descent is likely to include more of these foods than a more Americanized diet.

Another possible reason for the higher folate intake by Hispanic participants is that they reported higher supplement use than white and black women. For example, 43% of Hispanic participants reported taking a folic acid–containing supplement at least twice per month, compared to 38% of white and 36% of black women. Similarly, 33% of Hispanic participants reported the daily use of a folic acid–containing supplement, compared to 23% of white and 15% of black participants. Although our data do not explain why supplement use is more prevalent among low-income Hispanic women of childbearing age in California, it would be an interesting question to pursue in a future qualitative study.

Targeted nutrition education

Because more than half of our study participants had total SFA intakes below the Institute of Medicine's recommendation for reducing the risk of neural tube defects, low-income women of childbearing age in California may be at risk of suboptimal folate status. Previous studies have found that socioeconomically disadvantaged groups and some ethnic minorities have limited awareness and understanding of what folate is and why it is important (Kloeblen 1999; Chacko et al. 2003). Targeted nutrition lessons that include folate education could increase intake of this vitamin in low-income women in California.

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RESEARCH ARTICLE

Mineral balances, including in drinking water, estimated for Merced County dairy herds

by Alejandro R. Castillo, José E.P. Santos *and* Tom J. Tabone

Dairy producers must increasingly comply with environmental regulations at the federal, state and local levels. A key to many of the regulations is the development of manure management plans to protect air, water and soil quality. Information on complete nutrient balances and excretion is necessary to control or minimize the loss of nutrients to the environment. Data from 51 randomly selected dairy farms in Merced County, in California's Central Valley, was used to evaluate the impact of minerals in drinking water on nutrient balances and to characterize the mineral composition of manure from lactating dairy cows. We found that a lactating dairy cow producing approximately 66 pounds of milk daily might excrete 750 ± 117 grams of minerals daily, while the proportion of these minerals attributed to water ranged from 0.3% to 20%. On some dairies, controlling these minerals could reduce manure production and subsequent land applications.

New laws require dairy producers to control manure applications and limit the excretion of excess nutrients, in order to prevent pollution of the air, water and soils. Water is the most important nutrient for dairy cattle, but its mineral contents are not usually incorporated when animal diets are planned.

IN recent years, environmental regulations have been applied to the U.S. dairy industry. These regulations are aimed at protecting air, water and soils from excess nutrients excreted by cattle and manure applications. The National Pollutant Discharge Elimination System (NPDES) prohibits the discharge of pollutants into waters of the United States unless a special permit is issued by the U.S. Environmental Protection Agency (EPA). In 2003, this law was extended to confined animal feeding operations (CAFOs), including most dairies; many of the deadlines were extended to 2007.

In general, this permit system is enforced by California's Regional Water Quality Control Boards and various county ordinances. All dairy farms represent possible "discharges"; most producers have submitted Waste Management Plans to their regional board providing a complete evaluation of the existing dairy (facilities, animals, waste containers, flood protection, and so on) (US EPA 2004).

Furthermore, under guidelines developed by the U.S. Department of Agriculture's Natural Resources Conservation Service, certain animal feeding operations must develop Comprehensive Nutrient Management Plans (CNMPs). For dairies, the plans describe how management practices will be implemented to control nutrient losses from manure. The plans detail how dairy producers must apply manure, bedding or process water to the soil at agronomic rates, according to the chemical composition of the manure, local soil conditions and specific crop requirements. The enforcement and even application of CNMP rules vary by county, depending on local conditions (USDA 2003).

Given these new requirements, the American Society of Agricultural Engineers recently concluded that it is essential to improve predictions of nutrient excretion from dairy cattle, so that consultants and producers can develop nutrient management plans for individual farms (Nennich et al. 2005). Software developed by the National Research Council (NRC 2001) in conjunction with its *Nutrient Requirements of Dairy Cattle* report is considered one of the most current tools for estimating nutrient balances and excretion.

The importance of water

According to the NRC, water is the most important nutrient for lactating dairy animals. However, good-quality water is a scarce commodity in many areas of the United States and the world (Murphy 1992). In the United States, the availability of abundant, clean, drinking water may become a challenge in the future as dairy farms are forced to relocate away from population centers (Beede 2005). Water contaminants can also affect animal performance and health (Challis et al. 1987; Solomon et al. 1995; NRC 2001). Information is needed

The mineral balances and excretion of lactating dairy cattle on 51 Merced County farms were estimated based on analyses of the animals' diets and drinking water, using a model developed by the National Research Council.

on intensive and high animal production dairies systems in California.

