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Testing times:
The impact of mad cow disease

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Testing times:

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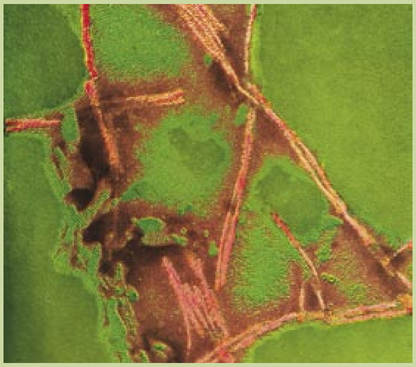

EM Unit, Veterinary Laboratories Agency/Science Photo Library

COVER: This colored transmission electron micrograph (TEM) reveals prion fibrils in the brain of a cow infected with BSE (bovine spongiform encephalopathy) or mad cow disease. The elongated orange fibrils are believed to be aggregations of the abnormal prion protein, which is the disease agent. Although it has only been confirmed in two U.S. cows, BSE has caused policy and regulatory ripple effects involving producers, processors and consumers both here and abroad.

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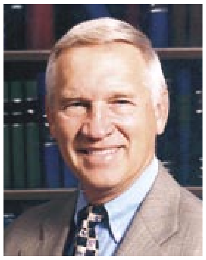

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UC expertise helps guide BSE response

ON Dec. 23, 2003, the U.S. Department of Agriculture announced the first U.S. case of bovine spongiform encephalopathy (BSE, or mad cow disease) in a cow from Washington state. Within days, 53 trading partners closed their borders to U.S. beef exports. Cattle futures plummeted, and consumption of beef declined as confusion and concern swept the country. The afflicted animal became known as "the cow that stole Christmas," as many experts from UC ANR joined others in responding during the holidays in TV, radio and print interviews.

BSE is a new disease first recognized in 1986; there is no evidence it existed prior to 1985. It joins a unique family of diseases, the transmissible spongiform encephalopathies (TSEs) that had been previously recognized, in some cases for hundreds of years, in other species. The emergence of BSE as a foodborne disease in the United Kingdom was linked to feeding cattle a protein supplement made from rendered cattle and sheep parts. It turned out that these were contaminated with a new and abnormal prion protein. The discovery of prion proteins represents a fundamental and revolutionary change in the dogma of infectious disease: no longer does an infectious agent need to rely on RNA or DNA to reproduce and create disease. TSEs are caused when small, naturally found proteins become misshapen from association with the abnormally shaped prion protein.

In the mid-1990s an association between BSE and a new disease in humans, variant Creutzfeldt-Jakob disease (vCJD), was identified as a foodborne zoonosis (a disease shared by humans and animals). Research has shown that interspecies transmission is inefficient and dependent on a number of variables. These include the level and age of exposure, TSE donor strain, and amino-acid sequence of the victim's normal prion protein. (All but one vCJD case have been in people homozygous for methionine at prion protein codon 129, a genotype found in 38% of the U.K. population.)

While the relative risks of vCJD from consumption of foods contaminated with the abnormal protein is extremely small, it is postulated that in the United Kingdom from the late 1980s to the mid-1990s, there was a massive level of abnormal prion contamination in certain meat products; as many as 800,000 diseased carcasses may have entered the human food chain prior to the emergence of the disease in humans. This combined with a cultural preference for food products that at that time were likely to contain the contaminated tissues. The tragic result is that since vCJD emerged in the 1990s, nearly 200 people worldwide have died from this disease.

Almost 200,000 cattle were killed as part of the effort to control the BSE outbreak. The impacts were huge, including the destruction of legacy herds representing generations of selective breeding, increased suicides among farmers who experienced the loss of their life's work, economic crisis in the U.K. farm community, and the eventual failure of former Prime Minister John Major's government due in part to the loss of public confidence. New BSE cases continue to decline and it appears that the aggressive response pursued in the United Kingdom will control this disease.

The Washington state animal, born and raised in Canada and imported to the United States as an adult, was likely exposed to abnormal prions in contaminated feed as a calf — Canada is known to have imported protein supplements from the United Kingdom during the danger period — and was in the long incubation phase before being imported. No additional cases were identified in an exhaustive investigation and testing of the majority of its herd mates and offspring.

Kevin P. Casey/Corbis

Cattle in Mabton, Washington, were quarantined by U.S. officials after a Holstein cow from the farm was found to be infected with mad cow disease. The finding had major implications for U.S. beef exports.

In 2005, a 12-year-old cow from Texas was confirmed with BSE. The investigation of the circumstances associated with this animal is ongoing. The cow was born prior to the U.S. feed ban and was likely exposed as a young calf. Key questions under investigation include whether the animal traveled outside of the United States or if it might have been exposed to imported feed supplements. The recently announced third suspect from an unidentified state had tests run in the United Kingdom and was found to be negative for BSE.

Yuriko Nakao/Reuters/Corbis

Above, a Japanese meat dealer examines cattle carcasses at a wholesale meat market in Tokyo. Between 1989 and September 2005, BSE cases were confirmed in Austria, Belgium, Canada, Czech Republic, Denmark, Finland, France, Germany, Greece, Ireland, Israel, Italy, Japan, Liechtenstein, Luxembourg, Netherlands, Poland, Portugal, Slovakia, Slovenia, Spain, Switzerland and the United States, as well as the United Kingdom (source: World Organisation for Animal Health [OIE]).

While BSE is unlikely to pose the significant threat to animal or human health in the United States that it did in the United Kingdom, due to our production systems and feed regulations, it appears BSE will be an issue for our beef industry and the U.S. economy for some time. Currently 59 countries have import bans or restrictions on U.S. beef or live animals, including several that represented major export markets for California beef. Whether BSE becomes a larger issue depends on what the investigation discovers about the origin and lifetime travels of the Texas animal, and whether any new positive animals are discovered. If sporadic cases occur in cattle in a manner similar to people, we can anticipate intermittent diagnoses of BSE from the surveillance program.

UC has been at the forefront of research on TSEs for many decades, trying to understand the nature and cause of the disease, and to discover potential diagnostic and therapeutic approaches. UC San Francisco neurologist Stanley Prusiner's groundbreaking work (leading to the Nobel Prize in 1997) identified a morphological change in prion proteins that are normally found as cell-wall components in the central nervous system. A change in the three-dimensional conformation of the protein creates the abnormal prion form, which is capable of inducing other normal proteins to become misshapen. This change in structure renders the altered proteins resistant to normal degradation processes, resulting in abnormal protein accumulation and subsequent interference with cellular function. TSE diseases represent a revolutionary concept — infectious agents that are out-of-reach of the victim's immune response. We will need equally revolutionary diagnostic and therapeutic methods to address them.

There have been and continue to be TSE-relevant activities across ANR's research-outreach continuum in campuses and counties. The California Animal Health and Food Safety Laboratory, managed by the UC Davis School of Veterinary Medicine for the California Department of Food and Agriculture, tests thousands of samples annually. The Center for Food Animal Health and its collaborators have funded projects looking for a rapid and accurate ante-mortem diagnostic test. Current tests rely on brain tissue samples harvested after death. UC scientists are also evaluating new methods to destroy prions, as well as technologies to monitor ruminant feed by rapidly identifying contamination by other-ruminant proteins (see page 212).

ANR faculty also provide science-based information for the public, policy-makers and industry on BSE and the other TSEs. Scientists in an array of disciplines provide expertise on the safety of the food supply, human and animal health, testing methods, animal tracking, disease surveillance and trade agreements.

Whatever the scope of the BSE problem proves to be, it has provided an opportunity to evaluate our food safety response systems. For instance, we have seen that animal trace-back and trace-forward are critical tools for investigating and controlling disease outbreaks. Effective tracing requires the ability to follow an afflicted host back to the likely source of infection, and to trace forward to identify others that may have been exposed. This is a formidable task when animals and animal products move through many hands, across multiple borders, all the while commingling with others on their way to their destination. An effective animal-identification system is an essential ingredient in protecting our food supply, and deserves ANR's multidisciplinary attention. Also, the loss of rendered protein supplements has created a demand for new protein sources equivalent to an estimated 3 million additional acres of soybean production nationally. An alternative safe use of ruminant protein that is no longer available to be recycled as feed supplements involves a number of researchable issues.

The emergence of BSE as a zoonotic disease represents just one reminder that we share the planet with many microbes and other potential health threats. ANR scientists represent the disciplinary breadth — as well as the combination of research, development and delivery — to play significant roles in finding answers to these challenges in the days and months to come.

A brief history of BSE

First recognized in 1986, bovine spongiform encephalopathy (BSE, or mad cow disease) is one of several similar but not identical diseases known as transmissible spongiform encephalopathies (TSEs). TSEs are characterized by progressive degeneration of the central nervous system. All have long incubation periods (4 to 8 years average in cattle, and up to 30 years reported in humans), evoke no immune response and are inevitably fatal.

