JANUARY–MARCH 2009 • VOLUME 63 NUMBER 1

California Agriculture

Injection protection:
Treatments for sudden oak death

University of California | Peer-reviewed Research and News in Agricultural, Natural and Human Resources

Setting agricultural science strategy in tumultuous economic times

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The international competitiveness and prosperity of U.S. agriculture depends on steady and rapid productivity growth fueled by public agricultural research and development (R&D). Agricultural science benefits consumers and the environment, not just farmers. Enhanced productivity as a result of agricultural R&D means that consumers have access to a more abundant, cheaper, safer, higher quality, and more diverse and convenient food supply, produced with less stress on natural resources and the environment. From a global perspective, productivity growth allows agricultural production to increase faster than demand; food has become much cheaper over time in spite of a rapidly growing world population with rising per capita incomes. In the future, continuing productivity growth will be necessary to meet the challenges of ever-increasing demand for food along with mounting pressures on the natural resource base, exacerbated by new demands for biofuels crops.

Long-term and sustained growth in productivity is mainly the result of technological innovations adopted by farmers. Some develop through tinkering and trial and error on farms, but the greater share of agricultural innovations can be traced to organized, scientific and industrial R&D efforts funded by government and the private sector.

Public investments in agricultural science have paid handsome dividends for society. Our formal analysis suggests that state-specific, benefit-cost ratios exceed 10 to 1, and are in many cases more than 20 to 1, for public agricultural research investments in the United States: $1 of research investment today will generate a stream of future benefits equivalent to an immediate dividend of $20 or more. These high benefit-cost ratios suggest that, as a state and nation, we have substantially underinvested in agricultural research, failing to capitalize on technological opportunities and foregoing potential large-scale, long-term net gains. Moreover, recent trends indicate that the extent of underinvestment in productivity-enhancing agricultural science may be worsening.

In 2006, public and private spending on agricultural R&D in the United States totaled $7.6 billion (2000 prices). For many decades, up to the 1970s, such spending grew rapidly. Since then growth has slowed and become quite erratic. In addition, public-sector research has drifted away from on-farm productivity enhancements toward investments emphasizing food safety and quality, human health and nutrition, and natural resources and the environment. Much of this research could have social payoffs comparable to those from farm productivity-enhancing research; but a slower rate of growth in total spending and the drift of research emphasis will result in slower rates of farm productivity growth and a decline in global competitiveness of U.S. agriculture.

Early warning signs of these trends are already apparent, but the full consequences of shifts of research support will not be immediately obvious. Successful agricultural research

Alston, Pardey and James

takes a long time to affect productivity. According to our analyses, it typically will take 10 to 20 years before the effects of a change in research spending implemented today will have their largest impacts, which may then continue for decades.

In California, aggregate agricultural production increased by more than 350% over the past 50 years even though the aggregate quantity of inputs increased by less than 70%, reflecting increases in purchased inputs and capital partly offset by substantial labor savings. This productivity growth fueled by R&D has been enormously valuable, saving resources worth more than $20 billion per year in recent years that would have been required otherwise to produce the increased output.

However, since 1990, the rate of agricultural productivity growth has slowed significantly in developed countries, including the United States and California. From 1949 to 1989, productivity in California agriculture grew by about 2.2% per year (slightly above the national average rate of about 2.1% per year). However, the growth rate slowed to 1.2% per year from 1990 to 2002 in California (slightly greater than the U.S. average of 1.1% per year). This measured slowdown is statistically significant, appreciable and economically important. A 1% compounding growth rate would result in productivity being 22% higher after 20 years; 2% compounding growth would result in productivity being 49% higher after 20 years. Applied to a U.S. industry with an economic value in the range of $300 billion per year, the difference between 1% and 2% compounding over time represents tens of billions of dollars per year even after only a decade or two.

California agriculture is large, diverse and different from that in other states and requires different kinds of research. California cannot rely entirely on others — whether in the private sector, federal government or overseas — to invest the amounts of money in the ways required to sustain an internationally competitive, environmentally sound and prosperous agricultural sector. The recent innovations in federal support for agricultural R&D, in particular an increased emphasis on competitive grant programs and the provision of new funds for specialty crops research (see page 6) may work to California's advantage, but may only have a minimal impact on the fundamental problem of systematic underinvestment. The current state budget problems, and recent cuts in support for the agricultural experiment station, will further undermine California's long-run prospects of sustaining an internationally competitive agricultural sector. Reinvigorated investment by the state government and the private sector, potentially in new funding partnerships, will be required if we are to reverse these disturbing trends.

(At press time, the research discussed was in preparation for publication. References will be posted at the *California Agriculture* Web site.)

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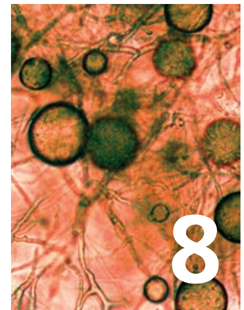
Photo: D.J. Schmidt/UC Berkeley

News departments



Research and review articles

California Agriculture is a quarterly, peer-reviewed journal reporting research, reviews and news. It is published by the Division of Agriculture and Natural Resources (ANR) of the University of California. The first issue appeared in December 1946, making it one of the oldest, continuously published, land-grant university research journals in the country. The circulation is currently about 15,000 domestic and 1,800 international.

Mission and audience. *California Agriculture*'s mission is to publish scientifically sound research in a form that is accessible to a well-educated audience. In the last readership survey, 33% worked in agriculture, 31% were faculty members at universities or research scientists, and 19% worked in government agencies or were elected office holders.

Current indexing. *California Agriculture* is indexed by Thomson ISI's Current Contents (Agriculture, Biology and Environmental Sciences) and SCIE, the Commonwealth Agricultural Bureau databases, Proquest, AGRICOLA and Google Scholar. In addition, all peer-reviewed articles are posted at the California Digital Library's eScholarship Repository.

Authors. Authors are primarily but not exclusively from UC's ANR; in 2005 and 2006, 14% and 34% (respectively) were based at other UC campuses, or other universities and research institutions.

Reviewers. In 2005 and 2006, 13% and 21% (respectively) of reviewers came from universities and research institutions or agencies outside ANR.

Rejection rate. Our rejection rate is currently 26%. In addition, in two recent years the Associate Editors sent back 11% and 26% for complete resubmission prior to peer review.

Peer-review policies. All manuscripts submitted for publication in *California Agriculture* undergo double-blind, anonymous peer review. Each submission is forwarded to the appropriate Associate Editor for evaluation, who then nominates three qualified reviewers. If the first two reviews are affirmative, the article is accepted. If one is negative, the manuscript is sent to a third reviewer. The Associate Editor makes the final decision, in consultation with the Managing and Executive Editors.

Editing. After peer review and acceptance, all manuscripts are extensively edited by the *California Agriculture* staff to ensure readability for an educated lay audience and multidisciplinary academics.

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UC research affects Mediterranean region

I was pleased to see that the entire 62-year archive of *California Agriculture* journal will soon become accessible online. Scientists and growers around the world use this publication as a source of agricultural research.

For instance, UC has a strong research presence in the Mediterranean area, where the climate is similar to that of California. In fact, UC and the state of California interact with most of the countries surrounding the Mediterranean including Egypt, Israel, Tunisia, Morocco, Syria, Greece, Turkey, Italy, France and Spain.

Sound agricultural research is paramount to the stability of this region. There is great economic disparity among the countries, with resulting political unrest and economic disadvantages to trade and to peaceful coexistence.

I would like to invite reader comments on "Egypt's Future Depends on Agriculture and Wisdom." It is an analysis, in draft form, of Egyptian agriculture, its trials, frustrations and opportunities. In it I have summarized the efforts that academics (including UC scientists), politicians, business leaders and farmers have made and continue to make to support the development of the economic potential of modern Egypt and many other developing countries. Go to www.cal-cat.com/Titlepage_04.htm.

Lowell Lewis, UC Coordinator
California-Catalan Programs, Barcelona, Spain

Editor's note: California Agriculture *journal plans to launch its redesigned Web site, including its entire archive of journals, in spring 2009. Lewis was ANR associate vice president for programs from 1981 to 1991.*

WHAT DO YOU THINK?

The editorial staff of *California Agriculture* welcomes your letters, comments and suggestions. Please write to us at 6701 San Pablo Ave., 2nd floor, Oakland, CA 94608 or calag@ucop.edu. Include your full name and address. Letters may be edited for space and clarity.

Science briefs

California salmonids face extinction

California's native salmonids are in precipitous decline and are teetering toward the brink of extinction, according to a new report by Peter Moyle, UC Davis professor and expert on California water systems and fish.

SOS: California's Native Fish Crisis, the first-ever comprehensive report on the status of California's 32 native fish species (salmon, steelhead and trout), was commissioned by the nonprofit organization CalTrout.

"The fish don't lie," says Moyle, who wrote the report with Joshua A. Israel and Sabra E. Purdy, all of the Center for Watershed Sciences at UC Davis. "The story they tell is that California's environment is unraveling. Their demise is symptomatic of a much larger water crisis that, unless addressed, will severely affect every Californian."

The November 2008 report included the following findings:

- If present trends continue, 65% of native salmonids will be extinct within 100 years.
- Sixty-five percent of the species headed toward extinction are found only in California.
- Of the state's 22 anadromous fish species (which spawn in freshwater and live most of their adult lives in the ocean), 59% are in danger of extinction.
- Of the state's nine living native inland fish, 78% are in danger of extinction.

All of the species studied support, or have previously supported, major recreational and commercial fisheries and provide economic and cultural value to Californians, Moyle says. Key stressors on fish include dams, agricultural and grazing practices, development, mining, railroads, logging, some recreational uses, illegal harvesting of native fish, reliance on fish hatcheries and invasive species. Global warming is also playing an important role, the report says, as salmonids are particularly sensitive to changes in water temperature.

Morgan Bond

▲ Central California Coast coho salmon face extinction.

Of the 32 taxa analyzed, one is extinct in California and 14 are listed as state and/or federally threatened or endangered. Pink and chum salmon, southern steelhead and coho salmon face the greatest immediate threat of extinction. Other species fighting for survival include both summer and winter runs of the Northern California Coast steelhead; Central Valley, South/Central California Coast and Central Coast steelhead; Little Kern golden, Lahontan cutthroat and Paiute cutthroat trout; and California Coast, Sacramento winter-run and Central Valley spring-run chinook salmon.

"This doesn't have to happen," Moyle wrote in the *Sacramento Bee*. "We have to leave more water for fish while protecting their diverse habitats. We need to engage in more large-scale restoration projects."

For more information:

www.caltrout.org

Jack Kelly Clark

◀ **Critical research often requires highly trained personnel, advanced instrumentation and updated laboratories — all requiring permanent funding support. *Left,* vegetative propagation of grape.**

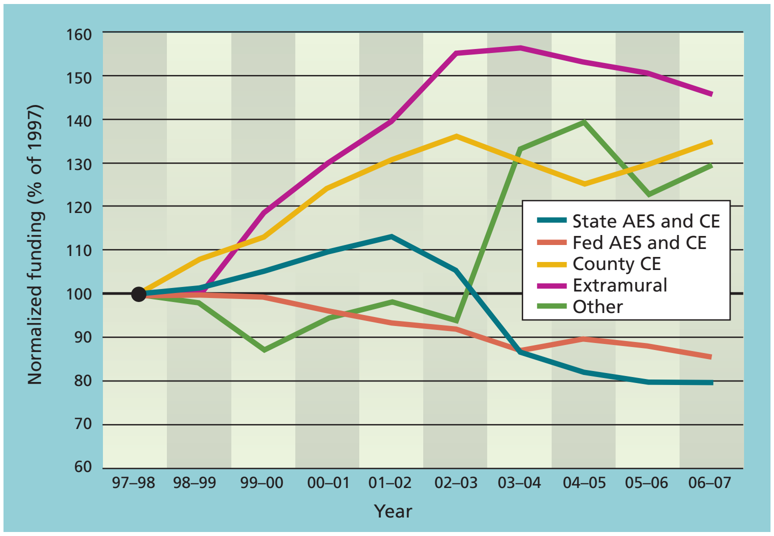
Sustained public investment needed for agricultural research

Ted Batkin, President, Citrus Research Board
Robert Curtis, Senior Manager, Almond Board of California

After 2 years of unprecedented debate and coalition-building, the U.S. Congress passed a landmark Farm Bill in June 2008. By the time it was enacted, the "Food, Conservation, and Energy Act of 2008" had sparked historic levels of public interest and redirected federal funding from established channels. Among other reforms, it strengthened conservation and environmentally friendly farming practices, provided support for local farmers markets and healthy diets, advanced responsible energy production, and increased assistance for families struggling with rising food costs.

Most notably for California, the Farm Bill also created a Specialty Crops Research Initiative to be administered by a newly formed National Institute of Food and Agriculture (NIFA). For the first time, a Farm Bill recognized the importance of specialty crops to the nation's health and quality of life, and the unique role that California plays in the nation's agricultural system.

NIFA's competitive research program is good news for California. Our state, especially the University of California, traditionally fares well in obtaining competitive grants. Already, through the unified efforts of commodity groups and the research community, California agriculture is benefiting from more than $4 million in new research funding for specialty crop needs awarded in FY 2008.



Real trends in UC Division of Agriculture and Natural Resources (ANR) funding sources, 1997–2007, normalized to 1997 funding base (represents 100% level). AES = Agricultural Experiment Station; CE = Cooperative Extension; county CE = funding from county governments; extramural = private and government contracts and grants; other = income generated by ANR.

Permanent funding in decline

Although welcome, this new source of competitive grant funding does not go far enough. It does not address the need for permanent funding to sustain UC faculty and staff who generate the continuous flow of agricultural science breakthroughs and new technologies. Our history of publicly funded research and extension has been the envy of the world and has contributed substantially to California agriculture — a $37 billion industry, producing more than 350 commodities, providing more than 50% of the nation's fresh fruit and vegetables, creating over 1 million jobs and exporting more than $10 billion a year in products.

Unfortunately, recent California trends show declining rates of productivity growth, as noted in this issue's editorial (see page 2). Agricultural economists at UC Davis, the University of Minnesota and California Polytechnic State University, San Luis Obispo, have linked this productivity slowdown to shifts in spending and declining public investment in agricultural research and extension (see figure). Since 1990, state and federal funds supporting the UC Division of Agriculture and Natural Resources (ANR) permanent base budget declined about 20% in real dollars. Today, these permanent funds represent less than 45% of total ANR dollars, and the result has been a 24% reduction in UC Agricultural Experiment Station (AES) scientists and Cooperative Extension (CE) academics over the past 2 decades. This loss of experienced campus- and county-based scientists is even more troubling because 52% of ANR academic staff — AES researchers and CE specialists and advisors — are expected to retire within the next 10 years. If they are not replaced, the consequences will be dire.

Conversely, competitive grant funding awarded to UC researchers and programs has increased by

49% since 1990 and now is roughly equivalent to base permanent funding in the Division. So-called extramural funding — from competitive grants, contracts and cooperative agreements — is critical to UC's research and extension mission. For example, the Gordon and Betty Moore Foundation, the U.S. Department of Agriculture, the California Department of Water Resources, commodity boards and private growers variously supported research in this issue. However, these dollars, otherwise known as "soft money," do not provide the long-term stability and continuity needed to sustain agricultural research, development and extension programs into the future. Why is this? Because base or permanent funding from federal and state governments is critical to supporting and retaining personnel.

Over 90% of permanent dollars in the ANR budget directly fund salaries for campus- and county-based AES and CE academics and support staff. While the substantial increase in extramural grant funding confirms the excellence of UC scientists, soft money can only go so far. Scientists and technicians are needed to ensure the continuity and rigor of long-term, technical research. Human resources (scientists, specialists, advisors, research assistants) are the University's most important resource, and the loss of permanent state and federal funds (in real dollars) reduces the capacity of faculty and staff to address current and future challenges facing specialty crop growers and our natural resource base.

Addressing critical needs

Agriculture in our state is unique — California grows hundreds of crops and is the nation's sole source of nearly 20 major commodities — and critical needs arise without warning, often requiring both a rapid response to control a problem and a more sustained response to resolve it. Examples are the recent discovery of the potentially damaging citrus pest, Asian citrus psyllid, in San Diego and Imperial counties; the decline in populations of honeybees essential to pollination of many California crops; and the continuing challenge of sudden oak death (see page 10). Research addressing these challenges is highly technical, requiring stages of basic and intermediate research, as well as applied field research. The investigations require long-term commitments of teams of scientists, often across disciplines.

Does ongoing investment in research benefit consumers and the broader community? Past history and accomplishments say yes. The current upward fluctuation in food prices is a reminder that we have enjoyed a plentiful, nutritious and relatively inexpensive food supply. However, California agriculture is now called upon to address much more than just productivity and quality. We must produce safe and high-quality food while safeguarding land, air, water and wildlife.

Strong, publicly funded research, development and extension programs at UC are critical for California agriculture to remain both competitive in global markets and sustainable and environmentally responsible at home. This is an investment in the future that provides measureable returns of $10 to $20 for every $1 in public funds (see page 2). Congress and the California State Legislature should be encouraged to increase public funding for agricultural research — few investments have such a high return these days.

But, public investment alone is not the answer. Agriculture, the environmental and natural resources communities and other sectors that benefit from UC research must increase their commitment to the Agricultural Experiment Station and Cooperative Extension, providing sustained funding for longer-term scientific investigations. Our industry's future, and the ability to ensure that "California grown" products will help feed the nation and the world in the future, is in the balance.

Photos: M.E. Rogers

▲ Statewide critical needs arise unexpectedly, such as the recent discovery of, *left,* Asian citrus psyllid in San Diego and Imperial counties. Adult psyllids damage plants directly through feeding and are efficient vectors of the bacterium that causes citrus greening. *Right,* a heavy psyllid infestation.

Photos: Jack Kelly Clark

UC ANR research is often highly technical, requiring stages of basic, intermediate and applied field research. *Left,* farm advisor Janet Caprille explains the procedure for releasing Trichogramma wasps, which control caterpillar pests. *Right,* honey bee hives in an almond orchard. European honey bees, which pollinate about one-third of the world's food, are declining in population. UC scientists are investigating several possible causes, including diseases, climate change and colony collapse disorder.

Science-based outreach helps stem sudden oak death

One of the biggest challenges in controlling sudden oak death is that prevention is the best treatment, but most efforts begin only after trees are already infected. "People don't deal with this disease except in crisis mode," says Janice Alexander, outreach coordinator for the California Oak Mortality Task Force. "We're trying to reach people on the leading edge of the pathogen before it's a crisis."

Caused by the water mold *Phytophthora ramorum*, sudden oak death was first found in California in Marin County during 1995. This pathogen thrives in cool, moist climates; spreads during rainy springs via air, water and soil; and is carried by more than 100 native and horticultural plant species. In California wildlands, *P. ramorum* kills tanoak trees and four types of oaks: black, canyon live, coast live and Shreve's. By 2006, about a million trees had died and another million were infected in California. Today the disease has spread to 14 coastal counties from Monterey to Humboldt, as well as southern Oregon.

The California Oak Mortality Task Force (COMTF) was formed in 2000 to unify efforts to control sudden oak death, ranging from research to management to education. Statewide about $250,000 per year is spent on prevention and outreach, which is funded by the U.S. Forest Service. COMTF members include government agencies, nonprofit organizations and university researchers, and outreach is coordinated by UC.

In 2007, prevention and treatment outreach included training sessions for coastal wildlands and nurseries, and community meetings in afflicted counties. COMTF also maintains a comprehensive sudden oak death Web site, and produces a monthly newsletter that includes updates on new host plants, regulatory changes and the latest research.

Oaks in infected groves can sometimes escape the disease, which spreads in weather-driven cycles. Infections peak during rainy years, and expanding outreach is key to preparing for the next wave of sudden oak death. "The disease is in a lull right now because the winters haven't been very wet," says Chris Lee, COMTF northern outreach coordinator. "We want to be ready when it starts to spread again."

Science-based guidelines

COMTF uses peer-reviewed science to help clear up misconceptions about sudden oak death. Because the pathogen can be transported in soil, sanitation is key to controlling the disease's spread. But people sometimes have taken unnecessarily extreme precautions after hiking in infested areas. "Some threw their shoes away right after hiking," says Alexander, who is based in Marin County. "Others put all their clothes in a paper bag, washed them right away and then burned the bag." But all hikers really need to do is to clean clumps of mud off their shoes during the rainy season, she says.

Another misconception is that people in susceptible coastal areas should avoid gardening with native plants. "Some see the host plant list as a prohibition list," Alexander says. "But aside from bay laurels, they all should be planted. Natives are better than nonnatives, which could introduce another pathogen." Bay laurel trees are the main host responsible for spreading *P. ramorum* in California wildlands.

Most recently, research has shown that sudden oak death infections are not affected by azomite, a mineral-rich powder that is mined from volcanic deposits (see page 10). While azomite is touted as a natural cure, "it's like treating pneumonia with orange juice," says study leader Matteo Garbelotto, a UC Cooperative Extension (UCCE) forest pathology specialist at UC Berkeley.

The only proven sudden oak death treatment is phosphonates, which boost plants' defense systems; this treatment is primarily preventative and is only feasible for individual or small groups of trees. "Azomite appeals emotionally to a lot of people," Alexander says. "Now we'll be able to tell them that it doesn't work."

Matteo Garbelotto Lab/UC Berkeley

Matteo Garbelotto of UC Berkeley demonstrates sudden oak death diagnosis and treatment for members of the media at the Berkeley campus.

"SOD Blitzes" monitor spread

Recent outreach efforts do double-duty by involving stakeholders in research on *P. ramorum*'s spread. Local communities help UCCE's Garbelotto monitor bay laurel trees, which are typically harbingers of infestation, at the edges of areas with sudden oak death. Participants in these weekend events, called "SOD Blitzes," learn how to identify diseased plants and collect bay laurel samples. Then they intensively canvas their areas, which include neighborhoods, open spaces and nature preserves, and give their samples to Garbelotto. After testing the samples for *P. ramorum*, he returns to the communities with maps of the collection points and any infected spots.

First held during spring 2008 with funding from the U.S. Forest Service's State and Private Forestry Office, SOD Blitzes have revealed new infections in the East Bay, the San Francisco Peninsula and Carmel Valley. Pinpointing the pathogen's locations helps communities manage the disease.