The lack of controlled research studies makes it difficult to evaluate the importance of water quality in dairy herds (Chase 2002; Socha et al. 2002). When formulating diets for dairy cows, some nutritionists ordinarily do not take into account the mineral content of water, because there is a belief that these minerals are of limited biological availability. However, minerals in water can, in some situations, be more biologically available than those in feeds (NRC 2001). Lactating animals generally get water from three sources: (1) drinking water consumed voluntarily, (2) water present in feeds and (3) water formed within the body as a result of oxidation processes. The first two are the most important; for practical purposes, together they represent total water intake.

Feeding certain minerals in excess of the cow's nutritional requirements may lead to environmentally damaging runoff and the application to land of animal wastes containing high mineral concentrations. The prediction of mineral excretion in dairy animals and the chemical composition of manure need to be considered as important as protein or energy dietary balances. This paper presents part of a survey on feeding management and nutrient balances carried out on dairy herds in Merced County. The aim of this work was to estimate mineral balances and mineral excretion in lactating animals, including the minerals in the drinking water, according to mineral requirements of the NRC (2001) for dairy cattle.

Dairy farm study

From February 2003 to March 2004, 51 dairy farms were randomly selected, and dairy producers were contacted by phone or visited directly. All dairies were visited one or more times to obtain information about nutritional management, herd characteristics and diet composition, and to sample water and concentrated feeds (grains, byproducts, minerals and vitamins mixes).

The NRC software for dairy cattle was used to calculate mineral balances. The final mineral balance to estimate daily excretion for each mineral was obtained as indicated by the software output on the difference between the total dietary supplies (TDS) and the total absorbed required (TAR) for pregnancy, lactation and growth. The TAR for maintenance components (fecal, urinary, sweat and miscellaneous losses) is removed from the body and under normal conditions is excreted daily and replaced with new dietary minerals.

The minerals in water consumed by the cows were estimated based on the water's mineral contents and daily drinking water intake, which was calculated using the formula recommended by the NRC (Murphy 1983). Mineral excretions were calculated for lactating animals in different production groups or diets (for example, fresh cows, first lactation, and low, medium and high milk yields) and by farm, according to the proportion of animals in each production group. The mineral composition of silages and hays was based on the NRC (2001) database. Samples of mixed-concentrate feeds (grains, byproducts, minerals and vitamin premixes) and water were analyzed for total soluble salts (TSS) and the following minerals (listed according to the amounts [grams and milligrams] that are needed): calcium (Ca), phosphorus (P), magnesium (Mg), potassium (K), sodium (Na), chloride (Cl), sulfur (S), sulfate, copper (Cu), iron (Fe), manganese (Mn), selenium (Se) and zinc (Zn); reference methods of the U.S. Environmental Protection Agency (EPA) were used.

Dietary characteristics

The average daily milk production per farm in this survey was 68 ± 11.7 pounds per cow (30.9 ± 5.31 kilograms per cow) ranging from 42 to 95 pounds per cow; and the average daily dry-matter intake (DMI) per farm was 48 ± 4.8 pounds per cow (21.8 ± 2.2 kilograms per cow), ranging from 35.8 to 57.6 pounds per cow. The average number of lactating animals per dairy was 809, ranging from 110 to 5,010, with a median of 523 cows.

In more than 75% of the farms, the diets of lactating cows were based on just five ingredients: corn silage, alfalfa hay, processed corn grain, whole

TABLE 1. Main feeds used for lactating cows on Merced County dairy farms (n = 51)

% farms	Forages	Grains	Proteins and byproducts
> 75	Corn silage, alfalfa hay	Corn grain, cotton seeds	Canola meal
50 to 75			Almond hulls
25 to 50	Wheat and/or oat hay, alfalfa haylage	Barley	Dry distillery grains, whey wet and permeate, rice bran, wheat middling and bran
< 25	Wheat and/or oat silage, sorghum hay and silage, pastures	Soybean seeds	Soybean meal, sugar beet pulp, soy hulls, corn gluten feed and meal, corn germ, citrus pulp, sunflower meal, bakery, raisins, grain screening