Different TSEs affect several different mammalian species including humans (Creutzfeldt-Jakob disease, Gerstmann-Straussler-Scheinker syndrome, fatal familial insomnia, kuru), mink (transmissible mink encephalopathy), sheep and goats (scrapie), and mule deer and elk (chronic wasting disease). About 15% of human TSEs are heritable, with 85% considered sporadic (about one person per million per year will be diagnosed with a sporadic TSE on every continent worldwide).

In the mid-1990s, U.K. scientists linked the consumption of food containing BSE-diseased cattle protein from specific organ systems to a new human disease, vCJD (variant Creutzfeldt-Jakob disease). As many as 900,000 U.K. cattle might have been infected with BSE between 1984 and 1995, with close to 800,000 carcasses entering the human food chain prior to recognition of the problem as a foodborne zoonosis (disease shared by humans and animals).

Studies implicated the feeding of meat-and-bone meal contaminated with the abnormal BSE prion protein to young cattle. Changes in rendering practices in the United Kingdom in the late 1970s and early 1980s may have allowed the infectious particle, a prion ("proteinaceous infectious particle," see page 206) to be amplified in the production of meat-and-bone meal. The export from the United Kingdom of rendered animal feed products contaminated with BSE prions is considered the source of BSE affecting native cattle in other countries. The use of meat-and-bone meal as a protein supplement for ruminants (four-stomached animals including cattle, sheep and goats) had been common practice for more than 50 years prior to the emergence of BSE. Historical tissues have been examined and there is no evidence that the disease existed prior to 1985.

The United States responded as early as 1989, well before there was any evidence that this was a zoonotic disease, with import bans designed to keep the prion contaminant out of the country. Between 1989 and the present, there have been several waves of regulation by the U.S. Department of Agriculture and the U.S. Food and Drug Administration, some in response to requests from livestock and professional animal-health organizations to minimize the nation's risks of importing this disease. Others, such as the slaughter surveillance program initiated in 1990, were designed to monitor the food chain.

After confirmation of the first U.S. BSE case in December 2003, the United States vastly expanded its slaughter surveillance program. While testing is mandatory for cattle condemned at slaughter, the majority of the program is voluntary. Surveillance now includes as many cows as possible from the highest-risk populations, such as downer cattle — those unable to stand and walk on their own. (While BSE-affected cattle become downers as the disease progresses, other downers are disabled for a host of reasons having nothing to do with BSE.) From June 2004 through September 2005, more than 470,000 samples had been examined with only one positive. Scientists in the California Animal Heath and Food Safety Laboratory at UC Davis perform more than 10% of these tests.

A key to containing the outbreak in the United Kingdom included the killing and stringent disposal of positive animals and a ban on using protein from ruminants in protein supplements for other ruminants. The United States banned ruminant-source protein in ruminant feeds in 1997 (following a voluntary ban in 1996).

One U.S. animal was confirmed with BSE in December 2003. This older dairy cow was imported to the United States from Canada and was likely exposed to prions while a calf in Canada. A second positive animal was identified in 2005 in Texas as part of the slaughter surveillance program. This beef cow was about 12 years old and investigations are under way relative to its source of exposure. All tissue from these animals was destroyed with no resulting contamination of the animal or human food chain.

— D.J. Klingborg and B.I. Osburn

J.P. Moczulski/Reuters/Corbis

Reuters/Corbis

***Left*, a Canadian rancher herded healthy calves in southwestern Alberta; Canadian authorities have reported three cases of mad cow disease since 2003. *Above*, on Dec. 30, 2003, protesters met a U.S. delegation arriving in Seoul to discuss mad cow disease with South Korean officials; South Korea banned U.S. beef imports following the discovery of the first confirmed U.S. case.**

New BSE cases limit U.S. beef exports, change cattle testing

The second case of mad cow disease (bovine spongiform encephalopathy, or BSE) in the United States has led to heightened scrutiny by critics and fine-tuning of the testing process, but has had little impact on domestic economics or consumer confidence. “The U.S. has gotten off more lightly than other countries such as Germany, which had only seven cases in 2001 but had a huge public outcry,” says Kate O’Neill, associate professor in the UC Berkeley Department of Environmental Science, Policy and Management.

BSE was first found in the United States in December 2003 in a Washington state cow that originally came from Canada. This case led to the collapse of the U.S. beef export market, which used to account for one-tenth of U.S. beef production. While some countries have since lifted their bans on U.S. beef, others have not and the export market is far from recovered.

In contrast, there has been little economic reaction to the second U.S. BSE case, which was confirmed in June 2005 in a Texas cow. “The impact of the second case has been pretty negligible,” says Donald Klingborg of the UC Davis School of Veterinary Medicine. For example, while Taiwan and the Philippines banned U.S. beef immediately after the second case was confirmed, these bans were short-lived. “However, the second case might still have a long-term economic impact if it keeps other countries from lifting bans that have been in place since the first case,” Klingborg adds. The most significant of these countries is Japan, which had been the most lucrative beef export market and accounted for $60 million in California and $1.5 billion nationwide. In 2004, Japan said it would reopen its market to U.S. beef in 2005. While this has yet to occur, Japan has said that the second U.S. BSE case will not affect the planned resumption of trade.

For more information:

Commonly Asked Questions About BSE in Products Regulated by FDA’s Center for Food Safety and Applied Nutrition (CFSAN):
www.cfsan.fda.gov/~comm/bsefaq.html

USDA BSE Testing Program:
www.aphis.usda.gov/lpa/issues/bse_testing/plan.html

USDA BSE Surveillance Plan:
www.aphis.usda.gov/lpa/issues/bse_testing/plan.html

Food and Safety Inspection Service fact sheet, Bovine Spongiform Encephalopathy — “Mad Cow Disease”:
www.fsis.usda.gov/Fact_Sheets/Bovine_Spongiform_Encephalopathy_Mad_Cow_Disease/index.asp

Debate over testing policy

Perhaps the biggest impact of the second case has been on the debate over U.S. policy on BSE testing (see page 203). “It has given strength to consumer group arguments that we’re going about testing all wrong,” O’Neill says. In 2004, the United States vastly expanded its testing program to assess the incidence of BSE nationwide. Called a surveillance plan, the expanded program was designed by an international group of experts to be able to detect one case of BSE in a million cows. This entails testing all identified cows from the highest-risk populations: downers, which can no longer walk, and cows older than 30 months with BSE symptoms such as emaciation and unusual behaviors, from agitation to kicking. Focusing on high-risk populations “is like using a canary in a coal mine,” Klingborg says.

To date, the United States has tested more than 470,000 cattle for BSE. Critics such as the Organic Consumers Association say this is far too few, given

— Continued on page 200

Feed tests, models helping to control BSE

Over the course of 2 decades, UC San Francisco neurologist and Nobel laureate Stanley Prusiner proved that the infectious agent in mad cow disease and related brain-wasting syndromes was a misfolded protein called a prion. Today, UC scientists in several disciplines continue to combat prion-caused diseases (or transmissible spongiform encephalopathies), working to control them through both prevention and treatment.

Prusiner, who won the 1997 Nobel Prize for his discovery of "proteinaceous infectious particles" (prions), demonstrated that these agents engender fatal brain diseases that occur in humans, cattle, sheep, elk, deer and other animals. "By showing that these misfolded proteins could be infectious agents, he redefined the long-accepted principles of infectious disease, creating the need for a new paradigm addressing its prevention and treatment," says Donald Klingborg, associate dean of the UC Davis School of Veterinary Medicine.

Among UC scientists focusing on mad cow disease is a team at UC Davis that has developed a new cattle-feed test to help keep the disease from spreading, and another team doing molecular-level modeling that could eventually yield treatments for the disease.

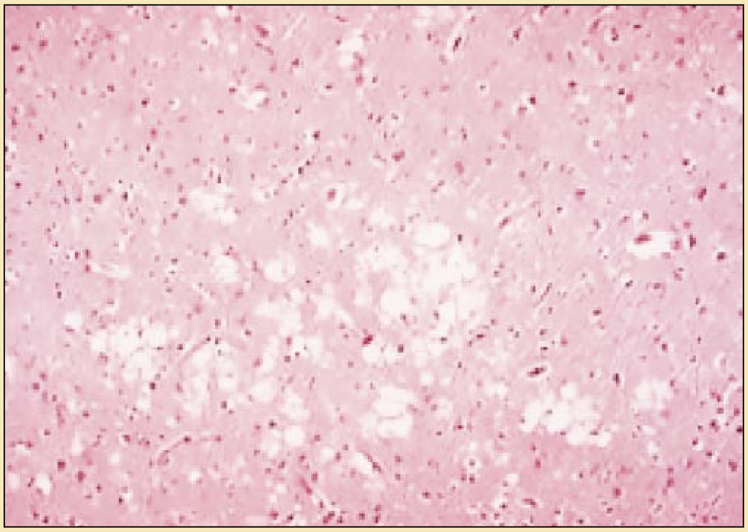
Feed test. Cattle can catch BSE from feed that contains byproducts from infected cows. While banned from cattle feed, byproducts from cows and other ruminants are allowed in poultry and swine feed. That means cattle feed can be contaminated accidentally if, for example, a feed mill is not cleaned properly between producing different types of feed.