"This is a wonderful way to do something when it's really needed rather than blanketing the landscape with a series of treatments that may not be necessary," Garbelotto says. The three newly infested areas will be "SOD Blitzed" again in spring 2009. "Changes in distribution from one year to the next should tell us a lot about the local potential for expansion of this pathogen," he says.

Working with tribes

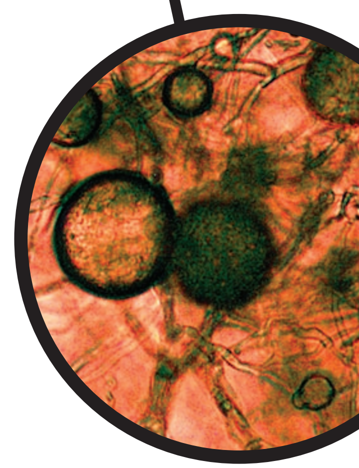
Similarly, Hoopa and Yurok tribes participate in a study that tracks sudden oak death throughout coastal Northern California watersheds, which is led by David Rizzo of UC Davis. *P. ramorum* spores can be carried far ahead of an infection edge in streams. The tribes monitor streams for the pathogen on their lands by using rhododendron leaf baits to attract any swimming *P. ramorum* zoospores. The tribal lands are in uninfested parts of Humboldt County, and so far the monitoring has not revealed new infections.

"We value our research partnership with the tribes," says COMTF's Lee, who is based in Eureka and is also one of the study's researchers. "They do all the baiting themselves. They are cautious over who has access to their land because they don't want it to become infested." Lee has also trained tribes to survey their lands for trees with sudden oak death symptoms.

Tribes are among those most affected by sudden oak death because many of the host plants are integral parts of their culture. "Acorns are an important source of food and bay laurels are important in ceremonies," Alexander says. To help tribes use plants safely, COMTF launched a Native Plant and Tribal Resources Web page in fall 2008. The page lists native plants associated with sudden oak death, with their traditional names and uses by various tribes, and provides guidelines for plant gatherers. Recommendations specific to tribes include burning any unused plant parts such as stripped bark, and boiling any water that has been used to soak twigs used to make baskets; both measures destroy the pathogen.

Trudy Paap, Murdoch University

Researchers use a mesh baiting bag filled with rhododendron leaves to "fish for *Phytophthora*." The bag is retrieved in a few weeks and symptomatic leaves are tested for the pathogen's presence. *Below*, *P. ramorum* chlamydospores grow on agar.



Treatment workshops

To make sudden oak death information even more accessible, Garbelotto recently began offering on-campus treatment workshops. "Many workshops are geared toward professional tree-care specialists," he says. "This lets us share our knowledge with the public in a regular fashion."

Offered monthly during the academic year, these informal 2-hour sessions cover preventative treatments for the disease. Participants learn how to choose trees likely to benefit from treatments, and get to observe field demonstrations of phosphonate treatments.

"We advise treating oaks with no sign of infection when symptoms show up on neighboring bay laurels," Garbelotto says. "We also advise that even if some oaks have died in a grove, it may be possible to protect those that have not been infected."

— *Robin Meadows*

For more information:

California Oak Mortality Task Force
http://www.suddenoakdeath.org

Matteo Garbelotto's laboratory
http://nature.berkeley.edu/garbelotto/english/index.php

UCTV 2008 SOD video
http://www.youtube.com/watch?v=_jWmg5PVK1I

Phosphonate controls sudden oak death pathogen for up to 2 years

by Matteo Garbelotto *and* Douglas J. Schmidt

Since its emergence in the late 1990s, sudden oak death has killed mature oak trees and tanoaks in 14 California counties. Treatment options are now available to safeguard these trees from infection by* Phytophthora ramorum*, the aggressive and exotic pathogen responsible for sudden oak death. We provide an update on current knowledge regarding this emergent disease in California, and present results from three controlled experiments of two chemical treatments to manage the disease in oaks and tanoaks. Phosphonate treatments, legally registered in California to control sudden oak death, were effective in slowing both infection and growth rates for at least 18 months. Conversely, an alternative method consisting of an azomite soil amendment and bark lime wash was always ineffective, and did not reduce either growth or infection rates.

Oaks and tanoaks in 14 California counties are plagued by sudden oak death, including, *above*, tanoaks in the Big Sur region. The tree disease is caused by the exotic pathogen *Phytophthora ramorum*.

Sudden oak death (SOD), the emergent forest disease plaguing four oak species and tanoaks in 14 California counties, is caused by the introduced, nonnative pathogen *Phytophthora ramorum* (Garbelotto et al. 2001; Rizzo et al. 2002). Trees are infected by the pathogen through intact bark, and death is caused by lesions girdling the tree's cambium and partly occluding its vascular system. The time from infection to death can range from a few months to a few years, depending on environmental conditions and genetics of the host and pathogen. Eventually, the majority of girdled trees experience sudden browning of the entire crown without thinning or the loss of foliage, giving the disease its name (Rizzo and Garbelotto 2003).

Sudden oak death epidemiology

Host plants. Although the disease kills large numbers of oaks and tanoaks, *P. ramorum* is a broad generalist capable of infecting more than 100 plant species and ferns (USDA APHIS 2007). On most hosts other than oaks and tanoaks, infection results in leaf blotches and branch diebacks that only occasionally kill infected plants. However, infected non-oak hosts are responsible for the disease's spread. In California woodlands, for instance, the surfaces of California bay laurel leaves and tanoak twigs produce large numbers of infectious airborne *P. ramorum* propagules, called sporangia. In commercial nurseries and European wildland settings, camellias and rhododendrons appear to play a key epidemiological role in spreading *P. ramorum*. Oaks are defined as "dead-end" hosts because they are seldom if ever infectious to other plant species (Garbelotto et al. 2003).

Infection propagules. While most *Phytophthoras* found in temperate forests are soilborne and waterborne (Erwin and Ribeiro 1996), *P. ramorum* is characterized by a predominant aerial phase (Davidson et al. 2005). During the rainy season, infectious sporangia are produced on the leaf surfaces or twigs of infectious hosts, and are released to the environment in wind or rainstorms. Once sporangia land on an area of a suitable host that is covered by a film of water, infection occurs by the release of motile zoospores

***Top left,* California bay laurel leaves typically become infected with *P. ramorum* on the tips of their leaves, where water and dew collect; bay leaves are believed to play a significant role in the disease's spread to oak trees. *Top right,* sudden oak death infections often produce weeping or bleeding cankers and may emit a thick, dark ooze from tree bark; this discharge is the tree's attempt to defend itself against infection. *Left,* in a mature coast live oak tree trunk, the black line shows the edge of the infection and marks the zone between living and dead woody tissue.**

(see photo, page 12) that swim until they can penetrate the host tissue. Epidemiological observations suggest that infection success may be highest when temperatures are warm. In unfavorable dry conditions, and at any time on some plant hosts, *P. ramorum* produces chlamydospores, which are survival or resting propagules characterized by a thick protective outer layer.

Dispersal. Large numbers of *P. ramorum* sporangia, zoospores and chlamydospores eventually end up either in the soil or in waterways. While the epidemiological role of pathogen-bearing soil and water has not been documented, their potential roles range from being short-distance infection sources (such as by infested soil splashing onto the main bole of a tree), to being agents of long-distance dispersal (such as by infested waterways carrying the pathogen downstream for tens or hundreds of miles).

Although the pathogen's exact introduction date and its area of origin are not known, a recent genetic analysis reconstructed the history of sudden oak death in California and elucidated the pathogen's dispersal ability (Mascheretti et al. 2008). *P. ramorum* appears to have escaped the plant nursery environment in Santa Cruz and Marin counties, probably in the 1980s. Humans further enhanced dispersal of the pathogen in the 1990s around the San Francisco Bay Area, presumably by moving infected plants, as indicated by the fact that identical pathogen genotypes were found at distances up to 100 miles, a range that exceeds the natural dispersal ability of this microorganism. Natural spread of the pathogen may be occurring very efficiently at distances up to 200 yards, and presumably in the presence of strong winds, up to 3 miles. Natural movement over 6 to 7 miles appears to be extremely rare.

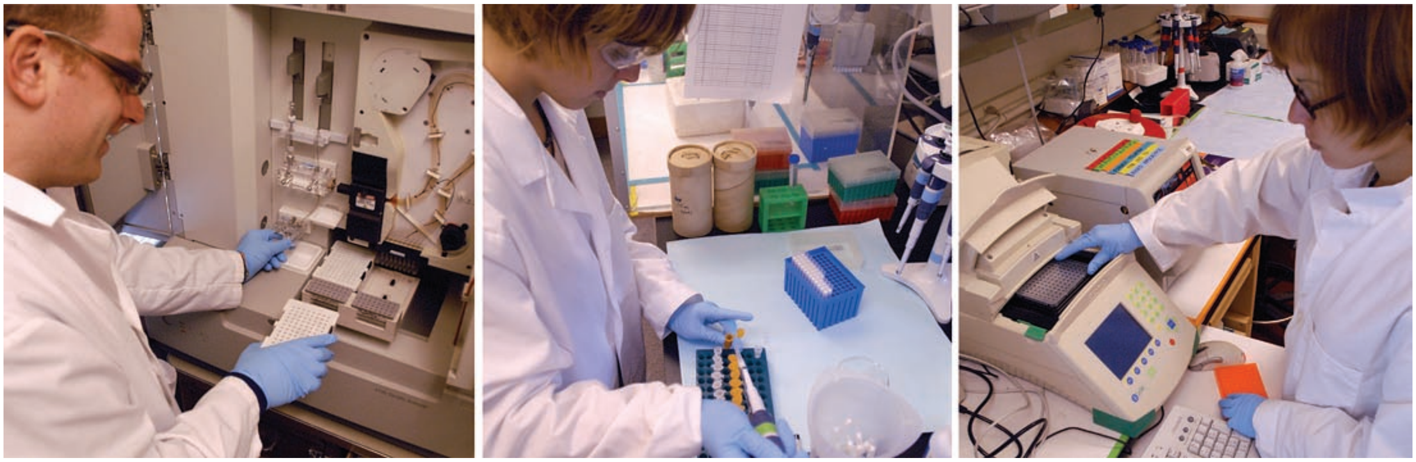
These inferences of the pathogen's spread are congruent with rates of spread for symptoms. Individual forest patches a few hundred yards long can be colonized by *P. ramorum* within one season, while movement of the pathogen between forest patches 0.5 to 3 miles apart can also occur at least once during the course of a rainy spring (Meentemeyer et al. 2004; Prospero et al. 2007). Long-distance spread is not well understood, but may rely on rare long hauls of propagules in strong winds or be linked to human movement.

Oaks and tanoaks. Although any plant subject to high levels of infection by *P. ramorum* may perish, oaks and tanoaks normally die in large numbers. The average mortality of oaks and tanoaks varies between 10% and 40%, but at the local level mortality levels over 50% for adult oaks and up to 100% for adult tanoaks have been recorded (Davidson et al. 2005; Maloney et at. 2005). Besides their ecological importance, oaks and tanoaks enhance residential landscapes and can significantly improve property values. As backyard trees, oaks and tanoaks have become part of the cultural and social heritage of California. As keystone species of coastal ecosystems, indigenous people revere them as sacred trees.

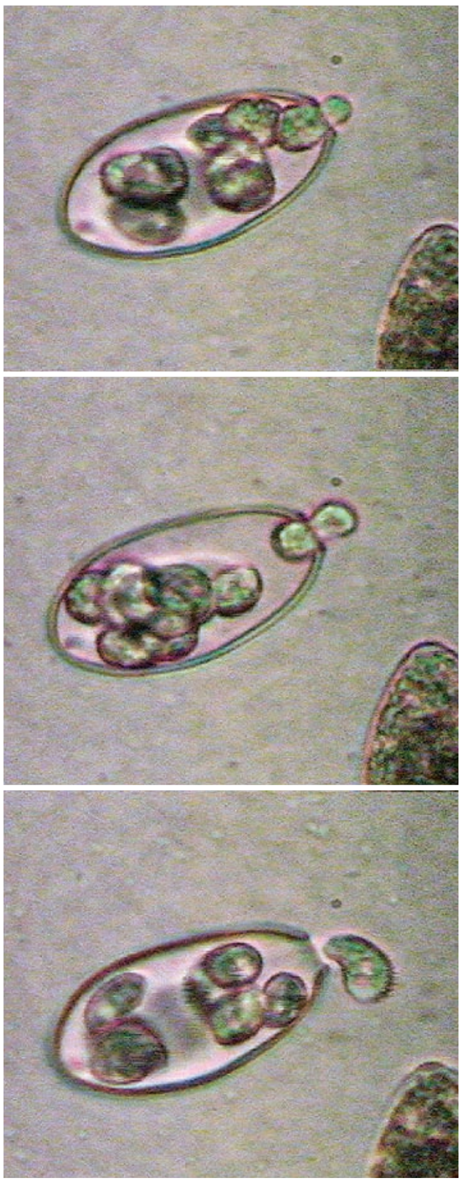
Chemical treatments tested

Phosphonate. Chemical phosphonate treatments are registered to control sudden oak death in oaks and tanoaks, and are most effective if administered preventively before trees are infected (Garbelotto et al. 2007). Phosphonates are environmentally friendly, narrow-spectrum fungicides that can be applied either by injection in the outer wood or topically on the bark. The topical treatments involve adding the organosilicate surfactant Pentrabark, which does not play any direct role in disease control but allows phosphonate to be absorbed through the bark.

Phosphonates are not toxic to animals, including fish and invertebrates, and do not affect beneficial microorganisms. Instead, these simple derivatives of phosphorous acid enhance the



▲ ***Left*, UC Berkeley postdoctoral researcher Noah Rosensweig uses a DNA sequencer to analyze DNA from *P. ramorum*; *middle*, UC Berkeley staff research associate Lydia Baker extracts DNA from *P. ramorum* and, *right*, uses real-time PCR to analyze it.**



Above, three stages of motile, swimming *P. ramorum* zoospores are released from infectious and airborne sporangia, which are normally produced by *P. ramorum* on the surface of California bay laurel leaves during the spring rainy season.

production of secondary metabolites that act as antibiotics (Garbelotto et al. 2008), helping treated plants to fend off microbial infections, including those by *P. ramorum*. The efficacy of phosphonate treatments has already been proven (Garbelotto et al. 2007), but such treatments were developed only relatively recently and no information is available on the duration of their efficacy.

Azomite and lime wash. Several unregistered and unproven treatments are also available to purportedly control sudden oak death in oaks and tanoaks. In particular, one alternative treatment involves amending soil with azomite and using a bark lime wash (see www.suddenoaklife.com). Azomite is an organic trace mineral added to soil as a fertilizer, while lime washes are meant to supply calcium to the plant. Supporters of this approach claim that these treatments decrease soil acidity in affected areas and provide nutrients to infected trees, thereby strengthening their defense mechanisms. The legality of this treatment is questionable because it lacks an official registration in California or elsewhere in the United States; furthermore, the absence of published scientific experiments makes it difficult to evaluate not only its merit and efficacy, but also the presence of potential side effects.

In this study, we present data from the first controlled experiment investigating the longevity of phosphonate treatments. We also present a novel way to test treatment efficacy on adult trees in the field without inoculating their main stems, and describe results of an experiment comparing the efficacy of the registered phosphonate treatment with that of the alternative azomite and lime wash treatment.

Longevity and efficacy studies

Potted oak experiment. We used 45 potted coast live oak trees that were 6 years old, 7 to 10 feet tall, and grown in 15-gallon pots filled with a 50:50 mix of sand and fir mulch. Trees were kept onsite in a lathe house at UC Berkeley for 2 months before the experiment started. On Nov. 30, 2005, 15 trees were injected with Agrifos (Agrichem, Queensland, Australia), the only phosphonate currently registered to treat sudden oak death in California. Another 15 trees were treated with a bark application of Agrifos and the organosilicate surfactant Pentrabark (Quest Products, Louisburg, KS), which is also registered for sudden oak death in California. The surfactant helps to even application of the phosphonate and enhances its adhesion on the tree trunk, so that the phosphonate can be progressively absorbed into the cambium. The remaining 15 trees were untreated controls.

Injections were applied with Chemjet injectors (Chemjet Trading, Queensland, Australia) each containing 10 milliliters of the phosphonate formulation at a concentration of 217 milligrams per milliliter. For the bark applications, we sprayed a solution of 310 milligrams per milliliter Agrifos and 1% Pentrabark on a 1-yard-high band of the tree main stems, until the bark surface was completely wet.

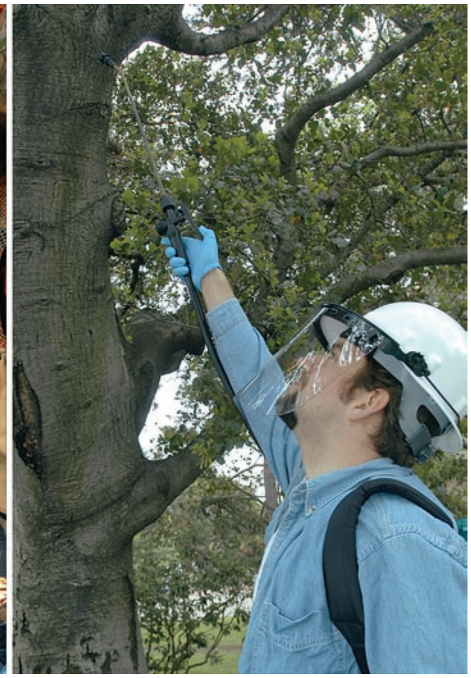
Approximately 150 milliliters of solution per square meter of bark was used for this application.

Pr102, a widespread *P. ramorum* genotype (Ivors et al. 2004), was inoculated under bark of the main stem of treated and positive control saplings as described in Garbelotto et al. (2007). As a negative control, each tree was also mock-inoculated by placing a plug of sterile agar on the opposite side of the stem and 5 inches higher than the pathogen inoculation point. The mock inoculation allows researchers to determine the size of lesions caused by wounding during the inoculation process, in the absence of the pathogen. For each treatment and control, five trees were inoculated 6, 12 and 18 months post-treatment.

Six weeks after each inoculation, we removed the outer bark and measured the extent of the necrotic lesions. To confirm that lesions were caused by *P. ramorum*, at least four isolations were taken by excising the margins of each visible lesion and plating them on a *Phytophthora*-semiselective culture media called PARP (Erwin and Ribeiro 1996). For each sapling, we calculated the cumulative linear lesion size by adding the maximum linear extent of the lesion along the stem to the maximum lesion extent across the stem's circumference. The data was then log-transformed and analyzed by ANOVA; multiple comparisons were performed with *t*-tests at *alpha* = 0.05. This experiment was repeated for phosphonate injections, but the results were analogous to the first experiment and are not shown.

Field study. We selected 15 mature coast live oak trees in Novato, Calif., whose trunks with diameters at breast height (DBH) ranged from 24 to 48 inches and averaged 31 inches. On Feb. 14, 2005, five trees were treated as recommended by the manufacturer with phosphonate and organosilicate surfactant on the bark (approximately 1.35 gallons of tank mix per tree; mix composed of Agrifos at 0.07 ounce per gallon and 1% Pentrabark). Another five trees were treated as recommended by the distributor by adding azomite (143 pounds per tree) to the top organic layer of soil from the tree trunk to the canopy drip line, and applying lime wash to the bark from the root collar to 5 to 6 feet up the trunk at approximately 2 gallons per tree. The remaining five trees were left untreated to serve as controls.

UC Berkeley staff research associate Ellen Crocker uses spring-loaded mechanical syringes to inject phosphonate compounds into a Marin County coast live oak.



UC Berkeley staff research associate Brett Voss applies a topical phosphonate spray to a coast live oak in Alameda County to study the treatment's effectiveness.

The efficacy of these two chemical treatments was tested at 6, 12 and 18 months post-treatment by inoculating 10 cut branches per tree with *P. ramorum* as described in Dodd et al. (2005). Terminal branches (0.8 to 1.2 millimeters diameter) were cut, immediately placed in water and transported to the UC Berkeley greenhouse. Cuttings were transferred to fresh water and kept in a mist chamber. The bark was slit with a scalpel, and a plug of agar recently colonized by *P. ramorum* isolate Pr102 was placed under the bark. Mock control inoculations were performed on one branch per tree by placing a plug of sterile agar under the bark. All inoculations were wrapped with parafilm (Pechiney Plastic Packaging, Menasha, WI) and aluminum foil.

Three weeks after inoculation, we removed the outer bark and measured the extent of necrotic lesions. To confirm that these lesions were caused by *P. ramorum*, at least two isolations were taken from the edges of each visible lesion and plated on PARP. For each cutting, the extent of the lesion upward toward the tip of the branch was added to the extent of the lesion downward toward the branch base. Data was then log-transformed and analyzed by ANOVA; multiple comparisons were performed by *t*-tests with *alpha* set at 0.05.

Efficacy testing. For most plant diseases caused by *Phytophthora* spp., there is a good correlation between the size of lesions caused by under-bark inoculations and susceptibility of the individual plant (Huberli et al. 2002). By wounding the inoculation site, under-bark inoculations bypass the infection process and thus test the rate of pathogen spread rather than that of infection. Because it could be argued that some treatments effectively prevent infection but do not necessarily slow disease progression, we designed a way to directly test the efficacy of treatments in preventing infection by *P. ramorum*.

To circumvent the limitations of under-bark inoculations, we used a more realistic inoculation test on 40 potted oaks like those used in the first experiment. Before treatment, the bark was gently scraped at the selected inoculation points to ease direct infection by zoospores. We treated 10 trees with a bark application of phosphonate and organosilicate surfactant (Agrifos and Pentrabark) and 10 with the azomite soil amendment and bark lime wash; 20 were left untreated. Fourteen days later, the 20 treated trees and 10

A plastic device developed at UC Berkeley is used to artificially infect trees with zoospores of *P. ramorum*.

The dark lesion in this Alameda County coast live oak may continue to grow until it girdles and eventually kills the tree.

of the untreated trees were inoculated with zoospores of Pr102 isolates. The other 10 untreated control trees were mock-inoculated using water without zoospores.

To produce *P. ramorum* zoospores, ten 1-square-centimeter plugs were cut from the margins of 2-week-old colonies and floated on sterile deionized water in 100-millimeter-diameter Petri dishes. The plates were incubated in the dark at room temperature for 72 hours (Erwin and Ribeiro 1996). At the end of the incubation period, the water used to flood each of the 10 plates was poured into a plastic bottle with a 250-milliliter-wide mouth (Nalgene, Rochester, NY). To obtain additional zoospore solution, all plates were rinsed a second time with

Phosphonate treatments reduce both colonization and infection rates of the sudden oak death pathogen.