TABLE 2. Mineral composition of water troughs on Merced County dairy farms (n = 51)

	Average	Stand. Dev.	Min.	Max.	Upper desired level*	Samples > upper desired level
	mg/L					%
Total soluble salts	592	367.3	74	2,200	1,000	14
Calcium	60	33.7	10	140	100	22
Phosphorus	0.1	0.06	0.01	0.45	ND†	ND
Magnesium	23	18.4	1.4	76	50	10
Potassium	3.25	1.94	1.0	8	10	0.0
Sodium	106	98.0	8.0	500	50	70
Chloride	83	85.4	3.2	390	100	31
Sulfur	24	34.3	1.0	160	ND	ND
Sulfate‡	53	48	4.0	210	50	39
Copper	≤ 0.005	ND	≤ 0.005	0.03	1.0	0.0
Iron	0.07	0.19	0.002	1.3	0.2	10
Manganese	0.13	0.26	0.01	1.1	0.05	43
Selenium	≤ 0.005	ND	≤ 0.005	0.06	0.05	< 2
Zinc	0.05	0.13	0.02	0.91	5	0.0

* Upper desired levels for lactating cows, which may increase mineral excretion.
† Not detected or not determined.
‡ Sulfate, n = 33.

cottonseed and canola meal (table 1). Between 50% and 75% of the dairies also used almond hulls. Almost 30 other different feeds (forages, grains and byproducts) were used in less than 50% of the dairies. Most of the dairy farms in Merced County were using only five dietary ingredients for lactating cows. An important proportion of animals were fed with different byproducts (table 1); this has important environmental implications because these byproducts cannot be used for human consumption but they are transformed into high-quality food (milk and milk products) by the dairy cows.

Minerals in water and diets

Drinking water. Dairy farms pump underground water for animal consumption. We used information from the NRC, U.S. EPA and World Health Organization to establish the upper desired intake levels in water for dairy cows; these levels should be considered a guideline, over which animals may be consuming and excreting excessive amounts of minerals. In our survey of 51 dairies, only 14% of the water samples were saline, with TSS greater than 1,000 milligrams per liter (mg/L). Eight minerals were in excess of the desired levels (table 2).

Our results for mineral concentrations from drinking water were similar to a previous survey of 101 samples collected from dairy farms throughout California (Socha et al. 2002). These authors indicated that the minerals of greatest concern in California were sodium and manganese, which exceeded the desired livestock levels in 64% and 41% of the water samples, respectively. We found a similar trend, but with greater values not only for sodium and manganese, but also for chloride and sulfates.

The results of this study on daily dietary mineral intakes, the contribution of minerals in the water and the final excretion for each mineral are presented in table 3, which shows the average daily dietary intake of each mineral for lactating dairy cows on the 51 dairies, the proportion of minerals consumed from the drinking water, and the estimated daily mineral excretion per cow.

Calcium. The average dietary calcium contents that we found were close to the requirement for cows producing 66 pounds (30 kilograms) of milk daily. The requirements for absorbed calcium that must enter the extracellular compartment for maintenance and production are fairly well known (NRC 2001); therefore, we expected the calcium excretion calculated in this survey to be close to the real calcium excretion. In our study, the average contribution of calcium from drinking water relative to total calcium excretion was low, about 4%.

Phosphorus. The concentration of phosphorus in water averaged 0.11 milligram per liter in our study, representing a small contribution to total phosphorus intake and excretion. These values for phosphorus content in the diet were similar to those found by Satter et al. (2002) and Dou et al. (2003) for U.S. dairy diets. The estimations of phosphorus excretion in table 3 are similar to values reported by Wu (2005) and Weiss and Wyatt (2004).

Magnesium. Magnesium levels in the diets of cattle that we studied were in the same range as Weiss (2004). Weiss compiled data from eight experiments with lactating animals under different feeding conditions, and measured magnesium digestibility using the total collection of feces and urine. The author concluded that the apparent digestibility of magnesium was 30% lower than the mean value calculated by the NRC model. The reason for this lower digestibility of magnesium was the high concentration of dietary potassium. Weiss observed that cows had to consume an additional 18 grams of magnesium per day for every 1 percentage unit increase in dietary potassium above 1%, to maintain the same intake of digestible mag-

Dairy producers often apply manure to fields, where excess nutrients can run off into surface- and groundwater. Better estimates of mineral balances can improve animal performance and protect the environment.

nesium as that consumed when dietary potassium is 1%. These results, and the mean concentration of potassium observed in our survey (1.6%), indicate that the magnesium excretion figures in table 3 should be taken with caution. We found that the average impact of magnesium in the water on magnesium intake was 3.2%.