The new test is DNA-based and can detect smaller amounts of ruminant contaminants than the current antibody-based test (see page 212). "The DNA test is 10 to 100 times more sensitive," says James Cullor of the UC Davis School of Veterinary Medicine (based in Tulare), who led the team that developed the new test. However, the antibody test is faster than the DNA test: the former takes only 25 minutes while the latter can take up to 6 hours for complicated feeds, which can contain grain, fruit, silage and even rejected M&Ms.

Now Cullor and his team are fine-tuning the DNA test, in part because the federal government wants it to work on European feed, which has smaller pieces of DNA due to processing differences. "We will keep refining the test to make it faster, better and less expensive," he says.

Prion models. Having a sensitive test for ruminant byproducts is critical because it does not take much to infect cows. BSE is caused by prions, an abnormal form of a protein found mostly on the surfaces of neurons and lymph system cells. Prions are folded incorrectly and can convert the normal proteins into more prions, which then stick together in aggregates called plaques. Much is still unknown about BSE, including why the incubation period is so long — up to 10 years in cattle and up to 30 years in people. It is also unknown how BSE crossed the species barrier to infect humans, an event first documented in the United Kingdom in 1996. While the human disease, named "variant Creutzfeldt-Jakob disease" is infectious, other diseases characterized by abnormal plaque accumulation are not. (These include Alzheimer's, Parkinson's and type II diabetes.)

UC Davis biophysicist Daniel Cox and his colleagues have developed models of prions, because these misfolded proteins are hard to study directly. Their prion aggregation model fits the actual incubation times for BSE derived from epidemiological data on about a million cattle from the United Kingdom. This model also fits the actual incubation times of laboratory animals experimentally dosed with prions.



Human brain tissue afflicted with Creutzfeldt-Jakob disease becomes riddled with holes (white areas). UC scientists have developed models of the abnormal prion that causes the fatal disease to aid in the search for treatments.

Another model supports work by other researchers, which suggests that it only takes three prions bound together (a trimer) to spread BSE. While the previous work did not explain what held the prion trimer together, the model by Cox and his colleagues shows that hydrogen bonding can do it. The fact that such a tiny prion dose can spread BSE argues against a proposed treatment. "Cutting up [plaques] has been suggested as a treatment but this would just provide more 'seeds' of the disease," Cox says.

Most recently, Cox and his colleagues have developed a model of how prions change shape when they bind to copper and other metal ions. "This could lead to a treatment that blocks other proteins from misfolding," he says. — *Robin Meadows*

New BSE cases — *continued from page 198*

that there are about 96 million cattle nationwide, of which about 36 million are slaughtered each year. In comparison, Japan tests every cow slaughtered and the United Kingdom tests a quarter of them. However, Japanese testing is driven by consumer demand and the United Kingdom has had more than 180,000 BSE cases altogether, neither of which applies to the United States. "BSE is at such a low level here that it doesn't make sense economically to test all cows," says Alex Ardans, director of the California Animal Health and Food Safety Laboratory System (CAHFS) at UC Davis, one of seven nationwide that screen cows for BSE.

Critics also call for testing cows before the 30-month cutoff because Japan has found BSE in two cows that were younger (21 and 23 months). However, the overwhelming majority of positive BSE cases are in cows older than 30 months, and there is a key distinction between the Japanese and U.S. testing programs. "The U.S. program is not food safety testing," Ardans says. Rather than determining whether cows slaughtered for human consumption are BSE-free, the goal is to assess whether the disease is present in the U.S. cow population and, if so, where and how widespread it is.

Eriko Sugita/Reuters/Corbis

Japan, the world's top buyer of U.S. beef, suspended imports in late December 2003, after the U.S. confirmed its first case of mad cow disease. On Dec. 24, 2003, a Japanese chef sliced imported U.S. beef at a Tokyo restaurant.

Changes in testing program

The second U.S. BSE case prompted important changes in the U.S. cattle-testing program for BSE. All along, the first step in this program has been a rapid screening test called an enzyme-linked immunosorbent assay (ELISA). This antibody-based test costs about $25 per sample, and the CAHFS lab at UC Davis can process up to 550 per day. If the ELISA result suggests that a sample may be BSE-positive, the next step is confirmatory testing at the National Veterinary Services Laboratory in Ames, Iowa. Between June 2004 and Sept. 18, 2005, only two of the more than 470,000 samples screened had gone on for confirmatory testing.

Originally, that meant doing an immunohistochemistry (IHC) test, which takes 4 to 7 days and has two components: examining brain tissue for the spongy-looking areas characteristic of BSE, and testing the tissue with antibodies. However, the IHC test failed to catch the second U.S. BSE case, which was ultimately confirmed by another, more sensitive antibody-based test called a Western blot. As a result, future confirmatory testing will include both the IHC and Western blot tests. The latter costs about $500 and takes about 2 days.

The second U.S. BSE case may also hasten parts of the testing program that are planned but have not yet been implemented. For example, in addition to testing downers and cows with BSE symptoms, the program is supposed to test 20,000 healthy-looking cows brought to slaughterhouses.

Livestock tracking several years away

The second U.S. BSE case also underscored the importance of being able to track individual cows. Federal investigators were unable to trace all the herd mates and offspring over the lifetime of this 12-year-old Texas cow, so the question of whether any of them also had BSE remains unresolved.

Livestock tracking is already required in countries such as Canada, the United Kingdom and Japan. Effective January 2009, the U.S. National Animal Identification System will also require U.S. producers to track all cows and other meat-producing livestock. For cows, tracking will likely be via radio-identification ear tags that send information automatically to a national database.

O'Neill says that while the risk of BSE may be small in the United States, the significance of the second case should not be downplayed. "BSE is indicative of larger problems in industrialized agriculture," says O'Neill, noting that avian flu and other diseases that spread among species could pose a larger health threat to people. "Economic integration brings other kinds of integration," she says. "Food and animals are shipped around the world, and countries need to work together better."

— Robin Meadows

Public school districts learning to reduce pesticide risks to children

Gone are the days when the school custodian casually reached into the broom closet for a can of insecticide spray to kill a column of unwanted ants slurping up a spilled soda. "California's school districts are making more intelligent decisions about how they control pests," says Chris Geiger, a former research scientist at the California Department of Pesticide Regulation (DPR) who conducted a study on integrated pest management (IPM) in California public schools (see page 235).

Recent research supports the need for IPM in schools. In July, the Journal of the American Medical Association (JAMA) reported a significant increase in the number of acute illnesses associated with pesticide exposure among students and school employees nationwide (from 1998 through 2002). It recommended implementation of IPM practices and other measures to ensure reduced exposure to toxic chemicals in school settings.

California is among 17 states that have already passed legislation to address the issue. The Healthy Schools Act (HSA) of 2000 (AB 2260) calls upon public school districts throughout the state to identify IPM coordinators, maintain pesticide use records, notify parents and staff, and post before-and-after warnings of pesticide treatments. Although the law does not specifically require IPM practices, their use is encouraged through an educational effort spearheaded by DPR, which frequently turns to the UC Statewide Integrated Pest Management Program (UC IPM) for expertise.

IPM training for schools

Mary Louise Flint, UC IPM publications director, says UC materials have been incorporated in DPR's curriculum and Web site for district IPM coordinators. At a spring 2005 workshop for nearly 50 IPM coordinators in Butte County, the UC IPM program debuted an interactive train-the-trainer presentation for ant control, a prevalent pest problem in schools. After a demonstration, each participating school district received a DVD containing presentation materials, templates for handouts and activities to train their own employees.

"We discussed ant biology, food preferences and management strategies, emphasizing simple changes in everyday activities that can prevent ant invasions in the first place," Flint says. "Participants learned how to set up bait stations and practiced caulking on props to simulate sealing up cracks to keep ants out of school buildings."

UC IPM will be taking its train-the-trainer efforts to school districts again next year with programs on IPM for weeds and cockroaches, and general IPM principles. Along with Flint and several advisory committees of experts, UC IPM interactive learning developer Cheryl Reynolds and computer systems manager Joyce Strand have been involved in the development of these materials.