10 milliliters of sterile deionized water, and second rinses were added to the first ones in the same bottles. The bottle cap was then secured without tightening it, to allow for air flow. We induced sporangia to release zoospores by placing the bottle on ice for 30 minutes, followed by 1 hour at room temperature. In order to visually verify that zoospore release had occurred, zoospore counts were performed using a hemacytometer (Hausser Scientific, PA).

Once zoospores were produced and their concentration adjusted to 1×10^4 per milliliter, 1.5 milliliters of zoospore suspension was added to an inoculation block placed around the inoculation point. The blocks, made out of polyurethane resin, had concave surfaces that conformed to the round bark surface. Each inoculation block had two openings: one in the concave side to allow delivery of zoospores to the inoculation point, and another on top to allow the operator to place the desired aliquot of zoospore suspension in the block. Grafting wax was used to ensure a perfect seal between the inoculation block and tree surface. Blocks were left for 24 hours with the upper opening corked. After the blocks were removed, the inoculation points were entirely wrapped with parafilm and foil.

Six weeks after inoculation, we removed the outer bark and measured the extent of necrotic lesions. To confirm that these lesions were caused by *P. ramorum*, at least four isolations were taken from the edges of each visible lesion and plated on PARP. For each sapling, we calculated the cumulative linear lesion size by adding the maximum linear extent of the lesion along the stem to the maximum lesion extent across the stem's circumference. The data was then log-transformed and analyzed by ANOVA; multiple comparisons were performed by *t*-tests with *alpha* set at 0.05.

Treatment comparisons

Phosphonate and potted oaks. Compared to the untreated trees, cumulative lesions were significantly smaller in the potted trees treated with phosphonate injections and bark applications of phosphonate and organosilicate surfactant (Agrifos and Pentrabark) for each post-treatment dataset (df = 2; $25 < F < 76.3$; $0.001 < P < 0.0001$), as indicated by ANOVA performed separately on the 6-, 12- and 18-month datasets (fig. 1). Treatment efficacy was also detected 1 and 3 months post-treatment (data not shown). In all cases, *P. ramorum* was isolated at least once from each inoculated tree, independent of treatment, confirming that the lesions visible in the phloem were caused by the inoculated pathogen. Negative mock inoculations never caused any lesions beyond the wound-induced one, and were excluded from the analysis.

Field study. The branch cuttings that we placed in water and kept in a mist chamber survived well during the length of each inoculation trial. *P. ramorum* was reisolated at least once from each inoculated branch, confirming that lesions visible in the phloem were caused by the inoculated pathogen. There was never a significant difference between the size of lesions in untreated trees and in those treated with the azomite soil amendment and bark lime wash, as indicated by ANOVAs performed on each individual dataset of inoculations at 6, 12 and 18 months after treatment (fig. 2). Conversely, the average lesion size in tree branches treated with bark applications of phosphonate and organosilicate surfactant was always significantly smaller than those in untreated tree branches (df = 2; $56 < F < 79.4$; $P < 0.0001$). Phosphonate efficacy was also detected 1 and 3 months post-treatment (data not shown).

Efficacy testing. Potted trees were successfully infected by zoospore suspensions applied through the inocula-

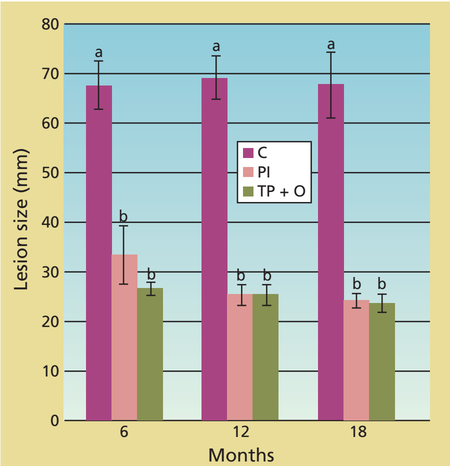


Fig. 1. Cumulative linear size of lesions (along and across stem) caused by under-bark inoculation of *Phytophthora ramorum* onto the main stem of potted trees at 6, 12 and 18 months after treatment. Treatments included injections with phosphonate (PI), topical bark application of phosphonate and organosilicate surfactant (TP + O) and untreated controls (C). Average lesion size and standard error in wounded but uninoculated negative controls were 16.6 and 0.6 millimeters, respectively.

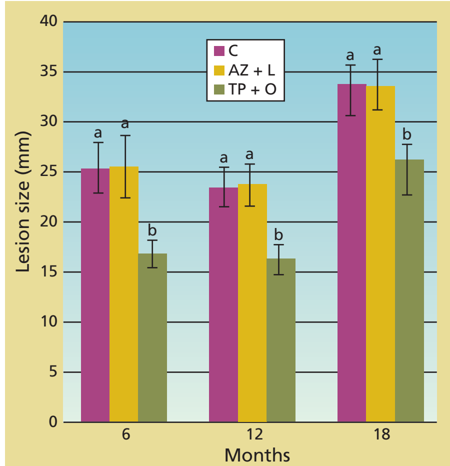


Fig. 2. Total linear size of lesions along stems of branch cuttings caused by under-bark inoculation of *Phytophthora ramorum* at 6, 12 and 18 months from treatment of parent trees from which cuttings came. Treatments included topical bark application of phosphonate and an organosilicate surfactant (TP + O), azomite soil amendment and bark lime wash (AZ + L) and untreated control (C). Average lesion sizes (standard error) in wounded but uninoculated negative controls were 14 (0.3), 13 (0.2) and 18 (0.3) millimeters, respectively, for the 6-, 12- and 18-month trials.

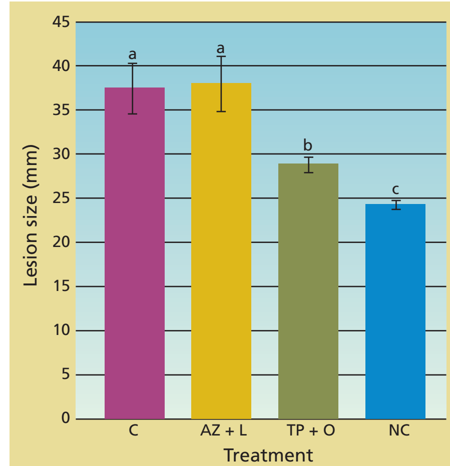


Fig. 3. Cumulative linear size of lesions (along and across stem) caused by applying zoospores of *Phytophthora ramorum* onto main stem of potted trees 42 days after treatment. Treatments included topical bark application of phosphonate and organosilicate surfactant (TP + O), azomite soil amendment and bark lime wash (AZ + L), untreated controls (C) and negative control in which bark was gently scraped but no zoospores were applied (NC).

tion blocks. Lesion sizes in trees treated with the azomite soil amendment and bark lime wash were not statistically different from those in untreated trees (fig. 3). In contrast, lesions in trees whose bark was treated with a topical application of phosphonate and organosilicate surfactant were both significantly smaller than those of untreated trees (df = 2; $F = 37.6$; $P < 0.001$) and significantly larger than those of the mock-inoculated trees.

Recommendations for SOD treatment

Introduced plant diseases represent one of the most serious threats to endemic plant communities in California and can lead to the decimation or even extinction of affected plant hosts (Wingfield et al. 2001). The high levels of mortality caused by sudden oak death among some oak species and tanoaks are a vivid example of the intensity and scale of damage that can be caused by an introduced microorganism. Genetic analyses confirm the exotic nature of the sudden oak death pathogen (Ivors et al. 2006; Mascheretti et al. 2008), intensifying the need for approaches aimed at both slowing down the epidemic and protecting tree species lethally affected by *P. ramorum*.

Phosphonates have long been used in agricultural situations, including avocado orchards in California to control other *Phytophthora* species (Guest et al. 1995), as well as in natural ecosystems, including the Jarrah forests of Western Australia (Hardy et al. 2001). Phosphonates are fungicides targeting a small but important group of plant pathogens called oomycetes. The action of phosphonates is often dual, with a direct contact effect augmented by an increased defense response in the treated plant, which may include a range of physiological changes from cell-wall thickening to the increased production of antimicrobial secondary metabolites (Guest and Grant 1991). However, *P. ramorum* is not particularly susceptible to direct contact with phosphonate (Garbelotto et al. 2008), and most of this treatment's efficacy hinges on the augmented production of defensive secondary metabolites.

While registered in California to treat sudden oak death in oaks and tanoaks, phosphonate treatments are not necessary for blue oak, valley oak, Oregon oak or interior live oak, all of which have never been reported as infected by *P. ramorum*. Because sudden oak death is an emergent disease with only a recent history in California, the longevity of efficacy for registered treatments is unknown, although controlled studies have shown that treatments are most effective if administered preventively before trees are infected (Garbelotto et al. 2007).

Phosphonate treatments have been effective for 2 to 6 years in other *Phytophthora* species, depending on the host species (Hardy et al. 2001). Our first potted tree experiment and the field experiment indicated that phosphonate treatments had a significant effect at 6, 12 and 18 months post-application; future testing will determine whether they are effective for longer. Because most *P. ramorum* infections occur in late winter and spring, 18 months of coverage will adequately protect trees for 2 years. However, in order to obtain the highest level of control using phosphonate treatments, a recommended schedule of preventive applications for oaks and tanoaks includes two applications the first year to ensure

The azomite and lime bark wash showed no efficacy whatsoever.

Top left, **UC Berkeley staff research associate Alex Lundquist uses a pole pruner to collect branches of a Marin County coast live oak.** ***Top right,*** **a small plug of agar is used to infect the branches with** ***P. ramorum*****.** ***Above,*** **UC Berkeley staff research associates Ellen Crocker and Lundquist inoculate oak branches in the laboratory. This study method allows researchers to safely infect oak wood with** ***P. ramorum*** **in the laboratory and test various sudden oak death treatments.**

full coverage (one in late fall and one in early spring), followed by one fall treatment every year. Fall treatments are recommended because they allow the plant to develop a full defensive response before infection spreads in the warm and rainy spring.

The choice of phosphonate application method (injection or bark spray) is to some extent a personal one. Injections release the totality of the product inside the tree without the organosilicate surfactant, presumably achieving efficacy faster; but specialized equipment and training are needed to drill multiple holes in the tree, and a certain number of injections may fail, especially in gnarly trees with abundant decay pockets. Although a single injection was applied to our potted trees, larger trees require multiple injections, approximately one for every 6 inches (15 centimeters) of tree circumference. Holes are drilled at a slight downward angle through the bark and cambium into the outer three to six growth rings of the wood. Absorption of the chemical appears to be fastest on warm sunny days, from the late morning to midafternoon.

Bark topical applications are rapid and easy; however, post-application efficacy takes longer, the organosilicate surfactant must be used in conjunction with phosphonate, the chemicals can disperse into the environment, and all types of foliage including moss are at risk of phytotoxicity upon contact with the chemical. It is best to mix the phosphonate and organosilicate surfactant on treatment day. Applications can be done with any kind of applicator or sprayer. The lower 10 feet of trunk must be thoroughly wetted, possibly including some of the lower main branches in the canopy.

The field experiment — in which adult trees were treated but efficacy was tested on cut branches rather than on the entire tree — was successful. This approach may provide a new tool to study treatment options for sudden oak death and other forest diseases without killing plants or using dangerous inoculum in the field. *P. ramorum* grew significantly slower in trees inoculated with phosphonates at 6, 12 and 18 months after treatment, while the pathogen's growth rate with the azomite and lime wash treatment was always the same as that of untreated controls. We found that the phosphonate topical treatment was effective up to 18 months from application, while the azomite and lime bark wash showed no efficacy whatsoever at any of the three time periods tested.

The second potted tree experiment was designed to directly assess the efficacy of treatments on infection rather than colonization rates of the pathogen. The results indicated that the phosphonate topical application significantly reduced the size of infection lesions caused by zoospores, but the azomite and lime wash treatment had no effect. Besides this lack of efficacy, we found that the sheer amount of material needed to treat each tree with azomite and lime wash was cumbersome and aesthetically unpleasant due to the large amounts of azomite on the ground around treated trees. Although this treatment is being touted as a holistic fertilizing approach aimed at enhancing the overall health of the tree, it is often and illegally prescribed to directly control sudden oak death. While true fertilization treatments do not require a registration, those used to directly control a disease do; as such, the azomite and lime wash treatments appear to be in a legal "grey zone."

Our results highlight the importance of continued testing approaches both to verify the efficacy of treatments and to better understand their real potential to control sudden oak death. Many aspects of phosphonate treatments are still unknown, including their longer-term efficacy and the percentage of oak and tanoak tree populations that will respond positively. Phosphonate treatments are one component of an integrated disease management approach that also includes sanitation and the elimination of infectious plant hosts. Because phosphonate is most effective when applied preventively before a tree is infected, one of the greatest challenges is identifying good candidate trees. Unfortunately, trees undergo a phase in which *P. ramorum* infection is latent.

Our improved understanding of sudden oak death epidemiology can help with the selection of good candidate trees, which may include asymptomatic oaks located in disease-infested groves. In tanoaks, the disease may affect large numbers of contiguous trees, while apparently healthy trees are most likely already infected. To circumvent this problem, we recommend extending the treatment of tanoaks at least 328 to 656 feet (100 to 200 meters) into the surrounding area that is free of the pathogen. Finally, given the novelty of the phosphonate treatment and limited knowledge of sudden oak death, treatment recommendations and guidelines will likely change as more research data becomes available.

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◀ A Marin County coast live oak was used to test an alternative sudden oak death treatment, in which bark is covered with a lime wash and an azomite soil amendment is applied around the tree. In this study, the unregistered treatment was not effective in stemming the disease's spread.

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New pistachio varieties show promise for California cultivation

by Craig E. Kallsen, Dan E. Parfitt,
Joseph Maranto *and* Brent A. Holtz

California pistachio growers have long relied on a single female ('Kerman') and single male ('Peters') cultivar. Despite their benefits, these cultivars present important production and marketing concerns. To evaluate new varieties for the pistachio industry, we conducted the first randomized and replicated pistachio variety trials in the San Joaquin Valley, where most U.S. pistachios are grown. After more than a decade of scientific evaluation, several varieties new to California (such as 'Kalehghouchi') or the world (such as 'Golden Hills') demonstrated commercial potential for the California pistachio industry and would complement the production characteristics of 'Kerman'.

The California pistachio industry, which grows more than 90% of the nation's crop, has historically been dominated by a single female variety, 'Kerman'. UC researchers conducted the first randomized and replicated pistachio trials, and two new female and one male variety were released in 2005. 'Golden Hills', shown at 9 years of age in June 2006, is similar to 'Kerman' but with a larger number of somewhat thinner scaffold branches.

Pistachio production has expanded rapidly in California, but varietal options for growers have not. In 2006, California growers harvested 112,500 acres of pistachio and planted another 40,100 nonbearing acres (CPC 2007; USDA 2007). In 2005 and 2006, the average yearly value of California's pistachio crop was approximately $518 million (CPC 2007). Unlike most crops, the California pistachio industry is almost completely reliant on a single female cultivar, 'Kerman', and a pollinating male, 'Peters', to produce pistachios. Aside from the potential genetic vulnerability to pests that is often associated with cultivating a single female cultivar, other problems became more apparent as pistachio acreage increased.

Alternate bearing is a prominent characteristic of pistachio, 'Kerman' included. The onset of alternate bearing in pistachio is a function of age. Fruit and nut tree age is typically measured in "leaves" as opposed to years: the age of a tree at planting is 1st leaf, and a tree does not become 1 year old until 2nd leaf. Usually, beginning in 10th-leaf trees (i.e., 9-year-old trees), 'Kerman' enters into an alternate-bearing cycle with a low-yielding year followed by a high-yielding year. For reasons that are not clear (weather is suspected of triggering the cycle), the majority of acreage in the San Joaquin Valley, where over 90% of the nation's pistachio crop is located, becomes synchronized so that production for an entire year is high or low, regardless of when a particular 'Kerman' tree (or orchard) was planted.

Since 'Kerman' is the dominant female cultivar grown in California, and most production acreage is located in a relatively limited region with similar growing conditions, the entire crop matures within a brief time period. Harvest begins in early September and ends by the second week in October. Most trees in the San Joaquin Valley are harvested within a 2- to 3-week period in most years. During "on" or high-yielding years, adequate harvest equipment, labor, processing and storage facilities are not available, while in "off" or low-yielding years some of these resources remain unused.

This is a particular problem for processing plants that hull, sort, dry, roast, store and package the crop. Processing capacity has an investment cost and if it is not used efficiently that cost must be absorbed by the processor-investor and passed along to the grower in the form of lower payments for the crop and to the consumer as higher prices for the product. Early- and late-maturing cultivars are available for other nut crops grown in California, distributing the harvest across a longer time period. For example, the commercial almond (*Prunus dulcis*) harvest in the San Joaquin Valley typically begins with the 'Nonpareil' cultivar about Aug. 1 and ends with the 'Fritz' and

As with most wind-pollinated plants, pistachio flowers are small and not showy, as with, *left*, the female 'Golden Hills' and, *center*, male 'Randy'. In California, male trees are commonly planted as every fifth tree in every fifth row to provide adequate pollination. *Right*, a 'Golden Hills' nut cluster demonstrates greater development of red color in the hull than 'Kerman'.

'Monterey' cultivars in mid-October. Reliance on a single, alternate-bearing cultivar has complicated pistachio marketing efforts, with prices declining in heavy-bearing years and increasing in low-yielding years.

While 'Kerman' has been a dependable producer of quality pistachios in California for 50 years, it also has some negative characteristics. For example, a load of 'Kerman' nuts arriving from the field to the processing plant at harvest includes a relatively high percentage of nuts with no kernels (called blank nuts) and unsplit nuts containing edible kernels (called edible, closed, in-shell nuts). A typical load of pistachio nuts delivered to a San Joaquin Valley processing plant may contain approximately 10% blank and 15% edible, closed, in-shell nuts.

The pistachio industry has attempted to address alternate-bearing, blank and closed-shell nuts through cultural practices such as pruning and irrigation management. Pruning can be conducted to reduce the nut bearing potential of the tree through leaf canopy reduction in a given year, which can mitigate the alternate-bearing cycle over a period of years (Ferguson et al. 1995). Deficit irrigation of the tree from May to mid-June has been shown to increase the percentage of nuts that split without reducing yield (Goldhamer and Beede 2004). California 'Kerman' growers are using both of these practices, developed by University of California researchers.

Pistachio industry boost

Pistachio was first introduced to California more than a century ago near Sacramento (Crane and Maranto 1988). The commercially grown pistachio species in California, *Pistacia vera* L., is native to a wide area of Central Asia, including parts of northeastern Iran, northern Afghanistan, southern Turkmenistan, and southeastern Uzbekistan, Tajikistan and Kyrgyzstan (Zohary 2006). This pistachio species is well adapted to Mediterranean climates typified by long, hot, dry summers and relatively cool winters.

Historically, Iran has been and continues to be the world leader in pistachio production, and in the 1960s competition from U.S. growers with the Iranian red-dyed pistachio nut was minimal. However, this changed with the imposition of U.S. embargoes on all Iranian exports as a result of the Iranian hostage crisis from 1979 to 1981. An unintended consequence of this embargo was the opportunity to establish a world-class pistachio industry in California. The California industry now produces high yields of excellent quality nuts in demand worldwide, and supplies over 99% of pistachios grown and consumed in the United States.

Most of this production occurs on deep, boric and calcareous soils of the southwestern San Joaquin Valley, where summers are characterized by hot temperatures, low humidity, low precipitation and light winds.

Dependence on one cultivar

The female cultivar 'Kerman', named in 1952, was produced from seed imported from Iran in 1929 through a U.S. Department of Agriculture (USDA) importation and evaluation program in Chico. 'Kerman' was introduced to growers in trials beginning in 1957. The 'Peters' male is reported to have been selected and named by A.B. Peters, a pistachio grower near Fresno, in the early 1900s. Pistachio trees are dioecious, which means female (nut-producing) flowers and male (pollen-producing) flowers are borne on different cultivars. Pistachio is wind-pollinated, and both male and female trees are required to produce nuts. The flowering period of 'Peters' overlaps with that of 'Kerman' well; few growers found other pollinating varieties necessary, and few other choices existed.

Through the 1980s, the state's relatively small pistachio industry expanded slowly and there was little interest or demand for new cultivars. A few growers had small acreages of cultivars such as 'Aria', 'Bronte', 'Red Aleppo', 'Trabonella' and 'Kalehghouchi', many of which originally came to California from the Mediterranean area and the Middle East before 'Kerman' was available. 'Kerman' proved well-adapted to the needs of the small pistachio industry, and there was little incentive for researchers to evaluate new or existing cultivars against it in randomized, replicated trials prior to the rapid expansion of the industry. Without the scientific evaluation of yield and nut-quality characteristics, growers were understandably hesitant to accept the

A single negative characteristic can render an otherwise useful variety with many exceptional characteristics unsuitable for commercial production.

risks associated with large plantings of new cultivars. Other cultivars widely planted elsewhere in the world, such as 'Damghan' and 'Rafsanjani' in Iran and 'Sirora' in Australia, have not been grown in California.

In the early 1980s a few growers planted 'Joley', which, like 'Kerman', was selected from seed collected in Iran. However, 'Joley' never gained acceptance among California pistachio growers because the nuts, while maturing early and splitting well, were relatively small and susceptible to staining. Many 'Joley' orchards were grafted back to 'Kerman'. Meanwhile, industry acreage planted with 'Kerman' has continued to expand.

To compare yields, older trees were harvested with a commercial, mechanical tree shaker and catching frame, while nuts from younger trees were knocked onto tarps with mallets and poles.

Pistachio breeding program

To address concerns about the industry's reliance on a single cultivar, in 1990 the California Pistachio Commission supported the creation of a UC-based Pistachio Breeding Program, conducted by UC Farm Advisor Joseph Maranto and UC Agricultural Extension Service Pomologist Dan Parfitt. This breeding program identified a collection of pistachio varieties from throughout California that provided a diverse base of genetic material for developing new varieties. Pollen from male trees was used to pollinate female trees in this collection in the springs of 1989 and 1990, resulting in seeds collected in late summer of each year. These seeds were germinated and the resulting seedlings were planted in three Central Valley locations: Winters, Kearney and Bakersfield. These locations provided different environments within Central Valley pistachio-growing areas. The trials were located at UC research and extension centers in Winters and Kearney, and on the property of a grower cooperator near Bakersfield.