Potassium. The daily excretion of potassium in this survey was estimated to be almost 300 grams per cow. This is 100 grams per cow lower than Grant (1997) found for cows producing 70.4 pounds (32 kilograms) of milk per day with 1.2% potassium in the diet. This can be explained by the differences observed in potassium dietary contents. The potassium in the drinking water was low, making an insignificant contribution to the diets and excretion (table 3).

Sodium and chloride. Daily intakes of sodium and chloride in the cow diets were high with respect to the NRC recommendations, and also highly variable. These variations may have been related to difficulties in obtaining good estimations of the salts that cattle are permitted to consume freely on some farms. However, the dietary concentrations of sodium and chloride in our study were comparable to those obtained in an extensive literature review by Sanchez et al. (1994).

Of all the minerals evaluated in water, sodium made the greatest contribution to the total daily excretion, averaging almost 17% of the mean sodium excreted. The daily excretion of sodium (64 grams per cow) in our survey was comparable to that found by Bannink et al. (1999), which estimated daily sodium excretions of 56 grams per cow from 10 feeding trials with lactating cows producing 55.4 pounds (25.2 kilograms) of milk per day.

The mean contribution of chloride in water to chloride excretion was 12%. In spite of the cows' ability to consume excess sodium and chloride with limited impacts on performance, the contributions of these minerals to the environment, such as soil salinization, should be considered. The NRC suggested that more research is needed to establish the requirements and appropriate concentrations of sodium and chloride in dairy cattle diets. Nonetheless, the interaction of sodium and chloride with other minerals should be considered when formulating dairy cattle diets, to substantially reduce the amounts of sodium and chloride supplemented and excreted (Sanchez et al. 1994).

Sulfur. The NRC set the sulfur requirement at 0.20% of dietary dry matter (DM), suggesting that the maximum tolerable level should remain at 0.40% of dietary dry matter, with higher concen-

TABLE 3. Estimates of daily mineral intake, drinking-water mineral contribution and net mineral excretion in lactating cows on Merced County dairy farms (n = 51)

	Daily intake*				Water contribution†				Excretion‡			
	Mean	SD	Min.	Max.	Mean.	SD	Min.	Max.	Mean	SD	Min.	Max.
	grams/cow/day											
Calcium	186	39.6	97	299	5.5	3.4	ND	15	150	36.3	72	247
Phosphorus	96	19.5	57	142	ND§	ND	ND	ND	69	17.1	39	114
Magnesium	71	14.7	41	112	2.3	1.9	ND	8	67	14.5	39	106
Potassium	338	51.6	236	520	ND	ND	ND	ND	297	48.8	211	485
Sodium	83	31.8	8	173	10.6	10.0	ND	51	64	30.7	26	153
Chloride	104	26.8	54	168	8.4	12.9	ND	83	71	26.8	15	140
Sulfur	59	11.2	40	87	2.4	3.2	ND	14	16	9.4	1	40
	mg/cow/day											
Copper	326	139.2	123	772	0.5	0.4	ND	2.3	322	138.9	119	767
Iron	4,232	985.9	1,657	6,534	10.8	49.1	ND	355.4	4,201	982.6	1,627	6,495
Manganese	1,457	491.5	573	2,459	10.2	22.1	ND	101.5	1,456	491.4	572	2,459
Selenium	8	2.8	4	15	0.5	0.1	ND	0.7	1.4	2.56	–3	9
Zinc	1,489	579.0	559	2,720	11.2	52.5	ND	377.4	1,375	569.3	480	2,592

* Total daily intake, including minerals in drinking water.
† Estimate based on mineral contents in drinking water and daily drinking-water intake, calculated using NRC (2001) recommended formula.
‡ Excretion (feces + urine) = total dietary supply – total absorbed required for gestation, lactation and growth (NRC 2001).
§ ND = not detected.