Less-toxic practices

Geiger's study, conducted in 2002, found that under the HSA California public schools are making progress toward an IPM approach, but he found differences between larger, urban schools and smaller, rural schools. In addition, preliminary results from a more recent survey conducted by DPR in 2004 show continued to progress. "The most important thing," Geiger says, "is that compliance has continued to increase."

Belinda Messenger, a DPR research scientist analyzing the 2004 data, says 64% of the districts surveyed are now in full compliance with the law, up from 50% in 2002. "We found really high compliance (92%) with use of warning signs before and after spraying," she says. "We also found 68% of the school districts have adopted an IPM program."

Messenger attributes the higher success rates to the ongoing workshops and pressure from parents. As of September 2005, about 39% of the state's approximately 1,000 school districts had sent personnel to a workshop. A faculty lounge poster is also being developed to encourage teachers not to use sprays on their own.

Geiger described the hands-on training program as very effective, especially given its small budget. "It's quality over quantity," he says. "The university's role is absolutely essential. UC IPM is the only program of its kind that provides a central repository of peer-reviewed, science-based information."

— John Stumbos

Photos: Cheryl Reynolds

The UC Statewide Integrated Pest Management Program (UC IPM) is working with the California Department of Pesticide Regulation to train school-based IPM coordinators in less-toxic pest control methods. *Top to bottom*, a school IPM coordinator applies boric acid powder to cabinet voids in the school kitchen, monitors for pests, places sticky traps for roaches and caulks to exclude ants.

For more info, go to:

www.schoolipm.info

Jack Marshall/CDF

In an effort to slow the spread of *P. ramorum*, UC Cooperative Extension joined Humboldt County and state agencies to remove and dispose of 77 infected California bay laurel trees. Soil, water and plants in the area are being monitored to determine if this procedure was effective in limiting the pathogen's spread.

Survey seeks to improve sudden oak death outreach

Established in coastal California counties from Monterey to Humboldt, the pathogen that causes sudden oak death prefers cool, wet climates and is spread by raindrops, infected plant material and people. "That's why it's so important that we get the word out about how to prevent the spread of this dangerous pathogen," says Janice Alexander, sudden oak death outreach coordinator for the California Oak Mortality Task Force (COMTF) and UC Cooperative Extension (UCCE).

Formed in 2000 and supported by state and federal agencies, COMTF is a nonprofit organization devoted to sudden oak death research, management, education and public policy. The organization has about 1,000 members from about 80 groups comprising other nonprofits, public agencies and private interests. Working together, COMTF and UCCE have developed educational resources that include a comprehensive Web site, training sessions and monthly newsletters.

To evaluate and improve sudden oak death education and outreach efforts, COMTF and UCCE Marin County conducted a statewide survey in April 2005. "We wanted to know how well we were reaching our audiences," says COMTF vice-chair Susan Frankel, manager of the U.S. Forest Service Sudden Oak Death Research Program in Albany.

Sudden oak death is caused by *Phytophthora ramorum*, an oomycete or water mold that resembles a fungus but is actually more closely related to downy mildews and potato blight. First seen in Marin County a decade ago, sudden oak death is now found in wildlands in 14 coastal California counties and one in southwest Oregon. In addition, the pathogen that causes the disease is found in nurseries across the United States and Europe. The disease gets its name from the fact that infected oak canopies can turn brown within weeks, and the trees can ultimately die. *P. ramorum* infects a variety of host plants that carry and spread the pathogen but are not killed by it, including California bay laurel, coast redwood and common nursery plants such as rhododendrons and camellias.

The outreach survey was posted online for a month and targeted people who are already concerned and knowledgeable about sudden oak death, including nursery professionals, arborists, homeowners, government resource specialists and UCCE Master Gardeners. "Part of the challenge in getting the word out is the diversity of those who need to know," Frankel says.

Overall, the response to the survey was positive. "It validated what we've already done," Alexander says. More than 90% of the 302 respondents said information about the disease was easily accessible, and the primary source of this information was the COMTF Web site. In addition, the training sessions were useful to nearly all of the 65% of respondents who had attended them. Moreover, based on what they learned about *P. ramorum*, nearly 90% of private sector respondents had changed their business practices (by, for example, disinfecting tools and other equipment after working with infected trees) and 80% also changed their personal practices (by, for example, washing their shoes and car tires when leaving infested areas).

The survey was used to identify further outreach needs. "We got lots of concrete suggestions," Alexander says. "It was like brainstorming with people." The suggestions included providing more information on how to distinguish *P. ramorum* from common look-alike diseases such as root rot and bacterial wetwood, and coordinating outreach efforts with state and local parks in infested areas.

Alexander also wants to increase outreach to nurseries. "The pathogen has a huge economic impact on nurseries because a positive find means destroying all the neighboring plants in the block," she says. So far, nurseries have destroyed 1.6 million plants nationwide due to *P. ramorum* infections.

Controlling the pathogen in nurseries is challenging because it thrives in the shady, moist conditions that are also preferred by the ornamental host plants. Sanitary measures include keeping host plants in smaller blocks and separating them with nonhost plants, making sure water does not drip from above and splash on leaves, and keeping plants on gravel instead of soil, where the pathogen can lie dormant for months.

However, these measures are not foolproof. "Some nurseries have had a hard time eradicating the pathogen and it's a big mystery why," says Alexander. "There's still a lot we don't know about how the disease spreads and establishes."

— Robin Meadows

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REVIEW ARTICLE

U.S. beef industry faces new policies and testing for mad cow disease

Kate O'Neill

Ed Lallo/ZUMA/Corbis

Two cases of bovine spongiform encephalopathy (BSE) have been confirmed in the United States since 2003, with broad implications for the U.S. meat industry. *Above*, a meat inspector looks over cattle carcasses in a Kansas slaughterhouse.

The years 2003 and 2005 were pivotal for the North American cattle industry. In May 2003, Canada announced its first case of bovine spongiform encephalopathy (BSE), also known as mad cow disease. This was the first time North America's indigenous cattle had been confirmed to have BSE. Seven months later in December, the U.S. Department of Agriculture (USDA) announced that a dairy cow in Washington state (born in Canada and brought into the United States in 2001, at about 4 years old) had also tested positive for BSE. Then, in June 2005 USDA confirmed another U.S. case, this time "home-grown," a 12-year-old cow from a herd in Texas. These events have resulted in vigorous debates over testing cattle for BSE in the United States, and several important new USDA regulations. The results of the United State's expanded cattle-testing program will be watched closely in light of differing risk assessments about the prevalence of BSE in the United States. Increased testing could also have serious impacts on both domestic consumption and export markets for U.S. beef. Even as USDA continues to implement and refine new testing and other regulations, challenges from other countries and watchdog groups may result in more rigorous and transparent testing procedures. Other groups, including the beef industry, oppose more rigorous testing as causing unnecessary alarm.

While bovine spongiform encephalopathy (BSE), known as mad cow disease, is prevalent in Europe, so far there have only been two confirmed cases in the United States. A dairy cow from Washington state tested positive for BSE in December 2003, and another from Texas in June 2005. Two cases amid 95 million U.S. cattle might appear insignificant. Certainly, two cases have few implications for public health.

Nor were these cases exactly a surprise. Three major scientific studies on the risk of BSE in the United States had argued that a few cases would not be unexpected (European Commission on Food Safety 2000; HCRA 2001; GAO 2002). Indeed, subsequent investigations discovered that the first infected cow was born in Canada, and most likely was infected there, technically allowing the United States to maintain its official BSE-free status, according to the World Organization for Animal Health Standards.

Similarly, the domestic consumer reaction has been muted. While consumer awareness of the 2003 Washington BSE case was high, 65% believed the nation's beef supply was safe and only 1% claimed to have given up beef for good, according to a January 2004 survey by the Rutgers University Food Policy Institute (Hallman et al. 2004). Furthermore, most consumers gave high marks to government officials for their handling of the case.

However, the international response was different. Fifty-three countries closed their borders to U.S. beef within days of Dec. 23, 2003, affecting the entire U.S. export market and accounting for 10% of U.S. production (*Food Chemical News* [FCN], Jan. 5, 2004). This reaction mirrors the experience of other countries such as Canada, Germany and Spain immediately after they announced minor outbreaks of BSE.

As a result of the North American BSE cases, and in response to demands from trading partners, the United States began reforming its BSE policy. This entailed tightening internal controls on slaughtering practices, tracking and BSE testing (for chronology see sidebar, page 204). These changes will be discussed in depth below.

California, as the fourth-largest cattle-producing (dairy and beef) state, will bear a strong burden of adjustment to new practices and policies laid down by the U.S. Department of Agriculture (USDA). Moreover, some California constituencies, especially consumer

Timeline of regulatory actions

Since the first reports of BSE in the United Kingdom in 1986, the United States has responded with import bans, testing programs, ruminant feed rules (to prevent the spread of disease in animals), slaughterhouse regulations (to protect the human food chain) and animal tracking proposals.