In 1996, Parfitt selected female trees with potentially valuable characteristics from the Bakersfield seedling plot, because warmer summer temperatures in this area generally promote more-rapid tree growth. Additional trees from Bakersfield and Winters were selected in 2001 and planted in 2002, in randomized, replicated experiments that will require additional years of evaluation. In summer 1997, budwood from the trees selected in 1996 was grafted onto *Pistacia integerrima* Stewart rootstocks that had been spring-planted in a grower cooperator's field, in a replicated and randomized trial near Lost Hills in northwestern Kern County. Rootstocks with *P. integerrima* heritage were necessary because this species is resistant to Verticillium wilt, a severe fungal disease of pistachio in the San Joaquin Valley.

The trial consisted of nine separate female selections compared to 'Kerman', and included the male pollinators 'Peters' and 'Randy'. Each female variety was randomly planted in groups of 10 trees in each of two separate blocks of trees. In 2005, based on the first 5 years of yield and nut-quality data, UC named and released to nurserymen the female cultivars 'Golden Hills' and 'Lost Hills', and the pollinating male 'Randy'. To date, these have been the only official UC releases of new pistachio cultivars bred in the United States.

Due to grower interest in Iranian varieties, a performance-evaluation trial was established in 1998 in northwestern Kern County a few miles from the UC cultivar trial described above, with the same cooperating grower and on a similar soil. This trial compared 'Kerman' to two cultivars, 'Kalehghouchi' and 'Aria', which were recommended by an interested grower based on their performance in limited plantings on his farm. 'Kerman', 'Kalehghouchi' and 'Aria', all of which originated in Iran, were grafted on *P. integerrima* rootstock, and each variety was replicated four times with 25 trees in each replication.

Evaluating alternatives

Table 1 compares important growth and production characteristics of 'Kerman' to those of the cultivars tested in the two trials described: the

TABLE 1. Relative growth and production characteristics of tested varieties compared to 'Kerman' in northwestern Kern County

	Cultivar			
Characteristic	Golden Hills	Lost Hills	Kalehghouchi	Aria
Bloom date	Earlier	Earlier	Earlier	Earlier
Harvest date	Earlier	Earlier	Similar	Earlier
Alternate bearing	Later onset	Much less	Less	Much less
Annual yield	Greater	Similar	Similar	Less
Lanky growth	Similar	Similar	Greater	Similar
Split-nut percentage	Greater	Greater	Greater	Similar
Closed shell percentage	Less	Less	Less	Less
Nut size	Similar	Larger	Larger	Larger
Shell-hinge strength	Similar	Weaker	Slightly weaker	Much weaker
Insect damage	Less	Less	Similar	Similar

UC-bred 'Golden Hills' and 'Lost Hills', and the additional Iranian cultivars 'Kalehghouchi' and 'Aria'.

Bloom timing. Since male and female flowers are located on different trees in pistachio, finding cultivars with overlapping bloom periods is critical for nut production. In these experiments, the approximate date of full bloom was determined by visiting the two trials at intervals of 3 to 4 days during bloom. The pistachio inflorescence is composed of a central axis, called the rachis, with lateral branches. These lateral branches have a terminal flower with a variable number (usually less than 25) of lateral flowers. Full bloom was defined as the date when trees had the maximum number of open flowers. Since each blooming inflorescence may contain several hundred flowers, and all inflorescences on the tree are not in bloom at the same time, the "full bloom" date represents the statistical mode of all the individual flower bloom dates.

The flowering periods for the female cultivars 'Golden Hills' and 'Lost Hills' overlapped well with those of the male cultivar 'Randy'. Also, full bloom for these cultivars was approximately 1 week earlier than that for 'Kerman' and 'Peters', the female and male cultivars most commonly planted in California today (table 2). Compared to 'Peters', 'Randy' had highly viable and durable pollen as measured by hanging drop slides in 1996. Initial pollen viability was 75% for 'Randy' compared to 45% for 'Peters', and pollen viability after 29 days in storage was 35% for 'Randy' compared to 10% for Peters.

Like the UC-bred female cultivars, the Iranian variety 'Kalehghouchi' bloomed earlier than 'Kerman' and the 'Randy' male overlapped this bloom period well (table 2). However, because the Iranian variety 'Aria' occasionally bloomed before 'Randy', earlier-blooming experimental male selections were also included in this trial to ensure that the earlier-blooming 'Aria' trees had sufficient pollen for adequate fruit-set.

Nut yield and quality. Nuts were knocked from 6th- through 8th-leaf trees with mallets and poles onto tarps, and were shaken from trees older than 8th leaf with mechanical shakers and catching frames. During harvest, two or three 20-pound (9.1 kilogram) samples were collected randomly from the harvest bins in each replication. These samples were transported to a pistachio processor on harvest day to begin nut quality evaluations. At the huller, each of the collected samples was weighed fresh, hulled, dried and evaluated by USDA-trained inspectors.

The pistachio nut consists of an outer hull that is removed early in processing, an outer shell, and the kernel or nutmeat. Evaluations consisted of quantifying the percentages of four categories of pistachios: (1) edible, split, in-shell nuts; (2) shelling stock (split nuts with a damaged shells that must be removed, or kernels that have separated from their shells); (3) closed-shell nuts (unsplit nuts with an edible kernel); and (4) culls (nuts with serious defects such as lacking a kernel or having insect damage) in each of the previous three categories. Culls were not included in yield determinations.

Sample results were averaged and used to calculate the percentage by weight, adjusted to 5% moisture on a per acre basis of the following: (1) total nut yield (total weight of shells and kernels); (2) edible yield (total weight of edible, split, in-shell nuts plus the weight of kernels from shelling stock and closed-shell nuts); and (3) edible, split, in-shell nuts. Nuts are typically stored at 5% moisture and growers are paid based on the edible yield adjusted to this level of moisture in the nuts. Drying all sample nuts to precisely 5% moisture for calculation purposes is impossible, and tables are used to adjust the yields appropriately for small variations plus or minus the required 5% moisture. Data were analyzed using a repeated measures ANOVA design. In addition to significant cultivar differences, significant differences in year and year-by-cultivar interactions were

TABLE 2. Estimated full bloom date for pistachio varieties evaluated in northwestern Kern County

Cultivar	2003*	2004	2005	2006	2007
Kerman	April 5	April 2	April 1	May 1	April 1
Golden Hills	March 31	March 29	March 26	April 24	March 26
Lost Hills	March 30	March 27	March 24	April 28	March 28
Aria	March 29	March 23	March 20	April 19	March 23
Kalehghouchi	March 31	March 24	March 24	April 28	March 23
Peters (male)	April 2	April 1	April 1	May 1	April 1
Randy (male)	March 29	March 23	March 24	April 28	March 25

* All trees in the two experimental trials were accidentally treated with dormant petroleum oil that advanced bloom in 2003.

On average, 'Golden Hills' produced 45% more edible, split, in-shell nuts than 'Kerman' in every year of the trial. Acreage of this new variety is slowly increasing in the San Joaquin Valley.

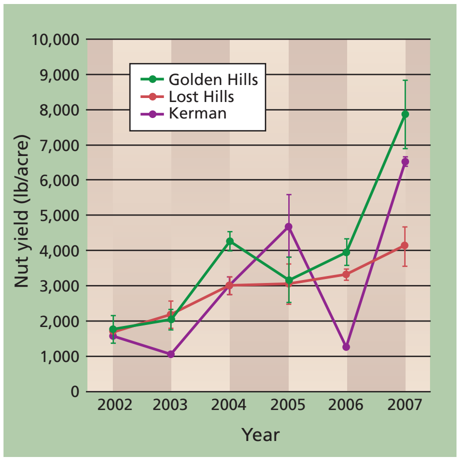

Fig. 1. Yield of nuts, minus culls and adjusted to 5% moisture, in northwestern Kern County for 6th- through 11th-leaf pistachio trees. Error bars represent ± one standard error of the mean (kg/ha = lb/acre × 1.1208).

TABLE 3 . Average characteristics of UC (6th through 11th leaf) and Iranian (5th and 7th through 10th leaf) varieties in northwestern Kern County

UC cultivars			
Characteristic	**Kerman**	**Golden Hills**	**Lost Hills**
Nut yield (lb/acre)*	3,036a†	3,849b	2,895a
Edible yield (lb/acre)	2,712a	3,655a	2,763a
Edible, split, in-shell yield (lb/acre)	2,389a	3,460b	2,631a
Edible yield (%)‡	30.2a	34.8c	32.6b
Edible, split, in-shell (%)§	67.8a	84.6b	85.5b
Total insect damage (%)§	1.8b	0.0a	0.2a
Loose shells and kernels (%)§	0.5a	0.6a	3.20b
Individual nut weight (grams)	1.24a	1.26a	1.47b
Harvest readiness date	Sept. 12	Aug. 29	Aug. 30
Iranian cultivars			
Characteristic‡	**Kerman**	**Kalehghouchi**	**Aria**
Nut yield (lb/acre)*	2,470b	2,539b	2,162a
Edible yield (lb/acre)	2,276b	2,430b	2,026a
Edible, split, in-shell yield (lb/acre)	2,080a	2,334b	1,880a
Edible yield (%)‡	29.4a	33.3b	30.2a
Edible, split in-shell (%)§	74.4a	84.8c	77.2b
Total insect damage (%)§	1.3a	1.1a	0.4a
Loose shells and kernels (%)§	0.5a	1.4b	5.2c
Individual nut weight (grams)	1.23a	1.44c	1.35b
Harvest readiness date	Sept.16	Sept. 14	Sept. 1

* lb/acre × 1.1208 = kg/ha.
† Values within the same row for each trial followed by different letters are significantly different at $P \leq 0.05$ by Fisher's protected LSD test.
‡ Based on percentage of total dry yield delivered to the processing plant.
§ Based on percentage of total nut yield adjusted to 5% moisture.

noted in the analyses as would be expected in alternate-bearing trees that were increasing in maturity and consequently yield.

In the UC-bred cultivar trial, yield of 'Golden Hills' was greater than that of 'Kerman' and 'Lost Hills' by all three measures (fig. 1, table 3). Compared to 'Kerman', 'Golden Hills' produced 27% more total nut yield, 35% more edible weight, and 45% more edible, split, in-shell nut weight on average every year from 6th through 11th leaf. The greatly reduced alternate bearing of 'Lost Hills' from 2002 through 2007 differentiated this cultivar from the others evaluated in these trials (fig. 1). In the Iranian variety trial, 'Kerman' and 'Kalehghouchi' had similar yields, while 'Aria' produced less than either of these cultivars (table 3).

The higher percentages of edible, split, in-shell nuts from 'Golden Hills' and 'Kalehghouchi' compared to 'Kerman' also means fewer shells and culls generated during processing, which means less waste for processing plants to dispose of. Compared to 'Kerman', the average annual percentage of a load of pistachios brought in from the field that was edible weight was 4.6% higher for 'Golden Hills' and 3.9% higher for 'Kalehghouchi' (table 3).

Earlier harvests. Perhaps the most valuable characteristic of 'Golden Hills' and 'Lost Hills' in these trials was their early harvest dates. Nuts of 'Golden Hills', 'Lost Hills' and 'Aria' were ready for harvest approximately 2 weeks or more earlier, on average, than those of 'Kerman' (table 3). This earlier nut maturity increased the San Joaquin Valley harvest period by approximately 30%. From 6th through 10th leaf, 'Kalehghouchi' was ready for harvest, on average, 3 days earlier than 'Kerman'. Earlier harvest would increase the efficiency of the industry-wide pistachio harvest by extending the harvest season and reducing peak demand for labor, harvesting equipment and nut processing facilities.

An earlier harvest can also reduce insect damage. Navel orangeworm typically causes the largest percentage of total insect damage that reaches the processing plant (see page 24). Navel orangeworm damage has the potential to increase greatly with each reproductive cycle in infested orchards. A fourth generation of this pest is often present before the 'Kerman' harvest, while 'Golden Hills' and 'Lost Hills' are harvested prior to its emergence. In the Kern County trial, both UC cultivars had less insect damage than 'Kerman', while 'Kalehghouchi' and 'Aria' had levels similar to 'Kerman' (table 3).

Shell-hinge strength and nut size. A successful pistachio cultivar will produce a nut that is split enough to open easily, yet has sufficient shell-hinge strength to prevent it from falling apart in hulling or later in storage, transportation or the retail store. A crude indicator of shell-hinge strength is the percentage of loose shells and kernels in nut samples taken at harvest. Loose shells and kernels result from nuts that fall apart during or after hulling but before packaging. Based on this measure, 'Golden Hills' and 'Kerman' had similar hinge strengths, even though 'Golden Hills' had a higher split-nut percentage (table 3).

While many consumers consider bigger as being better, larger nuts tend toward lower shell-hinge strength than smaller nuts. The nuts of 'Aria', 'Kalehghouchi' and 'Lost Hills' were larger than those of 'Kerman' (table 3). 'Kalehghouchi', characterized by a high edible split-nut percentage, showed only slightly reduced shell-hinge strength compared to 'Kerman'. However, poorer shell-hinge strength is more characteristic of 'Lost Hills' and 'Aria', which could potentially limit their commercial potential and acceptability at some nut processing plants.

Increasingly, shelled kernels are being sold as a product. However, in modern processing plants a larger percentage of kernels is recovered from shelling closed-shell nuts than from split nuts that fall apart during processing, where kernels are more likely to be lost or damaged during hulling.

Tree structure and growth. 'Golden Hills' produced more upright branches than 'Kerman' and 'Lost Hills', and as a result could potentially produce more flowers and nuts. Branches of 'Kalehghouchi' grew more vigorously than 'Kerman' during the growing season and, especially when young, produced numerous long, unbranched, low-hanging shoots (i.e., lanky growth) that had to be pruned prior to mechanical harvest. 'Aria' produced large clusters of nuts near the ends of branches, making the trees more difficult to prune and shake efficiently, and the nuts more subject to sunburning. 'Kalehghouchi' trunk diameter increased faster than that of 'Kerman', which may make this cultivar more difficult to mechanically shake as time passes. All of the varieties evaluated demonstrated a greater ratio of trunk-to-rootstock diameter than 'Kerman'.

Future cultivar development

Identifying suitable existing cultivars in other parts of the world may be difficult because pistachios are grown very differently elsewhere. The California industry produces pistachios with relatively abundant and good-quality irrigation water and fertilizers, excellent soils, and mechanized pruning and harvesting. The Central Valley climate is also characterized by winters in which the duration of cold temperatures is not sufficient to promote the rest period necessary for uniform flowering in the spring of some varieties (Ferguson 2006).

In contrast, because pistachios are still relatively undeveloped genetically compared to other tree and row crops, there is excellent potential for developing new cultivars to benefit the California industry. Breeding should remain an important component of any crop industry's research program. In most crops, new cultivars contribute significantly to insect and disease resistance. For example, the California pistachio industry would probably not exist without Verticillium wilt resistance, which was discovered in *P. integerrima* during rootstock trials. In addition, some pistachio varieties have varying resistance to Alternaria leaf blight, a serious disease of this crop. The possibilities of incorporating this leaf blight resistance into commercial pistachio cultivars remains undeveloped (Parfitt et al. 2006).

The potential for obtaining and breeding foreign cultivars for characteristics that might benefit niche markets remains almost untested and unlimited. For example, some Italian varieties with small kernel size are highly favored for cooking, and are considered to have a more intense taste and a greener and more attractive kernel than 'Kerman'. In addition, consumer demand is increasing for preshelled nuts. High-yielding cultivars could probably be developed that produce only closed-shell nuts for shelling. An advantage of closed-shell nuts is that they are almost immune from attack by navel orangeworm as well as contamination by aflatoxin, both major concerns of the pistachio industry.

Other breeding objectives that appear to be within reach are cultivars that produce commercially harvestable yields at a younger age (perhaps 4th or 5th leaf instead of the current 5th or 6th leaf) and cultivars that produce larger nuts than 'Kerman' and are ready for harvest 3 to 4 weeks earlier. Genetic variability also appears to exist for alternate bearing (Kallsen et al. 2007). Some varieties have been found to begin an alternate-bearing cycle the second year that they come into bearing, as opposed to others that had not yet initiated a cycle after 7 years of bearing nuts.

'Golden Hills' and 'Lost Hills' have been planted by California pistachio growers beginning with their releases in 2005, and there may now be as many as 800 acres under cultivation. However, since pistachio does not typically bear until the 5th or 6th leaf, the first commercial production is not expected until fall 2009. No additional UC-bred varieties will be released from the trial established in 1997, but some varieties in trials established in 2002 appear to have commercial potential. Unfortunately, only a single negative characteristic can render an otherwise useful variety, with many exceptional characteristics, unsuitable for commercial production. Sometimes negative characteristics may not appear for years, so existing trials must be conducted for several more bearing cycles before it is possible to release a new cultivar.

Whether new varieties result from breeding programs in the United States or importation from overseas, scientific evaluation will remain a critical component of developing suitable and accepted cultivars for the California industry.

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New navel orangeworm sanitation standards could reduce almond damage

by Bradley S. Higbee *and* Joel P. Siegel

The navel orangeworm (NOW), a primary pest of almonds and pistachios in California, is controlled in part by sanitation, with a current threshold of two mummy nuts or fewer per tree. However, almond and pistachio acreage has increased dramatically since the tree mummy threshold was established. This study addresses the impact of this expansion and the possible need for a more stringent standard. Beginning in 2002, the Paramount Farming Company conducted a series of large-scale studies reevaluating the current tree mummy threshold in almond orchards, as well as the impact of ground mummies and proximity to pistachio orchards. The data supports a more stringent threshold of 0.2 mummies per tree. In addition, a new threshold for ground mummies of four per tree for 'Nonpareil' almonds is supported in Kern County, although this needs to be validated in other regions. Proximity to pistachios was an important risk factor for navel orangeworm damage of 2% or less in almonds. Likewise, the influence of pistachios extended 3 miles from the center of the 10-acre almond orchard sections in our experiments to the margin of the nearest pistachio orchard.

Sanitation practices in almond orchards can have a significant impact on insect pest damage. Almond "mummies" remaining on the tree after harvest provide overwintering sites for navel orangeworm, which then infests the new crop.

Almond and pistachio plantings comprise more than 880,000 acres in the Central Valley (NASS 2006). Almonds account for about 83% and pistachios for about 17% of these plantings (730,000 and 153,000 acres, respectively). 'Nonpareil' is the most popular almond variety, comprising 37.7% of all standing acreage in 2006, and 'Kerman' comprises almost all pistachio plantings in California (see page 18). From 2003 to 2007, there has been unprecedented expansion in the acreage of both crops: 30% for almond and 31% for pistachio.

In 2005, the combined farm-gate value of almonds and pistachios was approximately $2.9 billion, according to the Almond Board of California. These crops contribute substantially to the U.S. export balance of trade. Approximately 67% of the almond (ABC 2006) and 49% of the pistachio crop was exported in 2005, according to the California Pistachio Industry Annual Report. Kern County had the single greatest concentration of both crops in 2005, with 20% of total standing almond acres (131,400) and 31.9% of total standing pistachio acres (48,770) (NASS 2006).

Navel orangeworm (NOW), *Amyelois transitella* Walker (Wade 1961), is the major pest of almonds and pistachios in California, and direct damage by this insect can exceed 30% in both crops. During the late 1970s through the early 1980s, navel orangeworm devastated the almond crop, causing average damage of 8.8% in 1978 (F.G. Zalom, personal communication). By the late 1980s, average damage in almonds was reduced to approximately 4%, due to the efforts of researchers at the U.S. Department of Agriculture (USDA) (Curtis 1979) and the University of California (Engle and Barnes 1983; Zalom et al. 1984).

This reduction in navel orangeworm damage was accomplished via a massive commitment to orchard sanitation, using a threshold of no more than two unharvested (mummy) nuts remaining in each tree, along with early harvest of the 'Nonpareil' crop and on-farm fumigation with insecticides after harvest (UC IPM Online 2007). These practices lowered damage by both reducing navel orangeworm populations and removing nuts before they could become infested by the large populations of navel orangeworm that occur from August through September.

While the 4% damage level was satisfactory for approximately 20 years, both food-quality standards and commodity values are dynamic, and today there is even less tolerance for damage. Since 2002, the almond industry's average

Jack Kelly Clark

Navel orangeworm control can be achieved in almonds by careful orchard sanitation, early harvest of the 'Nonpareil' variety and postharvest fumigation with insecticides. *Clockwise from top left:* a navel orangeworm adult; a fertile navel orangeworm egg laid on a mummy almond; a hatched egg; and an almond mummy infested with navel orangeworm larvae.

damage goal for navel orangeworm has been 2% or less. Factors contributing to this current threshold include the crop's increased value and the association of kernel damage by navel orangeworm with aflatoxin contamination, a major quality concern (Schatzki and Ong 2001; ABC 2006). In addition, the European Union — the largest market for California almonds — has imposed more-stringent import standards that have lowered the allowable level of aflatoxin B1 to 2 parts per billion (OJEU 2007).

In order to reduce navel orangeworm damage and increase almond quality, the Paramount Farming Company initiated research in Kern County in 2002 to evaluate the complex interactions between current sanitation practices, orchard damage history and proximity to an alternate navel orangeworm host (pistachios). Using pooled data from 2003 through 2006, we report on how 'Nonpareil' kernel damage is affected by numbers of both tree and ground mummies, as well as proximity to pistachios.

Post-sanitation studies

Between December 2002 and February 2006, a series of long-term, labor-intensive studies on mummy abundance following sanitation was conducted in ranches belonging to all divisions of the Paramount Farming Company in Kern County. More than 50 ranches were divided into 160-acre blocks, which were then subdivided into 40-acre plots, which in turn were quartered into the 10-acre sections comprising our sample units.