Dairy producers need information on the nutritional composition of cattle feeds, including complete mineral analyses, as well as access to analytical methods for estimating the minerals that cows may obtain from water.

trations being potentially detrimental to the absorption of copper and selenium. In our study, the mean dietary sulfur concentration was 0.27%, ranging from 0.20% to 0.40%. Ivancic and Weiss (2001) studied the dietary effect of sulfur and selenium concentrations in lactating dairy cows, and concluded that increasing sulfur in the diet (for example, by 0.21%, 0.41% and 0.70%) significantly reduced dry matter intake, as well as yields of milk, milk protein and milk fat. This negative effect was larger when cows were fed 0.271 parts per million (ppm) compared with 0.135 ppm of selenium. The mean water contribution of sulfur excretion in our survey was 15%. On some farms, sulfur from water must be included in the diet to decrease excretion, minimize interactions with other minerals such as selenium, and minimize possible negative effects on lactation performance. Our estimation of daily sulfur excretion was 16 ± 9.4 grams per cow.

Copper. Based on the zinc, calcium and sulfur contents of the cattle diets that we studied, some interactions with copper absorption were expected (NRC 2001; Spears 2003; Beede 2005). The mean dietary concentration of copper in rations from the farms analyzed in our study was 15 milligrams per kilogram. This concentration was 2.7 times lower than the established upper limit of 40 milligrams per kilogram, and 35% more than the NRC's suggested requirement (11 milligrams per kilogram). We found that the contributions of copper from drinking water and the diet to this mineral's overall excretion were low. However, daily copper intake and excretion were highly variable, ranging from 123 to 772 and 119 to 767 milligrams per cow, respectively.

Iron. Iron can interfere with absorption of copper and zinc when dietary levels are over 250 milligrams per kilogram dry matter (NRC 2001). In our study, the average concentration of iron was below 200 milligrams per kilogram dry matter, but about 10% of the dairy farms had high dietary levels of this mineral. The mean contribution of iron from drinking water to total iron excretion was very low. Daily iron excretion averaged 4,201 ± 983 milligrams per cow based on its coefficient of absorption, which the NRC set at 10% in feedstuffs for adult animals.

Manganese. Recently, Weiss and Socha (2005) estimated the maintenance requirements of dairy cows for manganese. The authors concluded that the dietary requirements were 1.6 and 2.7 times higher for lactating and dry cows, respectively, compared to those calculated with the NRC model. Daily manganese consumption in our survey averaged 67.1 ± 22.8 milligrams per kilogram dry matter (ranging from 23 to 142 milligrams per kilogram). These amounts can apparently support maintenance and production requirements for manganese with no negative effects on the animal. Despite the high concentrations of manganese in some water samples (table 2), the average contribution of manganese from water to total diet and excretion was insignificant or less than 1% (table 3). Daily estimated manganese excretion was 1,456 milligrams per cow, ranging from 572 to almost 2,459 milligrams per cow.

Selenium. Current regulations established by the U.S. Food and Drug Administration (FDA) limit selenium supplementation to 0.3 milligram per kilogram of diet. The mean values obtained in our survey were 20% over that limit. These differences could be explained by the lack of data on the selenium content of feeds. Assuming that most feedstuffs contain some selenium, it is expected that total mixed rations contain concentrations above the recommended level. Possible interrelationships between nutrients that may affect the absorption and metabolism of selenium would alter the requirement for this mineral (NRC 2001; Ivancic and Weiss 2001). The NRC concluded that data concerning the interaction between zinc and selenium is lacking. We found that the estimated average contribution of selenium excretion from water was 35%. The daily excretion of selenium was highly variable, averaging 1.4 ± 2.6 milligrams per cow. This value could also be related to the methodology used to estimate the efficiency of dietary selenium utilization by the animals.

The NRC found that the factorial approach is problematic for establishing selenium requirements because of how selenium is deposited in body tissues. As cows consume more selenium, its concentration in milk and the conceptus (the fetus and associated tissues) increases, indicating that selenium excretion in our survey was probably overestimated. A national meeting on selenium concluded that although minimum selenium requirements are well documented, continued research is needed to determine the optimum dietary requirements for humans and animals, to allow adequate function of the immune system and protect against infectious disease and physiological stress (Drake et al. 1995).

Minerals in the water may affect excretion of them, suggesting that water contributions must be controlled and incorporated when formulating animal diets.