Regulations now in place are subject to change when final rules are set, and the success of implementation varies. The U.S. Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) recently reported close to 1,000 violations of new slaughterhouse rules. Although policy-makers have proposed tracking systems, none has yet been implemented. (Consequently, 11 cows of the birth herd from the Dec. 23, 2003, BSE case were never located.)

1988: Rising BSE cases in United Kingdom prompt USDA to set up an interagency working group.

1989: United States bans imports of live cattle, cattle feed and beef products from the United Kingdom (or any country where BSE is found).

1990: U.S. BSE testing begins; 40 cattle brains tested.

1996: First cases of vCJD officially recorded in the United Kingdom.

1997: United States bans imports of live cattle, cattle feed and beef products from all of Europe.

United States bans feeding of "most mammalian proteins" to ruminants. Exceptions are mammalian blood and blood products and feed destined for nonruminants, such as poultry, which could later be rendered for cattle feed.

2002: U.S. cattle-testing program for BSE expands; 19,990 cattle brains tested.

May 2003: First Canadian BSE case confirmed.

December 2003: First U.S. BSE case confirmed.

January 2004: New USDA/Food and Drug Administration (FDA) regulations announced. Because feed restrictions proposed in November 2002 by FDA were stalled in the rulemaking process, FDA publishes an "interim final rule," which is subject to change when final rules are established.

USDA bans downer cattle and specified risk materials from entering the human food chain (see glossary, page 206). Ban extends to mechanically separated beef using AMR methods and air injection stunning. Both of these technologies can lead to specified risk materials entering the human food chain (*FCN*, Jan. 5, 2004).

March 2004: USDA begins testing as many cows as possible from the highest-risk population: downers and cows older than 30 months with BSE symptoms, such as emaciation or unusual behavior (agitation or kicking). USDA vastly increases annual testing rates, with the majority of the program voluntary; testing is mandatory for cattle condemned prior to slaughter. USDA also implements the "test and hold" policy, which prohibits downers from being processed until tests are confirmed negative. USDA introduces rapid screening tests used widely in the rest of the world, with inconclusive results subject to a slower immunohistochemistry (IHC) test (see page 206).

Yuri Gripas/Reuters/Corbis

On Dec. 23, 2003, then-U.S. Agriculture Secretary Ann Veneman (right) and USDA Undersecretary Bill Hawks briefed the media regarding the slaughter of an animal with BSE from rural Washington state.

June 2005: Second confirmed U.S. BSE case (first U.S.-born case). Testing problems come to light because it had taken USDA 7 months to reach a conclusive result, and this came only after USDA was ordered to perform the Western blot test. In the wake of criticism, USDA added a confirmatory Western blot to the second round of testing, in addition to the IHC tests (*New York Times*, June 25, 2005).

September 2005: Between June 1, 2004, and Sept. 18, 2005, just over 470,000 tests are completed, with one positive result. In addition to high-risk cattle, USDA plans to test a random sample of 20,000 healthy cattle over 30 months of age.

— *Editors*

groups and the organic agriculture movement, are calling for the state to take action above and beyond the federal mandate. In response, state senators Jackie Speier (D-S.F./San Mateo) and Mike Machado (D-Linden) introduced a bill that sought to test all cattle slaughtered in California for BSE. While this bill died in committee, as of July 2005 the California Legislature was considering three separate measures dealing with testing on farms, country of origin labeling and beef recall disclosure, respectively. However, the history of strong centralization in policymaking in this arena suggests that the USDA is likely to oppose these efforts.

British epidemic

BSE was first reported in the United Kingdom in 1986 and soon became epidemic among British cattle. It is largely accepted that these cattle were infected through being fed meat-and-bone meal (MBM) from BSE-infected sheep or cattle. BSE is one form of transmissible spongiform encephalopathies (TSE), diseases that destroy brain tissue, and cause disorientation, loss of motor and cognitive skills, comas and, quite rapidly, death. The human form of TSE is called Creutzfeldt-Jakob disease. TSEs are caused by prions (see sidebar, page 206) and currently there are no vaccines, cures or officially sanctioned live-animal tests, with the exception of third eyelid testing in sheep. Progress is, however, being made on developing live-animal tests (*London Observer*, June 6, 2004).

What was most disturbing about the British BSE epidemic was that the infectious prion causing the illness in cattle was able to jump species and soon infected the human population, an unprecedented event. In the early 1990s, the deaths of young people in the United Kingdom from a mysterious brain-wasting disease became a media scandal, and scientific evidence began to point definitively to a link between BSE and a new form of the human TSE, known as variant CJD (vCJD). However, it was not until 1996 that the British government officially acknowledged this link. This crisis led to the slaughter of millions of cattle, long-standing trade embargoes, and severe loss of public confidence in the governance of food safety in the United Kingdom and

The U.S. Department of Agriculture is phasing out several common slaughterhouse practices, to ensure that specified risk materials (such as brain and spinal cord tissue) do not enter the human food supply. *Above*, lines of workers process beef at a Kansas meatpacking plant.

across Europe (Jasanoff 1997; Powell and Leiss 1997).

Since first being reported in the United Kingdom, BSE has been reported in 23 other countries. Initially, many of these cases were in cattle imported from Britain, but were increasingly in indigenous cattle (OIE 2005a). In 2003, 1,390 cases of BSE were reported worldwide in 16 countries, including 612 in the United Kingdom alone. In 2004, worldwide incidence dropped, with 878 cases reported internationally, including 343 in the United Kingdom (OIE 2005a, b). As of August 2005, 150 deaths from vCJD had been reported in the United Kingdom, at a median age of 28.

The particular dimensions of the British crisis arose from a confluence of factors extremely unlikely to be replicated in the United States. Nonetheless, BSE is considered a threat to the U.S. cattle population, if only because of the extent of imports of cattle and beef products from the United Kingdom up until 1989. Responding to the threat of new diseases, especially ones like BSE and vCJD, is not easy for national governments, which must balance perceptions of risk against assessments of the likelihood of transmission, weighing in the potential costs of different courses of action.

Policy implications

The nature of the disease, surrounding uncertainties and its human impact have amplified risk perceptions of BSE as well as of vCJD, and in turn have necessitated strong policy responses by affected governments. To date, the U.S. government has framed BSE largely as an external or foreign threat, emanating first from the United Kingdom and then from Europe as a whole. Until the first North American cases were reported in 2003, this meant that U.S. BSE policy focused primarily on preventing both BSE and vCJD from entering the country from abroad. Internal precautions were more selective than those in BSE-affected countries (see sidebar, page 204), including a ban on the rendering of ruminants for ruminant feed and limits on the introduction of potentially dangerous meat products, such as spinal cord and brain tissue, into the food supply.

While many observers, particularly consumer groups, saw these policies as favoring the politically powerful U.S. beef industry, their design was not wholly influenced by industry interests. The U.S. BSE precautions were also strongly influenced — and justified — by risk assessments, particularly a 2001 Harvard Center for Risk Analysis study commissioned by USDA (HCRA 2001). This study, the cornerstone of USDA's BSE policies, argued that the risk of a BSE epidemic was low and characterized the major threats as chiefly external, validating a system of comprehensive external controls coupled with more-selective internal measures. Critically, in light of subsequent events, the study acknowledged but did not take into account the economic and policy implications of a minor outbreak of BSE in the United States. Subsequently, while the General Accounting Office's 2002 report gave a sobering account of the lack of institutional capacity to implement and enforce BSE policies, the USDA and U.S. Food and Drug Administration (FDA) largely dismissed its recommendations (GAO 2002). In particular, the GAO report cites loopholes and weaknesses in border controls, the absence of testing of cattle that die on farms, and, despite the existence of documented violations, relatively little follow-up on the part of

Glossary of terms

Bovine spongiform encephalopathy (BSE): One of several diseases characterized by fatal degeneration of brain and central nervous system. The infective agents are misfolded prions found in brain and other tissues. Prions can transmit the disease from the diseased animal to another host under certain conditions. BSE primarily affects cattle and develops when cattle eat feed contaminated with the infectious agent.

Variant Creutzfeldt-Jakob disease (vCJD): A fatal neurodegenerative prion disease in humans. Nearly 200 humans worldwide who ate beef or beef products containing the BSE agent have contracted this disease, first identified in 1996.

Immunohistochemistry (IHC) test: One of two confirmatory tests used when rapid-screening tests are inconclusive. IHC involves microscopic examination of an intact portion of the brain, the obex, to see if there are lesions (holes or a spongy appearance) characteristic of BSE, and use of a staining process with antibodies that detect the abnormal prion protein. It takes 4 to 7 days to run.