Abundance of mummies. Between January and mid-February, 2003 through 2006, we selected four adjacent trees from each of two consecutive rows (four 'Nonpareil' trees and four pollinizer trees) in each 10-acre section. (Almonds are not self-compatible and in order to achieve maximum yield, 'Nonpareil' must be pollinated by varieties other than itself. As a consequence, any block of almonds contains at least two different varieties.) Separate counts were made of nuts on the ground and those remaining in the trees. All of the fallen nuts from outside the drip line (or berm) between the eight trees were counted, and nuts in the trees were knocked down with bamboo poles (poling) and then counted. The average number of mummies per tree was calculated for both fallen nuts and nuts remaining in the tree for every year of our study. A total of 1,920 sections was used in this analysis, corresponding to 19,200 acres and 15,360 trees. In 2003 and 2004, all these mummies were collected and dissected, and data for the 2 years were pooled (233,821 ground mummies and 7,371 tree mummies).

Damage to kernels. In August and early September, 2003 through 2006, within 5 days of harvest, samples of 1,500 to 2,000 nuts were collected from these same 10-acre sections by walking a diagonal transect and taking 50 to 100 nuts at intervals of approximately 100 feet. A total of 2,596,008 kernels was obtained by a combination of hand-cracking and a small hulling and shelling machine. All kernels were examined using a lighted 3× magnifier, by personnel trained to identify common insect and cultural defects. On several occasions, subsamples were sent to a Paramount processing plant for independent grading, and the processor grades were in agreement with the laboratory grades.

Damage to kernels was scored and descriptive statistics including mean, standard deviation and pairwise correlations were calculated using JMP software (v. 7.0.1, SAS Institute, Cary, NC). In addition, relative risk, a statistic commonly used in epidemiology to evaluate the likelihood of a dichotomous outcome (one of two outcomes; in this study the outcome of interest was damage of at least 2%) was used to compare damage differences between the tree and ground mummies, and to assess differences in kernel damage by rounding the navel orangeworm damage to the nearest tenth and then contrasting all sections with damage of 2% or more with sections that had damage below this level (Kelsey et al. 1986). Distance in feet was calculated from the center of each almond section to the margin of the nearest pistachio block using ArcMap (ESRI, Redlands, CA) and the Paramount Farming Company GIS mapping database.

Damage higher in tree mummies

The average number of tree mummies was 0.7 (± 5.0 standard deviation [SD]) and the range was 0 to 69.7 per tree, while the average number of ground mummies was 5.0 (± 5.3 SD) and the range was 0 to 43.7 per tree. In the pooled dataset for 2003 and 2004, 13.64% of tree mummies and 7.91% of ground mummies collected were infested with navel orangeworm. The relative risk for tree-mummy compared to ground-mummy infestation was 1.72 (Chi square = 277, $P < 0.0001$), indicating that tree mummies were 1.72 times as likely to be infested as ground mummies. This infestation disparity is likely due to differential mortality between navel orangeworm in trees and on the ground, but we did not specifically address this in our study. A similar pattern exists in pistachios collected in February (Siegel et al. 2008), but the study did not specifically determine causes of mortality. The average distance from the center of the almond sections to the margin of the closest pistachio block was 8,600 feet (1.6 miles).

In order to properly sanitize an almond orchard in Kern County, it is essential to remove mummies from the trees and destroy them on the ground.

In this study, the average kernel damage per sample due to navel orangeworm was 1.6% (± 2.3% SD) and the range was 0 to 20.8%. The standard deviation was greater than the means for mummies and kernel damage due to the inclusion of sections with no navel orangeworm damage and/or no mummies.

The correlations among these variables using the parametric statistic, Pearson product moment coefficient (r), are summarized in table 1. Tree mummies were the most strongly correlated with navel orangeworm damage (0.46, $P < 0.00001$), followed by ground mummies (0.23, $P < 0.00001$). There was a negative correlation between navel orangeworm damage and distance to the pistachio margin (−0.29, $P < 0.00001$), indicating that damage decreased with distance. Tree and ground mummies were moderately correlated (0.39, $P < 0.00001$), indicating that when tree mummies were high in a section so were ground mummies, but there was considerable variation. Both tree and ground mummies were negatively correlated with distance to the pistachio margin (−0.09, $P < 0.0001$; −0.06, $P < 0.005$ respectively). These marginal correlations are statistically significant due to the large sample size, and they indicate that there was a slight tendency for fewer mummies to be recovered closer to the pistachio margins.

Mummies and new crop damage

Tree mummies. Damage in the new crop exceeded the 2% threshold when there were 0.7 mummies or more per tree in the winter (table 2), a reduction of 65% from the current guideline. However, further relative risk analysis supports a more stringent threshold of 0.2 mummies per tree. When sections containing 0.2 or more mummies per tree were compared to sections that had fewer than 0.2 mummies per tree, the relative risk was 2.15 (Chi square = 156, $P < 0.0001$), indicating that they were 2.15 times as likely to have kernel damage equal to or exceeding the 2% threshold. In addition, other factors beside the number of tree mummies

TABLE 1. Correlations among 'Nonpareil' kernel damage by navel orangeworm (NOW), mummies per tree and distance to nearest pistachio margin, 2003–2006

	NOW damage	Tree mummies	Ground mummies	Distance
NOW damage	1.00	0.46	0.23	−0.29
Tree mummies	0.46	1.00	0.39	−0.09
Ground mummies	0.23	0.39	1.00	−0.06
Distance	−0.29	−0.09	−0.06	1.00

TABLE 2. Relationship between average numbers of tree and ground mummies per tree and 'Nonpareil' kernel damage by navel orangeworm, 2003–2006

Tree mummies	Damage	Sections
avg. no./tree	*%*	*no.*
0	1.63	605
0.01–0.49	1.22	1,092
0.5–0.69	1.57	91
0.7–0.79	2.32	39
0.8–1.75	3.53	61
≥ 1.76	7.85	44
Ground mummies		
avg. no./tree	*%*	*no.*
0–4.9	1.39	1,272
4.91–7.9	1.57	300
7.91–8.9	1.72	67
8.91–9.0	2.78	44
≥ 9.1	2.72	238

clearly influence navel orangeworm damage, because in the sections that lacked tree mummies, the average kernel damage was 1.6%.

Ground mummies. In this study, the number of ground mummies per tree was also related to damage in the new crop (table 2). We found that kernel damage exceeded the current guideline of 2% when there were 8.9 or more ground mummies per tree. Use of the statistic relative risk indicated that a more stringent threshold of four ground mummies per tree is justified, because sections containing four or more mummies were 1.34 times more likely to have kernel damage exceeding the 2% threshold than sections with fewer than four mummies on the ground (Chi square = 13.6, $P < 0.0001$).

There is currently no established threshold for ground mummies. We suggest using an average of four ground mummies per tree for Kern County. We did not establish causality in this study, and mummies on the ground may harbor the overwintering navel orangeworm population, serve as a host for the first generation of the new crop year, or both. What is clear is that mummies on the ground were more than 36 times as prevalent as mummies in trees in the pooled dataset for 2003-2004, and these ground mummies may contribute to navel orangeworm damage due to their abundance. In order to properly sanitize an almond orchard in Kern County, it is essential to remove mummies from the trees and destroy them on the ground.

Proximity to pistachio

Damage caused by navel orangeworm decreased as distance to the nearest pistachio margin increased (fig. 1). The best fit was obtained using this quadratic equation: % 'Nonpareil' kernel damage = $0.0265156 - 0.0000016 \times \text{distance} + 0.00000000013 \times (\text{distance} - 8{,}889.8)^2$.

Although this equation is statistically significant (F ratio 112.3, $P < 0.0001$, $r^2 = 0.105$) it does not account for most of the variation, confirming that other factors also play a role in navel orangeworm damage. The relationship between damage and pistachio proximity declined with distance and ceased somewhere between 14,000 and 15,000 feet (table 3). Navel orangeworm damage was highest in the almond sections that were 0.25 mile or less from pistachios; there were 87 sections in this class and 55.2% of them had damage of 2% or more. At a distance of 3 miles or more from pistachios, there were 1,752 almond sections and 26.7% of them had damage of 2% or more. In contrast to sections inside the 3-mile limit, those beyond 3 miles (15,840 feet) were 25% to 50% less likely to have damage that exceeded the 2% threshold (data not shown).

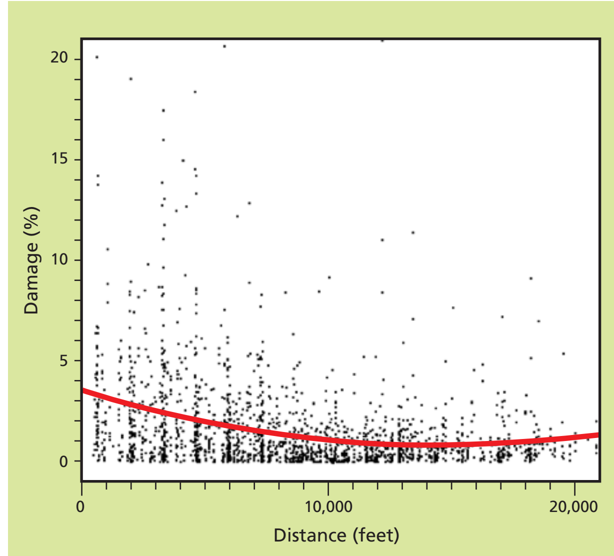

Fig. 1. Relationship between percent navel orangeworm damage and distance (feet) from center of almond block to nearest pistachio margin.

Reducing NOW damage to 2%

Mummy abundance. In order to meet a new threshold of 2% or less kernel damage in Kern County, the average number of mummies should be reduced to 0.2 per tree, and an additional threshold should be established of four ground mummies per tree after mummy destruction by flail mowing. In a 100-tree planting per acre, these new standards correspond to 20 tree mummies and 400 ground mummies per acre, leaving an acceptable total of 420 or more nuts per acre.

Sanitation. Assuming that an average 'Nonpareil' almond tree in a 1-acre planting bears between 12,000 and 18,000 nuts (UC 2006), and that the accompanying pollinizer varieties bear the same number of nuts, there is a potential load of 1,200,000 to 1,800,000 almonds per acre before harvest. Harvest operations and subsequent sanitation must remove or destroy 99.965% to 99.977% of these nuts in order to successfully meet the challenge of sanitation to ensure 2% or less kernel damage. Using these estimates, our current average sanitation efficiency ranged from 99.953% to 99.969%. Economic analysis is needed to establish a cost-benefit relationship between more stringent sanitation and economic return, in order to enable growers to determine the optimal amount of resources to devote to these practices.

Pistachio proximity. Pistachios as far away as 3 miles from the center of almond blocks may contribute to navel orangeworm damage. Further research

TABLE 3. Relationship between 'Nonpareil' kernel damage by navel orangeworm and distance to nearest pistachio margin, 2004–2006

Distance	Relative risk*	Damage ≥ 2%	Sections
miles		%	*no.*
≤ 0.25	2.27†	55.2	87
≤ 0.50	2.15†	48.5	233
≤ 1.00	2.61†	45.2	577
≤ 1.50	3.29†	39.4	961
≤ 2.00	3.14†	33.6	1,258
≤ 2.50	2.18†	28.6	1,562
≤ 3.00	1.66‡	26.7	1,752

* Relative risk values > 1 indicate increased likelihood of navel orangeworm damage ≥ 2%.
† $P < 0.0001$.
‡ $0.005 > P > 0.001$.

is needed to develop a coordinated strategy for managing this pest in both crops, as well as to determine whether additional measures are necessary to manage almonds in proximity to pistachios. Initial studies on the extent of navel orangeworm movement between almonds and pistachios indicate that in pistachios, male navel orangeworm can move up to 1,100 yards in 1 day while females moved up to 100 yards in 1 day (Burks and Higbee 2006).

Conditions vary throughout the growing regions of the Central Valley and there are likely to be differences that influence the factors identified in the Kern County study. Therefore it is essential to validate these findings in other Central Valley areas. Collaborative studies between USDA, UC, UCCE and Paramount Farming Company researchers are under way as part of a newly established areawide program for the control of navel orangeworm in almonds, pistachios and walnuts.

B.S. Higbee is Research Entomologist, Paramount Farming Company, Bakersfield; and J.P. Siegel is Research Entomologist, USDA Agricultural Research Service, San Joaquin Valley Agricultural Sciences Center, Parlier. *The authors thank the staff of the Entomology Laboratory at Paramount Farming Company, particularly Lori Smith and Mike Bryant, for technical assistance and gathering the tremendous amount of data required for this study. We also thank Scott Guseman and Lyndi Smith for their assistance with data management and Gary Schengel for his invaluable help in mapping the orchards. We thank Lawrence Lacey and Joseph Smilanick for their constructive comments on the manuscript.*

▲ A large-scale study in Kern County almond orchards found that navel orangeworm damage to nuts can be brought below 2% by reducing the average number of mummies per tree to 0.2 or fewer, and the average number of ground mummies to four or fewer per tree. By the time trees bloom in the spring, sanitation should be complete, since it is difficult to perform once new growth appears.

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Testing new dairy cattle for disease can boost herd health, cut costs

by Dale A. Moore, John M. Adaska, Gerald E. Higginbotham, Alejandro R. Castillo, Carol Collar *and* William M. Sischo

Dairy producers seldom test or examine incoming cattle, although these important biosecurity practices are recommended. This pilot project examined risk management decisions that producers make when faced with test-positive animals in purchased groups of dairy cattle, in order to provide information on disease risks and conditions that could affect animal health and performance. New arrivals to seven herds at dairy farms in four California counties were examined and tested for a range of conditions. The most common findings were bovine leukosis virus (33% of cattle purchased) and male reproductive abnormalities (16% of bulls purchased). Once testing results were known, producers made a variety of risk management decisions. Although testing costs for some conditions outweigh the benefits of finding an infected animal, an individual producer's decision to test new animals most likely depends on their knowledge of the pros and cons as well as their risk tolerance.

Every year, California milk producers import about 120,000 to 130,000 head of dairy cattle into the state, primarily heifers. Yet surveys have found that the vast majority of producers do not test incoming animals for economically important conditions and communicable diseases.

The farm is considered the front line of food system security, and biosecurity practices such as disease testing are the primary means to protect the farm. Attention to on-farm biosecurity for livestock has been spurred by current certification and eradication programs for Johne's disease and bovine viral diarrhea virus (BVDV), the foot-and-mouth-disease outbreak in British sheep and cattle in 2001, and the letter-borne anthrax bioterrorism attacks in 2001 (Sandvik 2004; Sockett 1996; US Senate 2006).

The dairy industry in the United States has not widely adopted biosecurity practices, particularly those related to purchased cattle. In Wisconsin, less than 50% of producers with recently purchased cattle asked about the herd of origin's disease status, and less than 20% did any testing of animals they purchased (Hoe and Ruegg 2006). In Idaho, 80% of herds undergoing expansion did not require health testing for new cattle, except for mastitis detection (Dalton et al. 2005a). In addition, only about 40% of producers purchasing bulls quarantined them on arrival and only about 25% required a breeding soundness examination (Dalton et al. 2005b). In the upper Midwest, nearly 60% of herds undergoing expansion obtained cattle with minimal health histories, and less than half required any health testing (Faust et al. 2001). Yet owners and managers involved in herd expansions indicated that herd health was compromised as a result of expansion. Similarly, Canadian farms that purchased replacement animals had more cattle testing positive for Johne's disease than farms that did not purchase animals (Chi et al. 2002).

There are approximately 1.7 million head of dairy cattle in California, which produced about 41 billion pounds of milk in 2007, generating an estimated $61.4 billion in economic activity in the state. The movement of dairy cattle into California is a risk to the state's herds. The state imports approximately 120,000 to 130,000 head of dairy cattle annually from other states and countries, a rate of about 10,000 to 13,000 animals per month. Although this includes mature cows and bulls, the trade is primarily in Holstein and Jersey heifers or young stock (6 to 24 months old). Each year, California imports heifers from as many as 35 states and from as far away as New Hampshire (M. Ashcroft, California Department of Food and Agriculture Animal Health Branch, personal communication, March 2006).

Dairy farm biosecurity

Good management practices for biosecurity focus on efforts to prevent the entry of diseases onto the farm as well as to prevent disease transmission within the farm (see box) (Buhman et al. 2000). An important element is the pre-purchase evaluation of cattle, because standard diagnostic laboratory tests or clinical examinations can detect many agents associated with clinical disease outbreaks that could be economically significant. These diseases include: bovine viral diarrhea virus (BVDV) persistent infection, which can spread from animal to animal and cause abortions or congenital defects; *Salmonella,* which can cause diarrhea in adult animals and young stock; mastitis or udder infections caused by *Mycoplasma bovis*, *Staphylococcus aureus* and *Streptococcus agalactiae*; and digital dermatitis, or foot warts, which causes lameness. Other diseases, such as bovine leukosis virus (BLV, a cancer-producing retrovirus) infections and Johne's disease, which can cause diarrhea and production losses, are insidious and so do not manifest disease in outbreak form. However, although insidious diseases initially have few if any detectable symptoms, they can eventually cause clinical disease and affect the marketability of animals. One of the diseases, *Salmonella,* can also infect people. Examining cattle and testing them for endemic diseases or other abnormalities upon arrival to the farm will not prevent the entry of all diseases, but it is the first step to reducing their introduction and provides a screening mechanism for diseases that could result in an epidemic.

Efforts by Cooperative Extension, animal agriculture organizations and others to educate cattle producers about biosecurity are extensive and include all the important disease prevention strategies. Nonetheless, many producers have not yet adopted testing for new herd additions. To better understand the decisions producers make about the testing and disposition of test-positive animals, we did a pilot project to develop a protocol for testing purchased cattle, in collaboration with UC Cooperative Extension (UCCE), herd veterinarians, Agricultural Experiment Station university scientists and the California Animal Health and Food Safety Laboratory.

Biosecurity practices recommended for cattle premises

- Know the health history of herds from which cattle are purchased.
- Know the health status of animals purchased or brought into the operation.
- Request that the herd veterinarian talk to the seller's veterinarian prior to purchasing animals.
- Never purchase unvaccinated animals.
- Never buy animals from a herd that has mixed-origin cattle.
- Transport animals in clean vehicles.
- Have a control program for outside animals that could spread disease (rodents, etc.).
- Load and unload animals and supplies in areas located at the perimeter of the operation.
- Provide an isolated pickup area for rendering trucks to pick up mortality, to prevent contamination of the operation.
- Limit the number of visitors who have access to cattle pens, feed mixing and storage areas, and treatment areas.
- Keep a record of visitors to the operation.

Adapted from Buhman et al. 2000.

Pre-purchase survey

Dairy farms enrolled in the study came from four California counties (Fresno, Kings, Stanislaus and Tulare) and were a convenience sample (not randomly selected) selected by UCCE farm advisors and practicing veterinarians. Eligible producers had to be actively engaged in purchasing animals and expect to purchase animals within 30 days of agreeing to participate in the survey. The herd owner completed a pre-purchase survey provided by the extension advisor or herd veterinarian. Survey questions focused on cattle purchased in the previous year, purchasing practices, disease testing, the examination of purchased cattle and the disposition of animals with specific disease conditions.

Seven dairy herds were enrolled, and all producers had purchased lactating animals the previous year. Five of the seven producers brought in new bulls and three bought bred (pregnant) heifers. None had information about specific disease history. Five of the producers knew the herd of origin but no testing was done. The exceptions were one producer who tested for Johne's disease, and four producers who checked for foot warts (digital dermatitis). Of the five farms purchasing bulls, two had breeding soundness exams done.

TABLE 1. Prevalence of test-positive animals in pilot study of newly purchased arrivals to California dairy farms

Condition	Animals tested	Test-positive
	no.	*no. (%)*
Bovine leukosis virus (BLV)	382	127 (33)
Bovine viral diarrhea virus (BVDV) persistent infection	382	0 (0)
Johne's disease	382	1 (0.26)
Salmonella spp.*	380	2 (0.53)
Mycoplasma spp. intramammary infection	373	0 (0)
Staph. aureus intramammary infection	373	3 (0.8)
Strep. agalactiae intramammary infection	373	0 (0)
Environmental *Staph*. spp.	373	10 (2.7)
Environmental *Strep*. spp.	373	8 (2.1)
Other intramammary infections	373	3 (0.8)
Abnormal bull genital findings	38	6 (15.8)

* *Salmonella* St. Paul and Mbandaka.

Five of the seven producers would not have purchased test-positive animals if they had known they were infected.

Five producers reported that they never isolate purchased animals upon arrival to the farm. Five producers did not cull animals positive for BVDV persistent infection or Johne's disease, but four usually culled cows positive for *S. aureus* mastitis.

Testing newly purchased cattle

The sample size for the number of cattle to be tested was based on an estimate of 120,000 animals purchased annually in California per 1.7 million head in the state dairy herd, or about 7.0% (CDFA 2005). If owners of the approximately 820,000 dairy cattle in the four-county study area reflect state trends, they purchase approximately 54,000 cattle per year. Detecting a 1.0% prevalence of the targeted diseases in these newly purchased cattle with 95% confidence would require a sample size of 298 animals. An extra 25% was added in case some cattle could not be found or subsequently tested, making a total of 372 animals to test and examine in the four-county area.

All new arrivals were examined by the project investigation team or herd veterinarian within 7 days of arrival. All cows, bulls and heifers received a general physical, and bulls also received a palpation examination of scrotal contents and seminal vesicles. A blood sample was obtained for the following infectious disease tests: BVDV antigen-capture ELISA (enzyme-linked immunosorbent assay), BLV antibody ELISA and, for cattle over 2 years of age, Johne's disease antibody-capture ELISA. Blood samples were processed by the California Animal Health and Food Safety Laboratory in Tulare. Milk samples from each quarter were obtained from all cows and post-calving heifers, and evaluated for common contagious mastitis pathogens (including *S. aureus*, *S. agalactiae* and *Mycoplasma* spp.) and for "environmental" pathogens like *E. coli* and *Streptococcus* species, by the UC Davis Veterinary Medicine Teaching and Research Center's Milk Quality Laboratory in Tulare. Fecal samples were evaluated for the presence of *Salmonella*.

Veterinarians examined 382 newly purchased animals in seven herds. The most common finding was bovine leukosis virus (33% of cattle purchased). Three of the seven producers surveyed decided to keep BLV-positive animals in their herds, but four out of seven would not have purchased the infected animals if they had known.

Within the first week of arrival to participating farms, 382 dairy cattle were examined and tested. Of these, 25% were pre-calving heifers, 65% were lactating cows and 10% were bulls. Most of the cattle (72.8%) came from private owner sales and the rest through a sales yard, cattle buyer or auction. In addition, 57% of the cattle had U.S. Department of Agriculture (USDA) ear tags placed in California, 21% had ear tags from other states (Hawaii, Minnesota, North Dakota, South Dakota, Colorado, Washington and Oregon) and 22% had no official USDA ear tag.