Zinc. Dietary zinc content in our survey was 68.2 ± 25.8 milligrams per kilogram dry matter. This amount is 5 milligrams per kilogram dry matter higher than the requirement set by the NRC for a cow producing 88 pounds (40 kilograms) of milk per day. In approximately 40% of the dairies that we studied, cows were fed more than 63 milligrams per kilogram dry matter, or 1,300 milligrams per cow per day of zinc. Also, in 12% of the dairies, zinc in the diet was too low, under the minimum recommendation (35 milligrams per kilogram dry matter). The mean content of zinc in drinking water was negligible, except at one dairy where 377 milligrams per cow was consumed daily from the water, representing 28% of the mean excretion. The estimated daily excretion of zinc ranged from 480 to 2,592 milligrams per cow.

Analysis of all minerals. Based on the minerals analyzed in this study, a lactating dairy cow producing approximately 66 pounds (30 kilograms) of milk per day might excrete 750 ± 117 grams of minerals per day, ranging from 451 to 1,019 grams per cow per day. The proportion coming from the water represented a mean of 4% ± 3.3% (ranging from 0.3% to 20%). On some dairies, controlling these amounts could reduce manure production and therefore minerals in land applications. The results of our survey indicate that minerals in the water may affect excretion of them, suggesting that water contributions should be controlled and included when formulating animal diets to manage mineral balances and reduce mineral excretion. When an unmanageable excess of minerals coming from water affects soil quality (for example, salinization) or animal performance, other methods to improve water quality should be analyzed (filtration, reverse osmosis and so on).

Obtaining accurate information

In order to obtain accurate estimates of mineral balances in dairy herds — to optimize animal performance and minimize the environmental impacts caused by the excessive excretion of minerals — more detailed information is needed about mineral concentrations in feeds, including the differences between forages, grains and byproducts. Better access to analytical methods for measuring trace minerals would help in formulating animal diets, as would the publication of nutrient compositions in feeds, with complete mineral analyses. For those minerals that receive a substantial contribution from water — such as, in this study, sodium and chloride — water analysis would allow nutritionists to minimize the use of supplemental sources such as free-choice salts.

Although selenium is the only mineral regulated by the FDA, little is known about its content in dairy cow feeds. This lack of knowledge may, in many instances, force nutritionists to not even consider contributions from dietary ingredients other than the supplemental source. A software application is needed that allows ration formulas to integrate minerals from drinking water, indicate the excess minerals consumed, measure potential interactions among minerals that could affect animal health and performance, and estimate the daily excretion of minerals in feces and urine.

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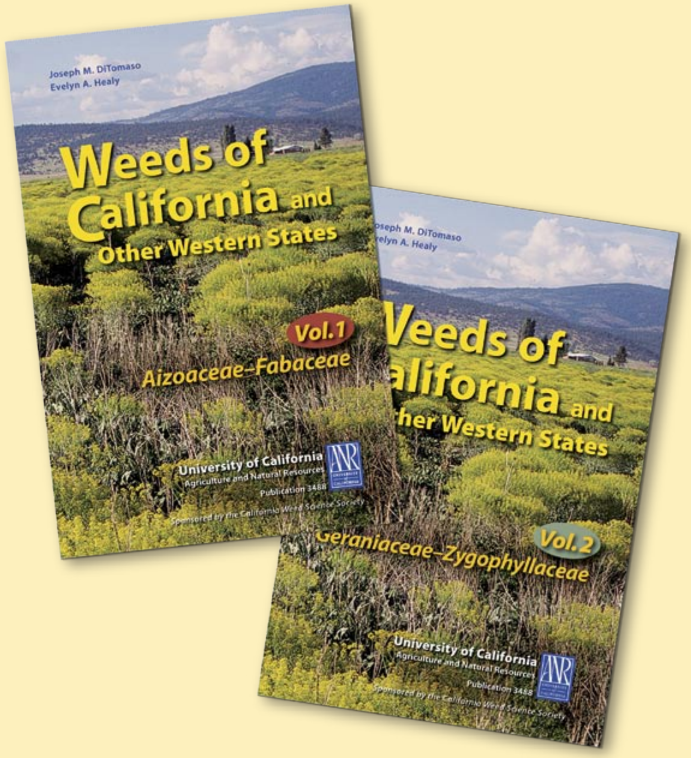

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