Prion: "Proteinaceous infectious particle," as defined by UC San Francisco neurologist Stanley Prusiner, who won the Nobel Prize for his discovery of this new biological principle of infection. All known prions are misfolded versions of normal cellular proteins. Prions accumulate in cells by influencing the normal, cellular prion protein to assume the disease-associated form. Misfolded prions resist digestion by enzymes that regularly "recycle" proteins. Aggregates of the misfolded protein build up and are associated with TSE infectivity and neurodegenerative diseases in both animals and humans.

Prion protein: The normal form of a protein found mainly in the body's nerve cells. Its metabolic pathway and physiological function are currently unknown. This protein is sensitive to digestion by enzymes.

Specified risk materials: In 2004, ruminant tissues deemed "specific risk materials" were banned from the human food chain in the United States (Federal Register 2004). Defined as skull, brain, trigeminal ganglia, eyes, vertebral column, spinal cord and dorsal root ganglia of cattle 30 months of age or older, as well as the small intestines and tonsils of all cattle.

Transmissible spongiform encephalopathy (TSE): All diseases associated with the presence of prions in central nervous system tissue. Prions from TSE-affected brain tissue are believed to transmit the neurodegenerative disease state from the affected animal to another host.

Western blot: One of two confirmatory tests used when rapid screening tests are inconclusive. Researchers use a large portion of obex brain tissue; the abnormal prion protein in brain material is concentrated by ultracentrifugation, and the sample is exposed to the enzyme protease to destroy any normal prion proteins that may be present. The remaining sample is then run through a gel to separate the abnormal prion protein components by molecular weight. After the transfer of the proteins to a membrane, proteins are stained using antibodies that can identify a specific banding pattern associated with prion diseases, including BSE. Scientists make diagnoses by recognizing three distinctive bands identified as a result of a reaction with the antiprion protein antibody.

Sources: Advancing Prion Science: Guidance for the National Prion Research Program (2004), National Academy of Sciences; USDA-APHIS, www.aphis.usda.gov/lpa/pubs/fsheet_faq_notice/faq_BSE_confirmtests.pdf.

federal agencies when firms — from feed mills to slaughterhouses — failed official inspections.

Despite the Harvard study's support for BSE being a "foreign" threat, consumer activist groups in the United States have continually challenged this perception. They argue that the national policy establishment ignored potential internal sources of infection as well as the probability that infectious prions were already circulating within the U.S. cattle system and potentially being transmitted to humans (Rampton and Stauber 1997). Prominent consumer groups tracking BSE in the United States include the National Campaign for Sustainable Agriculture, the Consumer Federation of America, Consumers Union, Public Citizen, and the Institute for Agriculture and Trade Policy.

Following the announcement of the Canadian BSE case in May 2003, U.S. officials started reassessing the country's vulnerability. In response to assessments by international experts and under pressure from Japan, a major importer of U.S. beef, agency officials began rethinking how the United States should approach BSE as a policy problem (Reuters, July 7 and 29, 2003). This process was accelerated by the Washington state BSE case that was reported on Dec. 23, 2003. On Dec. 30, 2003, USDA Secretary Ann Veneman announced new measures to control BSE, followed by the FDA on Jan. 26. The USDA measures included: banning all nonambulatory cattle (so-called downer cows) from the slaughter process; removing specified risk materials (SRMs), such as brain and spinal cord tissue, from meat entering the human food supply; and beginning to construct an adequate national system of animal identification (*FCN*, Jan. 4, 2004).

Cattle are classified as "downers" when they cannot walk, for any reason, from a broken leg to neurological damage. Europeans have long banned such cattle from entering the food chain, as they pose the highest risk of having BSE. In terms of SRMs entering the human food chain, two long-entrenched practices — air-gun stunning and advanced meat recovery (AMR, a technique used to take every possible scrap of meat from a carcass) — carry a risk of blasting SRMs into meat intended for human (or pet) consumption. Air injection stunning is banned and AMR is restricted under the new USDA regulations. Finally, cattle-tracking is important because, in the event of a positive test for BSE, authorities can trace the infected animal's movement from farm to farm, and to its birth herd and progeny.

The FDA also proposed — but later scrapped — feed rules that would have excluded blood and blood products, poultry litter and "plate waste" from restaurants in cattle feed (*FCN*, July 12, 2004). As of this writing, new feed rules are expected but have not been released.

Implementation of the new slaughterhouse regulations has been slow. According to recent data released after a Freedom of Information Act request from Public Citizen, the U.S. Food Safety and Inspection Service is still finding hundreds of violations of the new SRM rules at meat plants (*FCN*, Aug. 15, 2005).

These new measures imposed heavy costs on the beef industry. The industry itself estimated potential costs as somewhere between $183 million and $225 million (*FCN*, Feb. 16, 2004). However, they have satisfied neither consumer groups nor some important trading partners, notably Japan and other East Asian countries. As of August 2005, import bans or restrictions on U.S. beef and/or live animals and beef products remain in place in 59 countries (APHIS 2005).

Testing policies and programs

There are two main types of postmortem tests for BSE. Rapid tests can provide an almost immediate diagnosis, enabling testing of large numbers of cattle without delaying their use in food supplies. In the European Union, when a rapid test comes back with a positive or inconclusive ("presumptive positive") result, slower confirmatory tests are used, including the IHC and Western blot tests; the latter can be used on poorer quality tissue samples (see sidebar, page 206).

Prior to December 2003, USDA tested the brain tissue of slaughtered cattle for BSE solely via histological examination and immunochemistry. Known as the "gold standard" of BSE testing (APHIS 2004a), IHC tests are labor-intensive and the entire process takes up to 2 weeks, as opposed to the 24 hours it takes for results from the rapid tests approved by the European Union. The USDA Animal and Plant Health Inspection Service (APHIS) began testing cattle brains for BSE in 1990, testing 5,272 in fiscal year (FY) 2001 and 19,990 in FY 2002. In FY 2003, APHIS tested 20,543 brains, following a targeted testing strategy of focusing on the "higher risk" population: "adult cattle with central nervous system clinical signs and nonambulatory [downer] cattle" (APHIS 2004a). In contrast, the European Union's rapid tests allowed them to assess 18 million cattle in 2002.

Jack Kelly Clark

The scientific consensus is that BSE is transmitted via contaminated cattle feed. *Above,* livestock feed at the UC Sierra Foothill Research and Extension Center.

By 2003, the issue of cattle testing in the United States had already become a focal point of controversy (Tyshenko 2004). In light of E.U. studies attesting to the accuracy of rapid testing, it is unclear why the United States had not shifted to rapid testing before 2003 (Moynagh and Schimmel 1999). Consumer activists claimed that the APHIS was testing far too few cattle, and hinted, more darkly, that rapid tests were not used because they allowed more testing, increasing the likelihood that more cases might be found (Nelson 2001). Others have claimed that the rapid tests generate a higher number of false positives, which would then give rise to unnecessary alarm.

However, this is not an accurate perception (Moynagh et al. 1999). It is true that rapid tests for BSE are set to a high level of sensitivity, which means they readily pick up anomalies that may or may not be BSE. In countries that use these rapid tests, all such inconclusive results are then subject to further rounds of testing to provide the final confirmation of infection. Another factor to consider is that the cost of rapid tests is not insignificant: around $10 to $20 per cow. Although the federal government allocated an additional $47 million dollars to BSE-related activities in the FY 2005 budget, including $17 million for testing, it is possible that at least some of the additional costs are likely to be passed on to consumers.

Critical questions facing the U.S. policy establishment include which tests to use, how many cattle to test, which cows to test (downer cows, and/or all cows above a benchmark age), and whether to decentralize testing sites, and in particular, whether or not to allow testing on farms. Age is important because with two exceptions (in 21- and

23-month-old cows tested in Japan), BSE tests have never detected BSE in cattle under 24 months old. The European Union uses 30 months as its benchmark age above which all cattle destined for consumption must be tested at slaughter. Advocates of on-farm testing argue that currently, farmers may destroy and bury downer cattle without reporting their existence, thus potentially masking a wider outbreak.

In January 2004, USDA announced a 10-fold increase in cattle testing, to 221,000 animals annually, including 20,000 tests of healthy, aged cattle. Most of the surveillance program is voluntary. Goals have been established for testing 300,000 or more animals annually. This sample size is designed to allow for the discovery of BSE even if national prevalence is only one in 10 million adult cattle. To do this, USDA has licensed five rapid tests, four of which are produced by U.S.-based private laboratories, several of which are based in California (*FCN*, March 22, 2004). All these tests are already in use in the European Union, Japan and Canada. In addition, seven state laboratories, including the California Animal Health and Food Safety Laboratory System at UC Davis, will be allowed to carry out testing, with another five to be added over the next year. Protocols issued at this time stipulated a two-step process, with inconclusive results from the rapid screening test to be followed up with the IHC test to confirm results at the USDA's National Veterinary Services Laboratory in Ames, Iowa (APHIS 2004a).