The most common finding was evidence of BLV infection (33%) (table 1). Only one bull was BLV test-positive but over 35% of the cows and heifers were, making them 13 times more likely to test positive for this disease than bulls ($P < 0.001$). The proportion of BLV-positive purchased animals varied by destination farm, and ranged from 7% to 80%. Of 38 bulls evaluated, one was cryptorchid (right testicle not descended) and five had firm swellings of either a seminal vesicle or epididymus, parts of the male reproductive tract. Swellings in these structures can indicate previous or current inflammation that could impair fertility.

Producer reactions to tests

Participating producers received a standard report of the physical examination findings and laboratory results. A questionnaire, provided within 2 weeks of sampling, captured producer decisions for each test-positive animal: (1) marked the cow/heifer with, for example, a leg band and kept her in the herd; (2) kept her but moved or will move her to a separate string for cows with that kind of infection; (3) removed the cow/heifer from the herd; (4) treated the cow/heifer; or (5) have not yet decided what to do with the animal(s). The questionnaire also asked producers whether they would have purchased the animal had they known the test results beforehand.

When provided with test-positive results, most producers indicated that they would keep the animals in the herds rather than cull but would not have purchased the cow had they known that she had the disease. For the

TABLE 2. Decisions by dairy producers after receiving test-positive results for intramammary infections				
Dairy	Intramammary infection (no. cows)	What was done with animal(s)?	If knew animal infected before purchase	Decisions about infections
1	*Strep*. spp. (5) *Staph*. spp. (3) *Staph. aureus* (3) *Corynebacterium* (1)	Nothing	Would not have purchased	Ask veterinarian
2	*Strep*. spp. (1) *Staph*. spp. (2) *Corynebacterium* (1)	Kept but moved to a separate string for cows with same infection	Bought but asked for price discount	Make own decision
6	*Strep*. spp. (1) *Staph*. spp. (2)	Treated with intramammary antibiotics	Would not have purchased	Make own decision
7	*Strep*. spp. (1) *Staph*. spp. (3)	Treated with intramammary antibiotics	Would not have purchased	Make own decision

cow with suspected Johne's disease, the producer said he would make his own decision about what to do with her. For intramammary infections, the producers made the same decision for each of their positive cattle, regardless of the type of bacteria found (table 2). For the *Salmonella* St. Paul–positive cow, the producer said he would ask his veterinarian what to do. For the *Salmonella* Mbandaka–positive cow, the producer said he would make his own decision about what to do.

Decisions regarding BLV-positive animals were producer-specific. Each producer reported making the same decision for each of the animals testing positive for any condition in their herds (table 3). Five of the seven producers would not have purchased test-positive animals if they had known they were infected. The producer with affected bulls decided to remove all these animals from his herd, would not have purchased them knowing they were affected, and would make his own decisions about what to do with them.

Making biosecurity decisions

In this pilot study, decision-making by dairy producers varied. Knowing infection status before purchasing can provide information for decisions about treatment, isolation or culling, but the participating producers had different levels of risk-tolerance and said they would seek veterinary advice to varying degrees. Even though these producers were regularly purchasing animals and may have had infectious diseases in their herds in the past, these factors did not appear to influence their decision to require testing. In addition, new cattle were rarely isolated or quarantined: only two of the seven producers isolated some new additions on arrival, confirming the results of previous surveys (Buttars et al. 2006; Dalton et al. 2005a; Faust et al. 2001; Hoe and Ruegg 2006).

The perception of risk among farmers does not always translate into risk-tolerant or risk-averse behavior. In a study of swine producers, the perceived importance of a biosecurity practice was not necessarily associated with its implementation (Casal et al. 2007). The swine producers were more likely to implement biosecurity measures that affected disease transmission through people and wildlife than to implement measures for the most important risk for disease transmission: incoming replacement animals.

Costs and benefits of testing

The cost of examination and testing is a likely deterrent to producers purchasing large groups of animals. However, several results of this pilot project indicate that there is some value in examining and testing for certain conditions.

Intramammary infections. If the farm strategy is to keep *S. aureus* intramammary infections out of a herd and the probability of infection is 0.8%, the cost of milk cultures to find one infected cow in 100 is about $625. This is less than the cost of the average purchased cow minus her salvage value if she is tested after purchase (about $1,800 and $400 to $500, respectively). If the bacteria spreads and infects other cows, the costs due to clinical mastitis could average about 726 pounds (330 kilograms) of milk per lactation, about $120 (Shim et al. 2004).

Bovine leukosis virus. Evidence of BLV infection was the most common finding in our study. The consequences of BLV infection can include immunosuppression, premature culling, loss of salvage value if the animal becomes clinical and is culled, higher calving intervals and lower milk production (D'Angelino et al. 1998; Ott et al. 2003; Pollari et al. 1992, 1993). A few studies have found no influence of BLV infection on herd performance (Heald et al. 1992; Tiwari et al. 2007). However, a recent study that controlled for other factors associated with milk production found a significant relationship

TABLE 3. Decisions by dairy producers after receiving test-positive results for bovine leukosis virus (BLV) infections					
Dairy	Total purchased	Tested BLV-positive	What was done with animal(s)?	If knew animal infected before purchase	Decisions about infections
	no.	*no. (%)*			
1	61	22 (36.1)	Kept in herd	Other	Ask veterinarian
2	20	16 (80)	Kept in herd	Would have bought anyway	Make own decision
3	6	3 (50)	No response	No response	No response
4	83	15 (18.1)	Removed animals	Would not have purchased	Make own decision
5	14	1 (7)	Kept in herd	Would not have purchased	Ask veterinarian
6	80	15 (18.8)	Not yet decided	Would not have purchased	Ask veterinarian
7	118	55 (46.6)	Not yet decided	Would not have purchased	Ask veterinarian

between higher herd prevalence of BLV and lower milk production and annual value of production (a combination of milk production and annual value of calves at birth, minus the annual net replacement cost) (Ott et al. 2003). Given Ott's model, a herd with a BLV prevalence of 33% has 253 pounds (115 kilograms) less milk per cow in the herd (1% lower production compared to cows in herds without BLV).

BLV infects lymphocytes, resulting in a lifelong infection, and can result in lymphosarcoma or malignant lymphoma. If 0.1% to 5.0% of BLV-positive animals develop lymphosarcoma and the herd prevalence of BLV is 33%, as many as 1% of animals in the herd will be culled prematurely due to the development of lymphosarcoma (Pelzer 1997). Premature culling incurs losses due to the replacement of a cow with a heifer, loss of pregnant cows and loss of the cow's market value (Rhodes et al. 2003). Nationwide, 5,175,861 beef and dairy cows were sent to market in 2002 (USDA 2008). Of those, 2.77% (143,484) were condemned, and 17% (25,075) of those condemnations were for malignant lymphoma, resulting in no value to the producer or packer.

The laboratory cost for a BLV test is about $8.70 for 10 samples. In the case of BLV infections from purchased cattle, the risk is real and the potential consequences significant. Thus, BLV test results should be considered in dairy-cattle purchasing decisions.

Johne's disease. Only one Johne's disease test-positive animal was found in our study, resulting in less than 1% prevalence. However, the sensitivity of tests for this disease is notoriously low (Collins et al. 2006). The ELISA test on serum has a sensitivity of about 30% to 50%, which is the probability that a test is positive given that the animal is truly infected (Collins et al. 2006). As such, a negative Johne's disease test does not necessarily mean "not infected." Collins et al. (2006) provided a cattle purchase flowchart for Johne's disease biosecurity, which showed that the highest risk for buying infected cattle is from untested herd replacements.

The costs of Johne's-positive herds include reduced milk yield, body weight losses, a reduction in market cow beef value and early culling. About $60 to $90 of income per cow is lost in Johne's-positive herds compared to negative herds (Collins et al. 2006; Ott et al. 1999). The prevalence of Johne's-infected dairy cattle is estimated to be about 22% nationwide, but it varies by region (table 4) (Ott et al. 1999). Although the West appears to have a relatively low proportion of Johne's-positive herds, buying replacement animals can put Western dairies at risk for introducing the disease. Laboratory tests for this disease are about $3.60 for one and $5.50 for 10 samples.

Bull diseases. Replacement bulls can bring in disease as well as be poor performers. If it costs $50 to sample, test and conduct a 5-minute reproductive exam on a single bull, the 38 bulls in our project represented a total testing cost of $1,900, or about the value of one bull. With one cryptorchid bull and five bulls with evidence of reproductive tract problems that could affect fertility, the testing cost can be justified. Adding a test (about $8 each) for trichomoniasis, a disease spread venereally that can affect cow fertility, would also be an important biosecurity measure.

Testing as an insurance policy

Decisions to test cattle purchases for infectious diseases depend on both the risk of disease introduction and the risk aversion (or tolerance) level of each producer. Just as with making a decision about purchasing an insurance policy, individuals decide whether they can absorb the costs of some negative event or if they want to minimize risks associated with infectious diseases. Producers who decide not to test incoming cattle can employ three other strategies: (1) ask about the herd of origin and any disease information the sellers have, which will still not address carrier animals; (2) carefully examine cattle, particularly breeding bulls, to detect any obvious abnormalities before purchasing; and (3) provide an isolation facility where purchased animals can become acclimated to new surroundings and visually screened for abnormalities or illness for up to 3 weeks before adding them to the herd. These latter recommendations may help reduce the risk of disease introduction and would be first steps to help secure the herd's health. It would

Johne's disease can cause diarrhea and result in reduced milk yields, lower body weights and beef value, and early culling. It can be detected with a lab test costing about $5.50 for 10 samples.

TABLE 4. Herd prevalence of different diseases in U.S. dairy cattle (cows, bulls and heifers)

Johne's disease*	Prevalence
	%
Midwest	60.7
Northeast	26.5
West	8.3
Southeast	4.5
National	22.0 (cows, 5–10%)
BVDV persistent infection†	
Michigan dairy herds	15 (cows, 0.13%)
Bovine leukosis virus (BLV)‡	
Midwest	88.6
Northeast	86.6
West	88.6
Southeast	99.0
National	88.3 (cows, 41%)
***Salmonella* spp.§**	
Midwest	25.6
Northeast	6.7
West	42.9
Southeast	50.0
National	27.5 (cows, 7.3%)
***Staph. aureus* ¶**	
New York/Pennsylvania	9.1 (cows)
***Mycoplasma*#**	
Midwest	2.2 (bulk tanks)
Northeast	2.8
West	9.4
Southeast	6.6
National	7.9
Digital dermatitis (foot warts)**	
Midwest	46–60
Northeast	60.2
West	60–72
Southeast	30.0

* Source: Ott et al. 1999.
† Source: Houe et al. 1995.
‡ Source: Ott et al. 2003.
§ Source: USDA APHIS 2003b.
¶ Source: Wilson et al. 1997.
# USDA APHIS 2003a.
** Source: USDA APHIS 1997.

be prudent for producers to develop a protocol for testing risky animals when the herd of origin is unknown or health history is lacking.

Our results indicate that there are opportunities for dairy advisors, herd veterinarians and extension educators to emphasize the risks associated with new herd members and to work with clients on appropriate purchasing and testing strategies. Specifically, they can help producers to: (1) understand the consequences of specific infections, (2) identify and prioritize specific diseases they want to keep out of their herds, (3) assess existing disease conditions in their operation, (4) develop a testing plan for risky animals before or after purchasing (using table 4 and box below), (5) make the best decisions on what to do with infected animals based on available information and (6) assess the operation and facilities for potential within-herd transmission of diseases (CFSPH 2008). Keeping infectious diseases and other cattle conditions out of the herd can save money in the future.

Testing strategies for pre- or post-purchase of dairy herd replacements

All (bulls, cows, heifers)

- Examination for foot warts
- Blood sample for Johne's ELISA test (if over 2 years of age)
- Ear notch or blood sample for BVDV persistent infection test
- Fecal sample for *Salmonella* culture
- Blood sample for bovine leukosis virus (BLV) antigen-capture ELISA

Bulls only

- Breeding soundness investigation, including palpation of scrotal contents and seminal vesicles
- Preputial sample for *Tritrichomonas* testing

Cows only

- Milk sample for mastitis-pathogen culture

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RESEARCH ARTICLE

Sudex cover crops can kill and stunt subsequent tomato, lettuce and broccoli transplants through allelopathy

by Charles G. Summers, Jeffrey P. Mitchell, Timothy S. Prather *and* James J. Stapleton

Grass cover crops can be harvested for biomass or used as a surface mulch to reduce erosion, improve soil structure, suppress weeds and conserve moisture. There is concern, however, that such plantings may affect subsequent crops. We studied the effects of sudex, a sorghum hybrid used as a cover crop, on subsequent crops of tomato, broccoli and lettuce started from transplants. Within 3 to 5 days of being transplanted into recently killed sudex, all three crops showed symptoms of phytotoxicity including leaf necrosis, stunting and color changes. There was 50% to 75% transplant mortality in all three species. Plant growth and development, as determined by biomass measurements, were also significantly affected. Yields of mature green tomato fruit and marketable broccoli and lettuce heads were reduced significantly. Tomato, broccoli and lettuce should not be transplanted into sudex residue for at least 6 to 8 weeks, or until the residue has been thoroughly leached.

Sudex is a hybrid of sorghum and sudangrass that is grown extensively as a cover crop in the Imperial and San Joaquin valleys to reduce erosion, improve soil structure and suppress weeds. It appears to have allelopathic properties that can damage subsequent vegetable transplants. *Above*, sudex silage is harvested in Turlock.

Sudex, a sorghum-sudangrass hybrid, is grown as a cover crop in California to reduce erosion, improve soil structure and suppress weeds. Additionally, sudex (*Sorghum bicolor* [L.] Moench × *S. sudanense* [P.] Staph.) serves as a source of green manure (Weston et al. 1989), forage and silage (Chaudhry et al. 1997). Sorghum-sudangrass hybrids, including sudex and collectively known as sudan, are cultivated extensively in the Imperial and San Joaquin valleys. In Imperial County, over 55,000 acres of sudan hay is produced, while in the San Joaquin Valley an additional 25,000 to 35,000 acres of sudan, mainly for silage and winter forage, are produced annually (Frate 2001). Commonly, tomatoes are planted following late winter/early spring sudex, while broccoli and lettuce are planted after a summer crop of sudex. Sudex grows rapidly, producing large quantities of biomass, and can be harvested several times per season (Finney 2005). Sudex is also a candidate crop for ethanol production from lignocellulose, the woody portion of the plant, along with corn stover (*Zea mays* L.), switchgrass (*Panicum virgatum* L.) and *Miscanthus* spp.

There is, however, a potentially negative aspect of growing sudex as a rotation crop. Certain members of the grass family, including *Sorghum* spp. in general and sudex in particular, inhibit the emergence or development of nearby or subsequently planted annual and perennial plants (Geneve and Weston 1988). Using sudex extracts, Weston et al. (1989) found a significant reduction in the embryonic root, and elongation of tomato (*Lycopersicon esculentum* Mill.), garden cress (*Lepidium sativum* L.), foxtail millet (*Setaria italica* [L.] Beauv.) and barnyardgrass (*Echinochloa crus-galli* [L.] Beauv.). This negative impact of one plant on another is called allelopathy when it affects plants of a different species, and autotoxicity when it affects plants of the same species.

Allelochemicals have been isolated from all parts of the sudex plant (Ben-Hammouda et al. 1995a, 1995b; Einhellig and Souza 1992; Forney and Foy 1985). Whereas the impact of such allelochemicals on seedlings is well recognized, their impact on larger transplants is virtually unknown. In 1999, we observed significant mortality in tomato transplants set into a glyphosate-killed sudex mulch. We conducted experiments to determine if sudex was responsible for the transplant mortality, and here report the results of studies in which tomato ('Shady Lady') was transplanted into a

Tomatoes planted too soon after a sudex cover crop can suffer from mortality or yield reductions. *Left,* a healthy control tomato was planted in fallow soil. *Right,* a tomato transplant set 20 days after sudex roots and shoots were cut is stunted and shows evidence of necrosis (dead tissue) on the leaf margins.

TABLE 1. Mean number plants and mean dry weight of tomato, lettuce and broccoli shoots 5 weeks after transplanting, 1999

Treatment	Tomato	Lettuce	Broccoli
	mean no. plants		
Shoots + roots	3.5a*	0.3a	5.0a
Incorporated	5.0ab	4.0b	6.0b
Control	6.0b	4.5b	6.0b
	mean shoot weight (oz./yd.^{2})		
Shoots + roots	0.033a	0.003a	0.138a
Incorporated	0.121b	0.335b	0.697b
Control	0.211c	0.453b	0.647b

* Means followed by the same letter(s) are not significantly different according to LSD test, $P \leq 0.05$.

killed sudex crop during the summer. We also examined the impact of sudex on broccoli (*Brassica oleracea* var. *botrytis* L. 'Marathon') and lettuce (*Lactuca sativa* L. 'Cowboy') crops that would likely be transplanted in the fall following a summer sudex crop.

Transplanting into sudex

Studies were conducted at the UC Kearney Research and Extension Center in Parlier, on a Hanford fine sandy loam soil. Raised planting beds were formed, and fertilizer (15-15-15, 800 pounds per acre) was broadcast and incorporated.

Sudex treatments. On Aug. 6, 1999, sudex ('Green Grazer V') was drilled at 30 pounds per acre. Irrigation was by surface drip, and liquid fertilizer (17-0-0, 20 pounds per acre) was added through the drip system on Aug. 24 and Sept. 7 and 14. Sudex was shredded on Sept. 24, when the shoots were about 4.5 feet tall. Regrowth was sprayed 10 days later with 2% glyphosate (an herbicide) in 20 gallons of water per acre to provide complete coverage. Treatments, arranged in a randomized complete block design with four replications, consisted of: (1) sudex cut, sprayed and left on the soil surface; (2) sudex cut, sprayed and incorporated into the soil with a rototiller; and (3) fallow control, where no sudex was planted and plots were maintained weed-free by occasional rototilling. Sudex biomass (dry) was approximately 4,980 pounds per acre.

On May 1 and July 26, 2000, sudex was drilled into fertilized planting beds as described. The May 1 planting was shredded on June 27, when the plants were about 6 feet tall, and the July 26 planting was cut and shredded on Sept. 5, when the plants were about 6.5 feet tall. In both plantings, the sudex stubble was sprayed 10 days after shredding with 2% glyphosate in 20 gallons of water per acre. Treatments, arranged in a randomized complete block design with six replications, consisted of: (1) shoots + roots — sudex cut, sprayed and shoots left on the surface; (2) shoots only — a fallow bed that had not previously been seeded with sudex was covered with cut sudex shoots; (3) roots only — sudex shoots raked off; (4) incorporated — sudex cut, sprayed and then shoots and roots incorporated into the soil; and (5) fallow control. Sudex biomass (dry) was approximately 7,220 pounds per acre.

Vegetable transplants. In 1999, experimental plots were 3 feet long, and we hand-transplanted tomato, broccoli and lettuce (six plants per plot) into the sudex treatments on Oct. 14. While too late for the commercial production of tomatoes, this planting provided an opportunity to evaluate the impact of sudex on tomato transplant mortality. Irrigation was by surface drip, and liquid fertilizer (17-0-0, 20 pounds per acre) was added biweekly.

In 2000, each plot was 15 feet long, and all transplants were set into the sudex treatments in two rows of 10 plants each. Tomato seedlings (20 per plot) were hand-transplanted on July 17, 25 and 31 and Sept. 1, which was 20, 28, 36 and 67 days, respectively, after shredding. Broccoli and lettuce (20 per plot) were transplanted on Sept. 26 and Oct. 19, which was 21 and 35 days, respectively, after shredding. Irrigation was by surface drip, and liquid fertilizer (17-0-0, 20 pounds per acre) was added weekly.

In both years, cultural practices were standard for tomato, broccoli and lettuce production in the San Joaquin Valley (Jackson et al. 1996; Le Strange et al. 1996, 2000).

Plant mortality, biomass and yield. In both years, one of the two rows of transplants was selected at random, and plant mortality determinations were made 5 weeks after each group of transplants was set and again at harvest. Plants from the other row were used to determine shoot and root biomass at 5 weeks post-transplanting. To determine biomass, plants were cut at the soil level and the shoots placed in a paper bag. The roots

TABLE 2. Mean number tomato plants per plot 5 weeks after transplanting and at harvest, 2000

	Days between sudex shredding and transplanting			
Treatment	20	28	36	67*
5 weeks after transplant	. . . . *mean no. plants*			
Shoots + roots	5.0a†	4.0a	3.3a	9.8a
Shoots	7.5b	5.5b	4.5b	0.7a
Roots	8.0bc	7.7bc	8.8c	0.8a
Incorporated	4.0a	3.7a	4.8b	0.7a
Control	9.7c	9.8c	9.3c	0.8a
At harvest	. . . . *mean no. plants*			
Shoots + roots	4.8a	4.0a	3.2a	—
Shoots	5.3a	4.8a	4.3a	—
Roots	7.8b	7.7b	8.2b	—
Incorporated	3.5a	3.2a	4.8a	—
Control	9.0b	9.3b	9.3b	—

* Plants not taken to harvest.

† Means followed by the same letter(s) are not significantly different according to LSD test, $P \leq 0.05$.

were exhumed, washed free of soil and placed in a paper bag. Both shoots and roots were dried at 140°F and weighed.

Mature green tomatoes were harvested on Oct. 13, 20 and 27, 2000. Plants from the last tomato planting were not grown to maturity. Marketable broccoli and lettuce heads were harvested from December through January.

Statistical analysis. Data were evaluated by analysis of variance and means separated by LSD (Statistix 8, Tallahassee, FL, 2003).

Survival and mortality

Tomato. Sudex had a significant impact on tomato transplant survival. Five weeks after transplanting, the maximum tomato mortality was 95% in 1999 (table 1) and 67% in 2000 (table 2). In 2000, the combination of shoot + root material, shoots and soil-incorporated whole plants, resulted in significant mortality ($P \leq 0.05$) in transplants set 20, 28 and 36 days after shredding (table 2). Roots alone had no significant impact ($P \leq 0.05$) on plant mortality. By 67 days post-shredding, sudex no longer influenced tomato transplant survival. Plant mortality did not continue past 5 weeks. There was no difference in stand density between counts taken 5 weeks post-transplanting and at harvest. Whatever the causal factor in transplant mortality, it only affected the younger transplants.