California will bear a strong burden of adjustment to new BSE practices and policies laid down by the U.S. Department of Agriculture.

Then, in June 2005, it transpired that the second BSE-infected cow had only been confirmed as positive 7 months after its first, inconclusive rapid test. Flaws in the testing process, including two IHC tests with conflicting results, led the USDA's Office of the Inspector General to request that the sample be tested using the Western blot test — at that time, not an authorized test (*FCN*, July 4, 2005). Once this story came out, USDA testing procedures and protocols were heavily criticized: Michael Hansen, senior research associate of Consumers Union, referred to USDA's "triple firewall" defense against BSE as "more of a white picket fence" (*New York Times*, June 25, 2005). In response, USDA added the Western blot test to its testing protocols, and the department has vowed to correct procedural errors made in this case, including a failure to keep records, and mixing up parts from different cattle. Inconclusive results from rapid tests will now be subject to both IHC and Western blot tests.

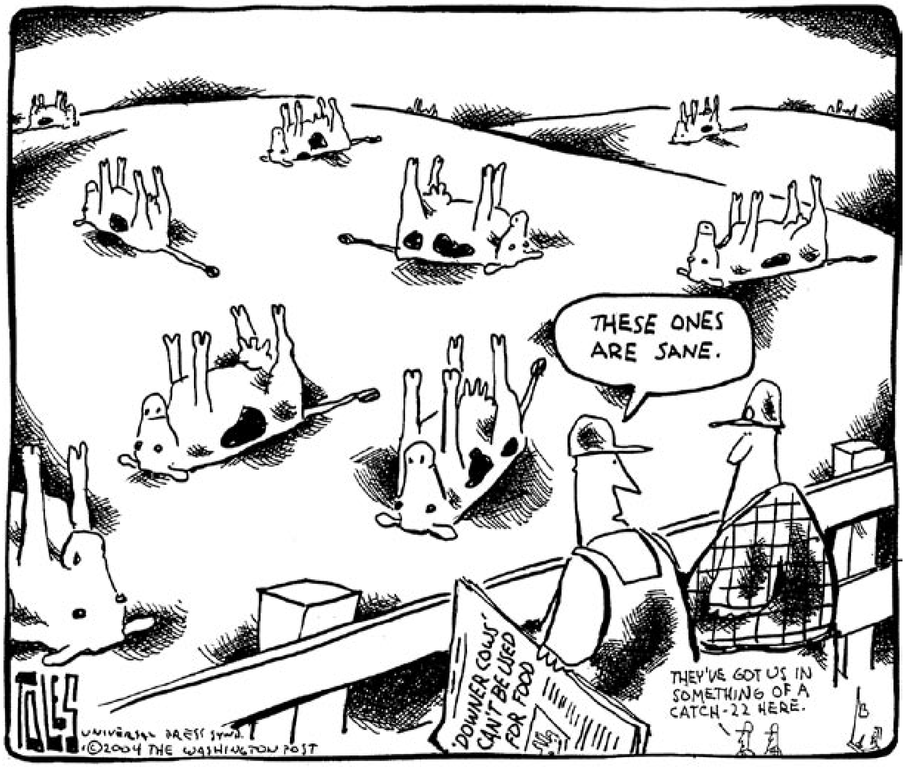
The decisions that the United States makes about cattle testing will signal to both its trading partners and internal critics how it intends to proceed in addressing BSE over the longer term. The stakes are high in the development of new testing standards. First, it is likely that the authorities will continue to find more cases. USDA chief veterinarian Ron DeHaven recognized as much in a March 15, 2004, briefing, admitting that "there is a chance that we could find more positive cattle," before assuring the audience that prevalence is low and the threat to public health minimal (*FCN*, March 29, 2004).

Ultimately, it is uncertain how many BSE-positive cattle will be found, nor is it certain how this could affect consumer confidence. However, it is clear that major export partners have a very low risk-acceptance threshold. Japan initially demanded that the United States test all cattle slaughtered for export to its markets, and Mexico is unwilling to accept imports of beef products from the United States that may contain any risk materials (*FCN*, April 26 and May 10, 2004). Further, the existence of new cases could disprove dominant official risk-assessments of the extent of BSE within the United States. USDA and FDA have strongly rejected the possibility, proposed by an international expert advisory committee in 2004, that BSE is now indigenous to North America and most likely circulating within both the United States and Canada (FCN, Feb. 9 and April 5, 2004; APHIS 2004b). These federal agencies are also deeply skeptical of possible indigenous sources of infection.

Experiences of other countries

The experiences of other countries that also initially found one or a few BSE cases are instructive. Germany's first seven indigenous cases, found in 2000, triggered a massive response from consumers and trading partners, and led to major reforms of German food safety policy. After fully implementing the European Union's BSE control regime, Germany started finding higher levels of the disease: 125 cases in 2001, 106 in 2002, 54 in 2003 and 65 in 2004 (OIE 2005a). Similarly, after finding two indigenous cases in 2000, Spain also found higher levels of BSE: 82 in 2001, 127 in 2002, 167 in 2003 and 137 in 2004 (OIE 2005a; Bird 2003). Japan found its first three cases in 2001, two in 2002, four in 2003 and five in 2004 (OIE 2005a).

In each of these cases, higher rates of testing led to more cases being found, albeit to differing extents and with differing results. Both Germany and Japan responded with fundamental reform of their policies to date. In Germany, consumer outrage led to a collapse in beef consumption, the resignation of prominent ministers, and a new ministry for Consumer Protection, Food and Agriculture (Imort 2001). Germany is now in full compliance with E.U. rules, which since 2001 have required testing of all symptomatic animals as well as animals older than 30 months sent for slaughter, and banned the feeding of mammalian proteins to all farm animals (SSC 2001). Spain also implemented E.U. policies, but beyond that, there were fewer institutional repercussions (European Commission 2002). Again, as in Germany, domestic beef sales collapsed (they were down by 50% soon after the initial outbreak), and farmers and bullfighters protested, demanding compensation (*New York Times*, Dec. 1, 2000). Japan, which also experienced a collapse in consumer trust in governance, until August 2005 required testing of all cattle sent for slaughter — the most precautionary reaction to date of any BSE-affected country (McCluskey et al. 2004). However, this is a far easier task than



Political cartoonist TOLES of the *Washington Post* skewered mad cow disease on Jan. 1, 2004.

in the United States, as Japan typically slaughters only around 1.2 million head of cattle annually, roughly 3% of U.S production. (MAFF, 2002, table 2.8).

BSE in the United States

Unlike in Germany, Spain and Japan, U.S. consumer confidence in U.S. beef has remained stable in the wake of the two BSE cases. However, it is by no means certain this would still be true if tens or hundreds of new cases were found, and the United States could also face the long-term loss of beef export markets. Experts on all sides are now holding their breaths for the results of the expanded U.S. BSE testing, but there is little consensus on what USDA is likely to find (*FCN*, March 22, 2004).

First, it is widely accepted that spontaneous TSEs emerge in older animal and human populations at rates of 1 in 1 million (HCRA 2001). This alone suggests the expanded program will lead to more cases being found, depending on the age of the cattle cohort. Second, a great deal depends on the proportion of at-risk stock, especially nonambulatory downer cattle older than 24 months, present in the testing sample, as these cattle have markedly higher rates of BSE prevalence (Bird 2003). Third, some worry that USDA's assumption that all animals testing positive will come from this high-risk group may be flawed. Hansen of Consumers Union has pointed out that BSE can also be found in apparently healthy animals, and that by largely excluding this population, authorities could underestimate the true BSE prevalence (*FCN*, March 22, 2004). Two authors of the Harvard study agreed with this assessment (Cohen and Gray 2004).

As important as the number of cases found over the next few years will be the trajectory of cases. It is impossible to tell whether the United States will mirror Germany (cases rising initially then falling significantly) or Spain (with new cases rising, then leveling off at over 100 per year). The latter scenario would likely be worse for consumer confidence than the former. Existing risk assessments vary in their predictions. The Harvard study (HCRA 2001) was highly optimistic about the ability of the existing U.S. system to dampen and eliminate BSE circulation. In contrast, the Geographical BSE Risk (GBR) study, which was commissioned by the European Union and published in 2000, was less optimistic. The GBR study concluded that if the United States had been exposed to cases originating from the United Kingdom before effective measures were put in place in the mid- to late 1990s, it is highly possible that by now these cases would have generated a number of second- or third-generation cases (SSC 2000).