Lettuce and broccoli. Fall-planted lettuce and broccoli responded much differently to sudex. In 1999, only plots containing shoot + root material resulted in significant transplant mortality (table 1). In 2000, when transplanted 21 days after shredding, lettuce and broccoli showed a significant increase ($P \leq 0.05$) in plant mortality in plots containing shoot + root material and shoot material only, but the other treatments (roots only, and soil-incorporated shoots + roots) were not significantly different ($P \leq 0.05$) from the control (table 3). At 35 days post-shredding, none of the sudex treatments had an impact on transplant mortality (table 3). As with tomatoes, the impact on lettuce and broccoli mortality appears to be confined to the period immediately following transplanting. Plant density at harvest was not significantly different from that at 5 weeks (table 3).

Transplant biomass

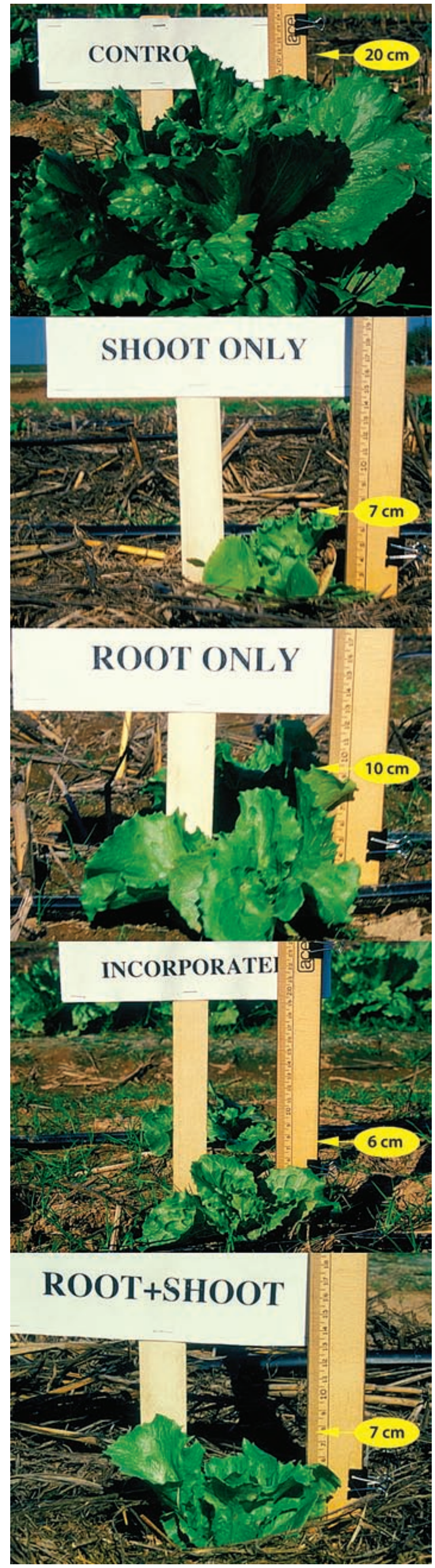
Tomato. Even in plots where mortality was low, tomato shoot and root weights were negatively affected by sudex. In the sudex shoot + root treatments in 1999, shoot weights of tomato transplants were reduced by 86% compared to the fallow control (table 1). In plots where sudex had been incorporated, tomato shoot weight was reduced by 44% even though there was no significant impact on plant mortality (table 1). Only plots containing sudex shoots + roots significantly reduced the shoot biomass for lettuce and broccoli (table 1). Similar results were obtained in 2000, with all sudex treatments producing less tomato shoot and root biomass than the control plots when transplants were set up to 36 days after shredding (table 4). Even at 67 days post-shredding, the shoot and root weights of transplants set into plots containing sudex shoot + root material were significantly lower ($P \leq 0.05$) than those in the fallow control (table 4).

Lettuce and broccoli. Lettuce and broccoli were less influenced by sudex than was tomato. At 21 days post-shredding, both lettuce and broccoli produced shoots and roots with significantly less weight in all sudex treatments compared to the control (table 5). By 35 days, lettuce shoot weights were still significantly less than the control in all treatments, but broccoli shoot

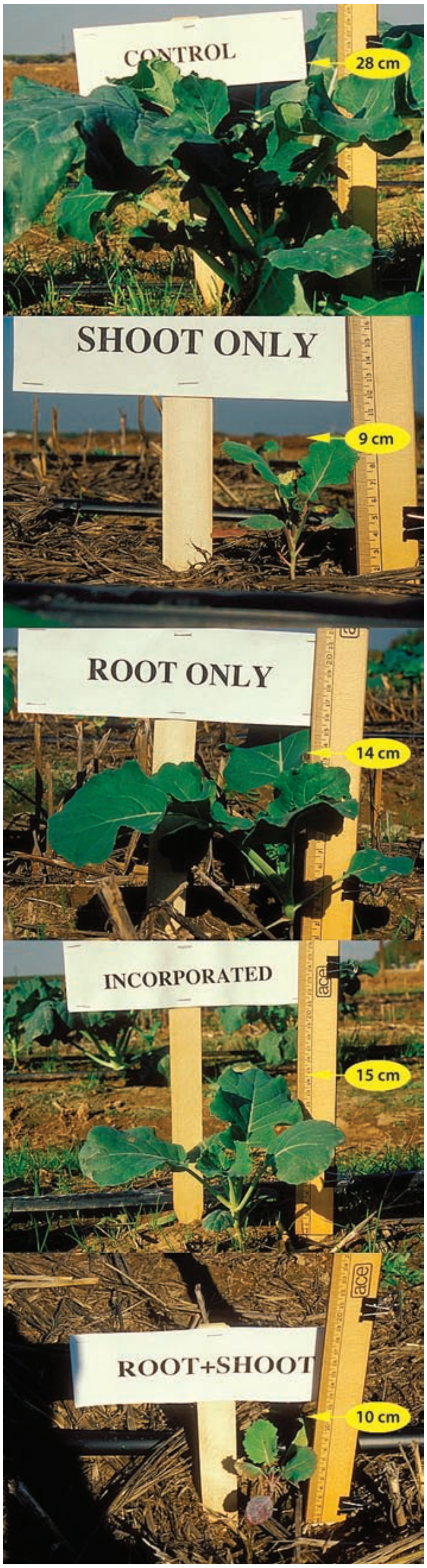
TABLE 3. Mean number lettuce and broccoli plants per plot 5 weeks after transplant and at harvest, 2000

	Days between sudex shredding and transplanting			
	21		35	
Treatment	Lettuce	Broccoli	Lettuce	Broccoli
At 5 weeks	. . . . *no. plants*			
Shoots + roots	8.17a*	8.17a	9.33a	9.83a
Shoots	7.50a	8.17a	9.50a	9.50a
Roots	9.83b	9.50b	10.00a	9.52a
Incorporated	9.67b	9.67b	9.83a	9.83a
Control	10.00b	10.00b	10.00a	9.67a
At harvest	. . . . *no. plants*			
Shoots + roots	8.17a	8.33a	9.83a	9.67a
Shoots	7.50a	6.83a	9.50a	9.67a
Roots	9.83b	9.17b	9.83a	9.50a
Incorporated	9.67b	9.00b	9.83a	10.00a
Control	10.00b	9.83b	9.67a	9.50a

* Means followed by the same letter(s) are not significantly different according to LSD test, $P \leq 0.05$.



Lettuce was a bit more tolerant of sudex than tomato, but those set 21 days after sudex was cut were all significantly stunted. However, lettuce transplants set 35 days after sudex was cut did not suffer any yield impacts.



Broccoli transplanted 21 days after sudex was cut showed varying degrees of damage and yield loss, depending on the treatment. This effect continued with broccoli transplants set 35 days after sudex was cut.

weights were significantly lighter only in the plots containing shoots + roots and shoots only. There was no difference in broccoli root weights among any of the sudex treatments (table 5).

Impacts on yield

Tomato. Yields of mature green tomato fruit from plots containing sudex shoots + roots were reduced by 96%, 83% and 74% when transplants were set 20, 28 and 36 days post-shredding, respectively. All sudex treatments produced significantly less ($P \leq 0.05$) marketable fruit than did the control (table 6). While producing significantly less yield than the control, plots containing only sudex roots produced more marketable fruit than any other sudex treatment.

Lettuce and broccoli. As with tomatoes, all sudex treatments had a significant impact on lettuce and broccoli yields. When lettuce and broccoli transplants were set 21 days after sudex shredding, all treatments resulted in significantly lower ($P \leq 0.05$) yields of marketable heads compared to the control. No marketable broccoli heads were produced in plots containing sudex shoots + roots and soil-incorporated sudex (table 7). Sudex plots consisting of roots only had no significant impact on lettuce yields from transplants set 21 days post-shredding, and these yields were not significantly less than those of the control. In transplants set 35 days post-shredding, none of the treatments had an influence on lettuce yields, but all treatments continued to cause a significant reduction ($P \leq 0.05$) in broccoli yields.

Sudex residue and transplants

The allelopathic impacts of a previous sudex crop on tomato, lettuce and broccoli transplants were noticeable almost immediately after they were set. Within 3 to 5 days, transplants began showing evidence of phytotoxicity, injury to a plant caused by a chemical. Tomato plants became chlorotic (yellow) with older leaves becoming necrotic (showing areas of dead tissue). Lettuce leaves had marginal necrosis and broccoli leaves turned purple, indicating a phytotoxicity of some kind. Plants were stunted, and those that were most severely affected failed to produce any new growth and died.

The combination of sudex shoots + roots was the most consistent treatment in reducing transplant survival in all crops, followed by plots in which the sudex shoots + roots had been soil-incorporated. Tomato transplants were more susceptible to sudex than broccoli or lettuce transplants. In tomato transplants set 36 days after sudex shredding, mortality remained significantly higher than in the control. In contrast, in broccoli and lettuce transplants set 35 days post-shredding, there was no difference in mortality among any of the sudex treatments and the control. In

TABLE 4. Mean tomato shoot and root biomass 5 weeks after transplanting, 2000

	Days between sudex shredding and transplanting			
Treatment	20	28	36	67
	. . . shoot weight (oz. per yd^2) . . .			
Shoots + roots	0.018a*	0.050a	0.102a	0.672a
Shoots	0.079a	0.038a	0.246ab	0.755ab
Roots	0.138a	0.170a	0.337b	0.897b
Incorporated	0.090a	0.284b	0.288b	0.851b
Control	0.434b	0.478c	0.658c	0.896b
	. . . . root weight (oz. per yd^2)			
Shoots + roots	0.005a	0.008a	0.010a	0.066a
Shoots	0.011ab	0.007a	0.024ab	0.089b
Roots	0.023ab	0.019b	0.036b	0.105b
Incorporated	0.009b	0.018b	0.028b	0.106b
Control	0.055c	0.034c	0.059c	0.093b

* Means followed by the same letter(s) are not significantly different according to LSD test, $P \leq 0.05$.

TABLE 5. Mean lettuce and broccoli shoot and root dry weight 5 weeks after transplanting, 2000

	Days between sudex shredding and transplanting			
	Lettuce		Broccoli	
Treatment	21	35	21	35
	. . . shoot weight (oz. per yd^2) . . .			
Shoots + roots	0.041a*	0.076a	0.019a	0.031a
Shoots	0.104ab	0.089a	0.019a	0.044a
Roots	0.173b	0.140b	0.096b	0.079b
Incorporated	0.085ab	0.146bc	0.042ab	0.080b
Control	0.410c	0.172c	0.425c	0.088b
	. . . . root weight (oz. per yd^2)			
Shoots + roots	0.061a	0.124a	0.023a	0.070a
Shoots	0.066a	0.122a	0.028a	0.068a
Roots	0.065a	0.118a	0.048b	0.054a
Incorporated	0.048a	0.136ab	0.028a	0.063a
Control	0.171b	0.169b	0.088c	0.093a

* Means followed by the same letter(s) are not significantly different according to LSD test, $P \leq 0.05$.

TABLE 6. Mean yield of mature green tomato fruit, 2000

Treatment	Days between sudex shredding and transplanting		
	20	28	36
	mature green fruit (lb per acre)		
Shoots + roots	1,721a*	3,282a	5,923ab
Shoots	6,442bc	4,707a	9,990ab
Roots	8,305c	10,636b	11,396b
Incorporated	3,684ab	3,351a	4,989a
Control	18,240d	19,664c	22,577c

* Means followed by the same letter(s) are not significantly different according to LSD test, $P \leq 0.05$.

TABLE 7. Mean yield of marketable lettuce and broccoli heads, 2000

Treatment	Days between sudex shredding and transplanting			
	21		35	
	Lettuce	Broccoli	Lettuce	Broccoli
	· · · · *fresh weight (lb per acre)* · · · ·			
Shoots + roots	344a*	0a	1,106a	112a
Shoots	495a	134a	1,269a	221a
Roots	1,611bc	405a	833a	354a
Incorporated	734ab	0a	597a	239a
Control	2,629c	1,865b	1,780a	1,597b

* Means followed by the same letter(s) are not significantly different according to LSD test, $P \leq 0.05$.

all three crops, transplants set into plots of sudex-roots-only did not differ in mortality from the control. Fertilization was adequate to produce healthy, viable plants and we observed no indication of disease or insect activity that could account for the mortality. Transplant shock was not an issue since the affected plants never recovered.

Abdul-Baki (1998) reported that allelopathy was severe on tomato and muskmelon transplants set into mulches containing rye (*Secale cereale* L.) but that full recovery was attained 3 weeks after transplanting. Norsworthy and Meehan (2005) found that the leaf margins of tomato and bell pepper (*Capsicum frutescens* L.) transplants into soil amended with wild radish (*Raphanus raphanistrum* L.) residue were necrotic for 2 and 9 weeks, respectively, but that injury was transient and both eventually recovered. This clearly was not the case in our sudex experiments. Not only was there substantial transplant mortality, but also most of the surviving plants were severely stunted and failed to recover and produce yields comparable to the controls; the exception was lettuce set 35 days post-shredding. Similarly, Finney (2005) reported that when grown following a sudex cover crop, cabbage (*Brassica oleracea* var. *capitata* L.) had reduced head weights and increased time to maturity.

In our study, the impact of allelopathic chemicals was reduced as the sudex residue aged, probably due to leaching. By 35 days post-shredding, there was no further increase in plant mortality for both broccoli and lettuce and no additional loss of yield for lettuce. Diab (2003) reported similar results on the germination of lettuce seed following the removal of allelochemicals from rye by leaching. Holmes and Mayberry (1996) found significantly fewer lettuce plants started from seed in sudangrass plots that had not been either leached by flooding or allowed to decompose for at least 22 days prior to planting. Our results were similar in that transplants set between 21 and 35 days post-shredding showed no increased mortality. This period involved several irrigations, which could have leached the active allelochemicals from the sudex and moved them beyond the vegetable root zone.

Effects of allelochemicals

Allelochemicals derived from sudex apparently affected transplant mortality and ultimately yields in all three crops. The active allelochemicals operating in this system were not determined, as this was outside of the scope of our field study. However, a number of inhibitory compounds have been identified from *Sorghum* spp. These compounds include prussic acid (Dover et al. 2004) and *p*-hydroxybenzoic acid, *p*-hydroxybenzaldehyde and dhurrin (Weston et al. 1989), the latter of which converts to cyanide. Seigler (2005) noted that cyanide, derived from cyanogenic glycosides, may be responsible for the allelopathic activities of *Sorghum* spp., but that the active toxicants may actually be benzaldehyde or *p*-hydroxybenzaldehyde. However, in addition to its negative impact on animals, cyanide is also known to be detrimental to plants. Morita et al. (2005) reported that hydrogen cyanide inhibited both radicle and hypocotyl growth of lettuce seedlings.

Ben-Hammouda et al. (1995a) found that aqueous sorghum extracts contain five phenolic acids (*p*-hydroxybenzoic, vanillic, syringic, *p*-coumaric and ferulic) that were all allelopathic to wheat. Another *Sorghum* spp.–derived compound that has been implicated is sorgoleone, a photosynthesis inhibitor that is a potent allelochemical (Czarnota et al. 2001; Geneve and Weston 1988; Weston et al. 1989). However, it is not likely that sorgoleone is the allelochemical responsible for plant mortality in our studies. Sorgoleone is produced by the root hairs and is secreted into the soil, and our studies showed that the root portion of the sudex plant was likely not responsible for the mortality: plant mortality in plots containing sudex roots only was not significantly different from that in the fallow control. But sorgoleone could have played a role in reducing shoot and root weights, leading to plant stunting; in all three crops, weights were significantly reduced in the sudex root-only plots for several weeks after transplanting. Also, while plant mortality was not affected in the sudex roots-only plots, plants were still injured as indicated by the significantly reduced yields.

Ben-Hammouda et al. (1995a) also found that water extracts of sorghum stems were the most inhibitory to wheat seedling growth, followed by extracts from leaves and roots. Similarly, in our studies, sudex shoots (including leaves), both on the surface and soil-incorporated, appeared to be most toxic to all three vegetables studied. These sudex treatments had the greatest impact on both transplant mortality and vegetable shoot and root weights. Del Moral (1975) made the significant observation that allelopathy is seldom due to a single chemical, but rather to the interaction of similar compounds or, sometimes, unrelated compounds.

Not only was there substantial transplant mortality, but most of the surviving plants were severely stunted and failed to recover and produce yields comparable to the controls.

He further states that bioassays of individual chemicals may lead to erroneous conclusions. We think that a number of allelochemicals are interacting to produce the toxic effect found with sudex and vegetable transplants, but further research is needed to elucidate the exact allelochemicals involved.

Transplant recommendations

None of the vegetables studied should be transplanted into a sudex cover crop that has been shredded unless an adequate time interval (6 to 8 weeks) is allowed and measures are taken to leach the active allelochemicals from the sudex residue. Flooding, sprinkling, surface drip or multiple precipitation events can accomplish leaching. It is apparently less risky to plant any of these vegetables into sudex stubble following removal of the crop for forage, silage or biomass, since these plots did not result in significant transplant mortality. However, there was sufficient injury and stunting to these transplants, with significantly less yield than those planted into fallow soil. Transplants of other vegetable crops may react the same way, so caution should be practiced. The same rule of thumb for a shredded sudex cover crop should also be followed for stubble that remains after the biomass has been removed. Care should also be taken that there is no sudex regrowth following final removal of the standing crop and setting transplants, as this will only add to the problem.

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A time interval of 6 to 8 weeks, coupled with adequate irrigation or precipitation, can facilitate the leaching of allelochemicals from the soil, making it safe to plant the vegetables studied.

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REVIEW ARTICLE

Biomass crops can be used for biological disinfestation and remediation of soils and water

by James J. Stapleton *and* Gary S. Bañuelos

Many plants that are candidates for refining into biofuels also possess qualities that make them potentially useful for managing soilborne pests, reclaiming polluted soils, supplementing animal feed and other purposes. Phytoremediation with these plants may provide a practical and economical method for managing the movement of trace elements into water tables, surface- and tail-water runoff, and drainage effluent. Mustards (Brassicaceae) are of particular interest for biodiesel, and grasses (Gramineae) for bioethanol production. These plants, as well as others such as certain members of the onion family (Alliaceae), also possess properties that could make them effective natural biofumigants for soil. Some of these crops have high allelopathic activity and must be employed carefully in rotations to avoid damaging subsequent crops.

Plants in the mustard family (Brassicaceae), such as 'Ida Gold' at Red Rock Ranch near Five Points, are of particular interest for biodiesel production. Chemicals produced by these plants appear to have pesticidal activity that may also be useful for soil disinfestation.

Recent interest in the production of biofuels from agricultural feedstocks has resulted in considerable controversy. On one hand, biofuels offer partial relief from societal demand for petroleum, and their combustion products may contribute less to global climate change than fossil fuels. On the other hand, widespread production of biofuels from staple crops raises prices and may result in food scarcity. Also, increased cultivation of biomass can hasten degradation of environmental features, such as soil quality and water availability, and increase destruction of wildlands for conversion to cropping. As issues of biomass production are debated in California, the "value-added" sustainability of candidate feedstocks for biofuels must be considered.

Many plants that are currently or potentially useful as biomass crops for biofuel production also possess properties that may be exploited for other purposes, such as managing soilborne pests or reclaiming polluted soils (in addition to traditional uses such as soil-building and nutrient management). A number of candidate species for biofuel production are taxonomically grouped into two plant families: mustards (Brassicaceae), of particular interest for biodiesel, and grasses (Gramineae), of wide interest for bioethanol production.

Editor's note: An upcoming 2009 edition of *California Agriculture* will feature a special collection on biofuels research and policy.

Both have a long history of scientific study and characterization of their various bioactive properties.

Devising processes that take advantage of not only their primary crop value but also their biofumigation, phytoremediation or other properties, may optimize the usefulness of these "multitasking" biomass plants. For pest disinfestation, simply incorporating raw or residual plant materials into soil may be sufficient. The amended soil, however, may need a sealing cover or heating in order to derive maximum benefit. Conversely, phytoremediation of soils requires the long-term presence of growing plants to actively scavenge unwanted trace elements or compounds from the soil. Plant materials enriched with trace elements must then be collected and pro-

Glossary

Allelopathy: Deleterious effects of chemical constituents of one plant species on (an)other species.

Bioactivity: Effect on, or response of, an organism or living tissue upon exposure to a substance or agent.

Biofuel (biodiesel, bioethanol), bioenergy: Alternative fuel/energy produced from biological sources such as plants, animal oils or fermentation.

Biofumigation, soil: Intentionally using bioactive plants and other organic materials to aid in reducing populations of plant pests in soil.

Biomass crop: Plants grown for conversion into fuel or other nonfood commodities.

Disinfestation, soil: Reduction or elimination of harmful organisms from soil by physical, chemical and/or biological means.

Phytoremediation: Use of plants/trees to manage high levels of unwanted trace elements or compounds by accumulation, volatilization or stabilization.

Phytotoxicity: Quality or extent of producing deleterious effects on plants by means of a toxin or poisonous substance.

At Red Rock Ranch in the west-central San Joaquin Valley, UC and USDA-ARS researchers are studying the use of biomass crops to remediate residual salts, including those containing selenium. Some "multitasking" biomass crops may function as feedstocks for biofuels as well as help manage soilborne pests or reclaim polluted soils.

cessed during the biofuel conversion process, and reutilized if possible.

Biofumigation of soils

Since ancient agriculturists began managing crops seasonally, plant residues left in the field after harvest have offered both benefits and challenges. While beneficially contributing organic material and nutrients to the soil after decomposition, or remaining on the soil surface as moisture-conserving mulch, plant residues also sometimes harbor destructive pests and disease-causing organisms ready to attack the next crop. This is especially harmful if the susceptible plants are grown in a monoculture over and over again in a particular field or region.

Growers learned that following a particular crop with a taxonomically different crop (one not susceptible to the same pests) often eliminates carry over pest problems and sometimes even results in unexpected growth and yield increases. However, certain crops were also found to inhibit the growth of subsequent crops. These observations form the basis of the modern agricultural strategy of crop rotation, or sequencing, in which thoughtful crop scheduling — in terms of both biology and economics — can provide maximum advantages for the cropping continuum. The commercialization of potent soil fumigation chemicals from the 1950s to the 1970s diminished the apparent value of crop rotation as a pest control tactic. However, after environmental and safety problems became associated with many of the soil pesticides, interest in crop rotation was sparked anew.

In recent years, interest has grown in the cultivation of biomass crops for liquid fuel production (Jenkins et al. 2009), although serious global concerns have been raised regarding the sustainability of switching from food production crops to biofuel feedstock cultivation (Gomez et al. 2008). Interestingly, many of the plant taxa being used or tested, including members of the grass and mustard families, also possess bioactive properties that make them useful as biofumigation residues.

***Brassica* spp.** Much of current interest in the Brassicaceae revolves around a constituent class of nitrogen- and sulfur-containing compounds called glucosinolates. These compounds, which are responsible for the spicy-hot flavor in mustards and radishes, have been widely studied as possible anticancer agents as well as for antimicrobial properties (Rosa et al. 1997). Upon hydrolysis, glucosinolates break down into a number of bioactive compounds, including isothiocyanates, some of which are synthetically manufactured for use as soil pesticides (Morra and Kirkegaard 2002). The term "biofumigation," first coined to describe the particular use of Brassicaceous cover crops or soil amendments for isothiocyanate release, has become associated with the more general practice of intentionally using bioactive plants and other organic residues to aid in soil disinfestation (Stapleton et al. 2000).

Apart from the glucosinolates, studies have demonstrated that the pesticidal activity of *Brassica* spp. is likely due to mechanisms other than, or in addition to, isothiocyanate release following glucosinolate hydrolysis. Much of the biofumigation research has focused on the role of isothiocyanates as being primarily

German Perez uses a press to extract oil from mustard and canola seeds, which were irrigated with water high in salt, boron and selenium. Under experimental conditions, the press can process up to a ton of canola seed per hour with an oil-extraction efficiency of nearly 90%.

responsible for the pesticidal effects. But additional chemical compounds arising from nonglucosinolate pathways — including aldehydes, acids and other sulfur- and nitrogen-containing compounds released during plant growth or decomposition in soil — also have significant pesticidal activity (Kelly and Baker 1990; Gamliel and Stapleton 1993; Bending and Lincoln 1999). Furthermore, besides chemical activity, alterations in microbial activity (Gamliel and Stapleton 1993) that are deleterious to pest organisms (Hao et al. 2003) have also been associated with *Brassica*-mediated soil disinfestation.

In California, published results on biofumigation with *Brassica* spp. range from spectacular to insignificant. Experimental work in the Salinas Valley reported that broccoli (*B. oleracea* var. *italica*) rotations were effective in controlling certain soilborne fungal pathogens, such as *Verticillium* and *Sclerotinia*, in vegetable crop rotations (Koike and Subbarao 2000; Hao et al. 2003). In contrast, cropping and subsequent soil incorporation of rape/canola (*B. napus*) and certain mustards (*B. juncea* and *Sinapis alba*) had no significant effects on the soilborne pests of processing tomatoes in the Sacramento Valley (Hartz et al. 2005).

Several studies in California have demonstrated that covering and heating soil containing *Brassica* residues can produce deleterious effects on soilborne fungi and nematodes far exceeding those where the residues were simply incorporated into natural field soil (Ramirez-Villapudua and Munnecke 1986; Gamliel and Stapleton 1993; Stapleton and Duncan 1998; Ploeg and Stapleton 2001). During plant residue decomposition, concentrations of volatile chemical compounds in the soil tend to increase with increasing temperature (fig. 1). This is important because toxic effects are a function of the toxicant concentration multiplied by the duration of exposure. The liberation of volatile compounds from decomposing crop residues generally occurs within a few days after their incorporation in moist soil (fig. 2). Manipulation of the system via soil covers and/or heating to maximize biofumigant concentrations can be the difference between the effective and ineffective management of pests.

Grasses. Members of the grass family (Gramineae) also produce a rich diversity of bioactive chemical compounds, including phenolics, glycosides, benzoxazinones and amino acids. Although many are primarily known for their allelopathic activity against other plants (Putnam and DeFrank 1983), they may also possess properties deleterious to a broad range of fungi, bacteria, nematodes and insects in soil. For example, residues of several Gramineous crops of agronomic importance, including cultivars of barley (*Hordeum vulgare*), wheat (*Triticum aestivum*), triticale (*X Triticosecale*) and oats (*Avena sativa*), all demonstrated significant, deleterious effects on soilborne nematodes during their decomposition

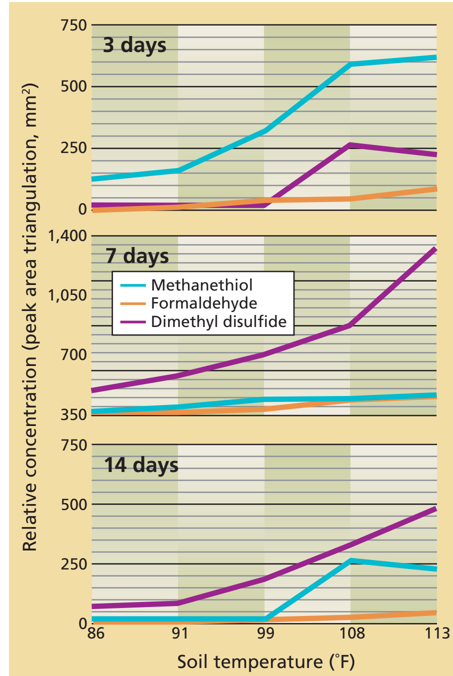


Fig. 1. Effects of soil temperature and time on relative concentration dynamics of three volatile chemicals in soil during a laboratory study. The chemicals are nonglucosinolate-derived decomposition products of cabbage plant residues, which were incorporated in soil microcosms 3, 7 and 14 days prior to headspace sampling and analysis by gas chromatography (adapted from Gamliel and Stapleton 1993).

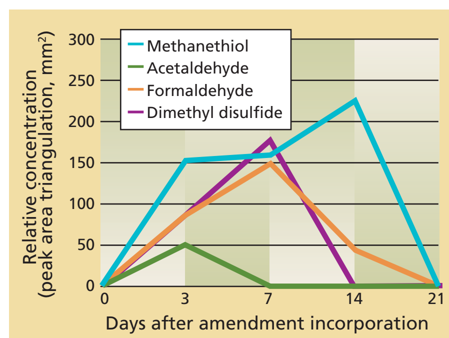


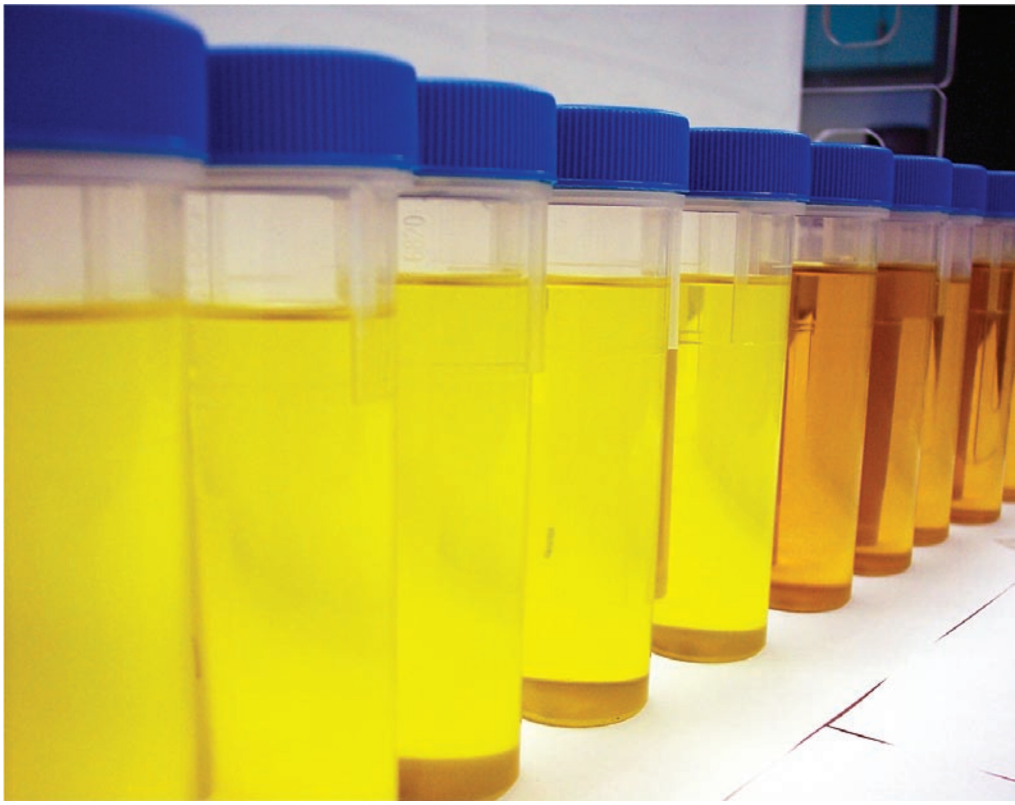
Fig. 2. Relative soil atmosphere concentration dynamics of four volatile chemical compounds produced during cabbage plant decomposition in a field study. The compounds, not derived from glucosinolate hydrolysis, were liberated from plant residues incorporated at day '0' in moist soil, which was then subjected to diurnal solar heating (solarization) prior to headspace sampling and analysis by gas chromatography (adapted from Gamliel and Stapleton 1993).

in soil. Phytotoxicity was evident in many test plants when they were subsequently established in the soil shortly after amendment with the residues (Stapleton 2006).

A similar phytotoxic effect was even more pronounced when field and greenhouse studies were conducted using sudex, a hybrid of sorghum and sudangrass (*Sorghum bicolor* × *S. sudanense*), as a cover crop. Severe allelopathic effects occurred on subsequently planted tomato, broccoli and lettuce transplants, unless a waiting period of at least 6 weeks was observed between the incorporation of sudex residues in soil and planting of the following crop (Summers et al. 2009; see page 35).

Garlic and onions. Yet another group of bioactive plants are those in the onion family (Alliaceae). Garlic and onion, especially, have been known for their bioactive properties since ancient times. Feasibility studies examined the decomposition of garlic and onion residues in moist soil, as related to the seed inactivation of four important agricultural weeds: black nightshade (*Solanum nigrum*), common purslane (*Portulaca oleracea*), London rocket (*Sisymbrium irio*) and barnyardgrass (*Echinochloa crus-galli*). The inhibitory and herbicidal effects of the Alliaceous residues were generally mild or inconsistent when tested at soil temperatures of 73.4°F (23°C). However, at 102.2°F (39°C), which by itself was mildly inhibitory to weed seed germination, the activity of the decomposing residues was far more potent (Mallek et al. 2007).

Rotational crops as pesticides. Today, there is great interest in developing rotational crop cultivars that can act as natural pesticides, and also in identifying, purifying and synthesizing bioactive compounds. The question of biocidal or inhibitory activity stemming from soil amendment with crop residues is one of great importance when considering crop sequencing. The desired result is to produce a biofumigation effect on targeted pests without harming or retarding the following crop. The old adage "the dose is the poison" comes into play here, and rotation crops with high allelopathic activity must be incorporated sparingly, or soil must be leached or fallowed between subsequent crops.



Freshly extracted canola (left) and mustard oils (right) from Brassicaceous plants can be grown for the phytomanagement of soluble selenium in the soil.

Many plants that could potentially produce biofumigation effects during their decomposition in soil have activity that is mild, inconsistent or both. Combining incorporation with a soil sealant such as plastic film or a water layer may intensify the pesticidal effect, especially if the plastic film is applied to moist soil during warm weather for solarization (Ramirez-Villapudua and Munnecke 1986; Gamliel and Stapleton 1993; Stapleton and Duncan 1998; Ploeg and Stapleton 2001).

The soil environment is complex and not clearly understood. Biofumigation, as with other approaches to the biological control of soilborne pests, cannot be expected to perform in every field or geographic area uniformly or consistently. Factors affecting the performance of organic amendments used for soil disinfestation include soil type, texture, chemical composition, temperature and moisture content, composition of native soil microflora and cropping history (Stapleton et al. 2000; Stapleton 2006; Mallek et al. 2007).

Apart from the plant taxa mentioned here, many others possess properties, metabolites and decomposition products that may be useful for managing soilborne plant pests. Additional studies will be needed to optimize their rotation with the high-value specialty crops that are important and unique to California's agricultural landscape. Scientists and growers will be looking carefully at crop sequencing, with an eye toward employing specific crops as soil disinfestants that can provide additional usefulness and sustainability to their cultivation.

Phytoremediation of soils and water

Excess trace elements such as arsenic (As), boron (B) and selenium (Se) can cause significant soil and water pollution. "Phytoremediation" uses accumulator plants or tree species with deep rooting systems to scavenge and collect mobile trace elements residing in contaminated soils. This plant-based technology may provide a practical and economical method to slowly manage the movement of trace elements into water tables, surface- and tail-water runoff, and drainage effluent. For example, in the central San Joaquin Valley's west side, phytoremediation has been tested by several growers for managing soluble selenium by using cropping systems in conjunction with microbial activity to extract, accumulate, volatilize and stabilize the offending pollutant.

Managing selenium. Although field research on selenium phytoremediation is still in the nascent stage (Bañuelos 2000), intensive and long-term field studies are key to developing sound strategies for detoxifying soils and sediments. Growing crops on a sustained basis to manage soluble selenium requires knowledge of a wide range of site-specific factors, including the: (1) impact of high soil salinity when coupled with phytotoxic concentrations of additional elements, such as boron or arsenic; (2) presence of competitive ions, such as sulfate (SO_4^{2-}), which affect selenium uptake and volatilization by plants; (3) utilization of groundwater and drainage water management strategies under saline conditions; (4) consumption and infestation of phytoremediation crops by wildlife and pests; (5) development of sustainable cropping systems; (6) production of viable products from crops used for phytoremediation; and (7) acceptance of phytoremediation as a management technology by the public and growers in regions known to have selenium contamination.

Crop selection is an important factor for successful field management of selenium. Bioremediation crops should be compatible in rotations with other agronomic crops, such as cotton, wheat, tomatoes and sugarbeets, which are typically grown in the saline soils of central California (Shennan et al. 1995), or in rotation with other crops used in phytoremediation (Bañuelos 2002). For the last 2 decades, researchers from the University of California, California State University (CSU) and U.S. Department of Agriculture Agricultural Research Service (USDA-ARS) have studied the use of selenium-tainted agricultural drainage water as an alternative source of irrigation water in central California (Suyama et al. 2007). Two multifaceted *Brassica* crops are now under serious consideration for water reuse strategies where selenium is present: canola and mustard (*B. juncea*) (Bañuelos et al. 2000; Bañuelos 2006). On-farm experiments near Five Points and Firebaugh successfully demonstrated that the two species accumulated and volatilized selenium from polluted drainage water reapplied for irrigation, hence minimizing the buildup of soluble selenium in the soil (Bañuelos 2002; Zayed et al. 2000).

Since ancient agriculturists began managing crops seasonally, plant residues left in the field after harvest have offered both benefits and challenges.

Canola and mustard. In order for the *Brassica* species to play an important role in remediation of California soils, viable economic uses must be developed for their harvested plant products. Worldwide, canola and mustard are mainly grown for their seed, which has a high oil content, 35% to 40% (Carr 1995). Canola and mustard oils also have high energy content per unit weight and are two of the most efficient sources of bioenergy in terms of British thermal units (BTUs) per acre planted. These attributes have led to interest in the adoption of canola and mustard oils as sources of biodiesel fuel (McDonnell et al. 2000).

Since 2002, USDA-ARS scientists, in cooperation with Red Rock Ranch in Five Points, have been investigating the production of biodiesel from oil extracted from canola and mustard plants irrigated with selenium-tainted water. Initial efforts have resulted in the capability to process up to 1 ton of canola seed per hour, using an Insta Pro 1500 Oil Press and a 2000 RC Extruder (Insta Pro International, Des Moines, IA), with an ideal oil extraction efficiency of almost 90% under controlled experimental conditions. Seed yields were estimated as high as 1.7 tons per acre when derived from hand-sampling a multitude of 10.8-square-foot (1-square-meter) microplots prior to major harvest. In general, about 1 ton of seed per acre was mechanically harvested from canola grown with soil and water containing an average sulfate-dominated salinity of 7 deciSiemens per meter (dS/m), 150 parts per billion (ppb) soluble selenium and 5 parts per million (ppm = milligrams per kilogram) soluble boron. From seed yields determined under field conditions, an optimal production of 100 gallons (380 liters) of 100% biodiesel (BD100) made from canola and mustard oil, or 500 gallons (1,900 liters) of BD20 biodiesel (a mixture of 20% vegetable oil and 80% petrodiesel) per acre could be achieved.

Selenium-enriched seed meal. Another potential byproduct from *Brassica* phytoremediation crops is the residual, selenium-enriched seed meal remaining after oil extraction. Canola meal is one of the most widely traded protein ingredients in the world; its use in animal feed rivals soybean meal because of its high nutritional quality in terms of fiber, protein and fat. In phytoremediation experiments on the San Joaquin Valley's west side, the selenium concentration in residual canola meal was almost 2 ppm of dry matter (Bañuelos 2006).

The benefit of selenium in canola meal is that it is a component of the animal enzyme glutathione peroxidase, an antioxidant capable of reducing the cell-damaging free radicals produced during metabolism or from oxidant stress (Gladyshev and Hatfield 1999). Based upon these nutritional characteristics, collaborative feed trials with Holstein and Jersey dairy cows are in progress at CSU Fresno, whereby the selenium-enriched canola seed meal (at 2 ppm of dry matter) is provided as a selenium source instead of the more expensive selenized yeast or the inorganic form of selenium that would normally be added, as needed, to daily feed rations.

Similarly, in earlier research Bañuelos and Mayland (2000) improved the selenium status of animals by carefully mixing selenium-rich vegetative canola plant material with other animal feedstuffs. An organic source of selenium may be more bioavailable than inorganic sources (Muñiz-Naveiro et al. 2006), but selenium absorption by animals will always depend on the animal species, duration of feeding, composition of diet and ruminal microbial population (Koenig et al. 1997).

Animal requirements for selenium are generally low, between 0.1 and 0.3 ppm of the diet dry matter (Mayland

1994), so it is important that selenium concentrations in organic sources of selenium, such as canola seed meal, be constantly monitored to ensure that excessive selenium levels do not occur. Irrespective of the selenium source used to maintain healthy dairy production, excessive selenium provided to animals in synthetic or inorganic forms is not only potentially toxic to the animals but also may increase the environmental burden of selenium cycled back into soil and water from animal manure. The incorporation of selenium-enriched canola seed meal in mixed animal diets can provide growers in high-selenium regions with an additional and valuable use for otherwise-discarded seed material after oil has been extracted. In contrast to canola, mustard seed meal after oil extraction tends to contain high concentrations of glucosinolates (approximately 300 ppm in mustard compared to 21 ppm in canola), which is too high to be used in animal feed rations. Glucosinolates break down into toxic aglucones, and their bitter taste also results in reduced feed intake.

Biomass crop sustainability

The future extent, scope, sustainability and economics of biomass crop production, particularly with respect to biofuel feedstocks in California, cannot be predicted. Regardless, the coupling of biofumigation, phytoremediation, animal feed enrichment and other uses, along with primary commodity harvesting, may provide California growers with unique opportunities to increase the environmental and economic sustainability of these cropping systems. These and other value-added benefits should be identified, tested, incorporated and utilized in the widest possible range of crop taxa for maximum benefit and flexibility within cropping sequences. The early results reviewed here with certain cultivated members of the Brassicaceae, Gramineae and Alliaceae plant families can provide opportunities for additional work.

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2008 Index

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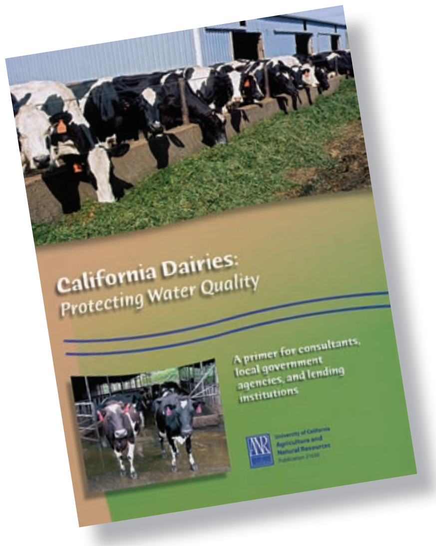

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SPECIAL COLLECTION: Climate change and California

The Intergovernmental Panel on Climate Change published its fourth assessment report in 2007, summarizing recent global climate change and projections for the next century. The next issue of *California Agriculture* journal explores how California agriculture and natural resources will be affected by climate change, possible means to address these impacts and future directions for research. In California, projected impacts range from decreases in winter chilling (necessary for many fruit and nut crops), to more extreme air pollution and more frequent coastal flooding. Winter snowpack is expected to further decline (*above*, Shasta Reservoir is currently at 58% capacity), and elevated carbon dioxide concentrations and increasing temperatures are expected to affect crop performance and plant-insect interactions.

The California Global Warming Solutions Act of 2006 (AB32) mandates reductions in California's greenhouse gas emissions to 1990 levels by 2020. Carbon trading markets are being developed, and alternative practices such as winter cover-cropping and more efficient fertilization and dairy feed management can decrease gases. Likewise, carbon sequestration techniques can exploit the role of agricultural soils as carbon sinks.

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