The GBR study assessed the BSE risk of close to 60 countries, based on how well they could both prevent the disease's introduction across their borders (external challenge) and reduce its spread within their borders (internal stability) (SSC 2000). The study's assessments of Germany, Spain, the United States and Canada were all published in 2000, before any of these countries had reported BSE, and placed the North Americans and the Europeans into different categories of risk. As of 2000, the United States and Canada fit into GBR Level II (BSE unlikely but not excluded), while Spain and Germany fit into GBR Level III (BSE likely but not confirmed). On the face of it, this implies that Spain and Germany are far more vulnerable than the United States and Canada, therefore predicting that incidence of BSE in North America would be far lower over time.

However, the difference between these two groups was based on levels of external challenge: Germany and Spain, as European Union members, faced a far greater threat of BSE transmission from Britain, Portugal and other highly affected countries. In contrast, the picture looked very different in terms of internal stability: the United States, Canada and Germany were categorized as "neutrally stable" (neither amplifying nor reducing circulating BSE-infectivity over time), while Spain was categorized as "stable" (eliminating BSE over time). (Note that these assessments turned out to be at variance with actual outcomes over the following 4 years.) Each of these countries had moved from "highly unstable" in the early 1990s (before BSE-prevention measures had been put in place) to its 2000 position. If it turns out that the United States' external challenge was higher than initially assumed or has risen in recent years, the United States could turn out to look more like Germany or Spain in terms of BSE cases, with the ultimate outcome (numbers rising or falling over time) dependent on the effectiveness of and compliance with, internal measures put in place since May 2003. In fact, in August 2004, the SSC upgraded both countries to GBR Level III (BSE confirmed at a lower level: likely but not confirmed) (*FCN*, Aug. 30, 2004).

While these scenarios, based on the German and Spanish experiences, are hypothetical, they demonstrate that the underlying assumptions of expert risk assessments are vulnerable to challenge and reinterpretation as well as to error. Public debates between members of the international advisory panel commissioned by USDA to look into U.S. BSE policies and safeguards in January 2004, and authors of the Harvard study, indicate the gulf that can exist between groups of similarly qualified experts (*FCN*, Feb. 9, 2004). More generally, uncertainties remain about potential paths of BSE transmission — whether from other ruminants such as sheep or goats, or heredity — and about the institutional capacities of the U.S. policy establishment, in terms of closing regulatory loopholes in the face of resistance from the beef industry, or generating adequate enforcement of new requirements.

Certain stakeholder groups within the United States are concerned that USDA and FDA are dragging their feet over new controls, and that these measures may not be adequate, either in finding or preventing both existing and new BSE cases. USDA has, in response, been active in ensuring that it maintains centralized control over setting and implementing testing standards. For example in 2004, Creekstone Farms Premium Beef, a Kansas meatpacking firm that specializes in preparing beef for export to Japan, wanted to test all its cattle for BSE on-site. This bid to privatize testing standards was rapidly denied by USDA, which argued that there was no scientific justification for 100% testing (*FCN*, April 12, 2004), despite public and media support for the firm's actions (*FCN*, April 26, 2004). In California, state senators Machado and Speier sponsored legislation in March 2004 that would allow licensed slaughterers in California to voluntarily test all cattle carcasses for BSE. USDA and the beef industry both opposed this measure, and it died in committee. In addition to claiming that such measures are not justified, USDA claims that different standards across states would undermine competitiveness. Likewise, ranchers and beef producers are concerned about the costs of universal testing, and the fair implementation of the proposed California standard. At this stage, it looks as if USDA will maintain federal control over BSE testing, over and above the objections of several stakeholder groups. Nonetheless, California lawmakers remain active on this issue. As of July 25, three new bills stood before the California Legislature. One, SB 611, sponsored by Speier, deals with meat and poultry recall. A second, SB 905, sponsored by Machado, would allow independent testing by California ranchers. The third, AB 1058, sponsored by Assemblyman Paul Koretz (D-West Hollywood), would impose country-of-origin labeling for unprocessed beef.

Consumers and trading partners

Finally, scientific risk assessments do not tell us how results will be perceived or how consumers and trading partners will react. So far, U.S. consumers have been far more stoic about two cases of BSE than have U.S. trade partners, and there is some evidence that U.S. consumers demonstrate far more trust in government food safety regulation than do Europeans (Vogel 2003).). Further, the economic impact on the U.S. beef sector has been far less than on its Canadian counterpart (O'Neill 2005).

The U.S. administration is currently engaged in actively wooing back former import partners, particularly Mexico and Japan. After months of negotiations, in October 2004 the United States and Japan announced they had reached a tentative framework agreement that would allow the beef trade between the two countries to resume within 12 months (*New York Times*, Oct. 24, 2004). Part of this deal involved Japan ending its policy of blanket testing for BSE, a move opposed by many Japanese consumers. But this deal remains vulnerable. Although the second U.S. BSE case did not change Japan's position, no timetable has yet been set for this ban to be lifted. New U.S. BSE policies, most particularly the strengthened feed and SRM ban, remain heavily contested by the beef industry, and, although the new testing program has been put in place, other protective mea-


ANR Communication Services

So far, the two confirmed U.S. BSE cases have not significantly shaken consumer confidence in the safety of homegrown beef.

sures are yet to be fully implemented. If more BSE cases are found over the next few years, will actions taken to bolster consumer and foreign market confidence be in vain? Worse, is there a threshold over and above which consumer opinion will shift against U.S. beef?

U.S. regulatory authorities are facing some tough decisions about risk mitigation and communication, particularly in light of high economic stakes and strong opinions held by domestic and international constituencies. The decisions made about cattle testing in the wake of two BSE cases reflect both U.S. policy culture, and the policy-makers' desire to avoid the mistakes made by British counterparts in the 1980s. So far, USDA and FDA have done little to prepare the U.S. population for the possibility of longer-term economic impacts of finding new BSE cases, although, to their credit, they have done a good job of communicating their policy decisions and processes. Some, however, fear they have been *too* transparent over the issue of testing since the new program was implemented in June 2004, by disclosing every tentatively positive case prior to confirmatory tests. Other groups believe the USDA and FDA have not been transparent enough, for instance, in not disclosing their sampling procedures, including the geographic location of sampled cattle, their age and disease status. How USDA and FDA continue to walk this tightrope between transparency and reassurance over the next few years will be highly instructive, not only to the California agricultural community and elsewhere, but also to scholars of risk assessment and communication.

Although the incidence of BSE in the United States may seem like a minor problem at present, the responses of policy-makers and regulators provide useful case studies that put the magnifying glass to the workings of our regulatory, food production and distribution, and trade systems and relationships. We can use this BSE outbreak to examine the strengths and weaknesses in our responses to food safety issues, and glean important lessons for meeting future challenges that will undoubtedly come.

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PCR and antibody methods:

Research compares two cattle feed tests that detect bovine byproduct contaminants

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Aldridge/UCDavis News Service

Gerald Johnson, manager of the UC Davis feed mill, advises researchers and supplies them with custom-milled feeds required for their projects. The feed mill, built in 1959 by the California Feeder Council and numerous other donors, is still in daily use.

Preventing the spread of mad cow disease through contaminated cattle feed is a major concern of beef and dairy producers, regulators and consumers around the world. Routine testing of cattle feeds for the presence of banned substances is a critical control point in assuring animal health and food safety. We compared the results of two test procedures (a real-time polymerase chain reaction [PCR] assay and a commercially available ruminant antibody detection kit) on five cattle rations spiked with bovine meat-and-bone meal, or with bovine dried blood. The real-time PCR consistently detected these contaminants at lower levels in each of these diverse cattle rations.

Bovine spongiform encephalopathy (BSE), also known as mad cow disease, has now been found in 26 countries including Canada and the United States. The consumption of meat from BSE-infected cattle is believed to have caused the deaths of close to 200 people worldwide, from a disease called variant Creutzfeldt-Jakob disease (vCJD) (GAO 2005). Furthermore, BSE can have devastating effects on a country's beef industry. More than 5 million cattle were killed in an effort to control BSE in Europe; in the United Kingdom alone, almost 4 million head of cattle were destroyed through January 2004, costing the British economy as much as 5 billion pounds (the equivalent of up to 8 billion dollars).

When a single BSE-infected cow was found in the United States on Dec. 23, 2003, major foreign markets prohibited the entry of U.S. cattle. The immediate effect was that the beef industry lost more than 80% of its export trade, or an estimated $2 billion dollars. A second U.S. case was confirmed on June 24, 2005, leading to extensions of the feed bans. Fifty-nine countries have now imposed import bans or restrictions on American beef, virtually wiping out a $3 billion export market (see page 203).

The transmission of BSE in cattle most probably occurs through the ingestion of feed that contains rendered byproducts of BSE-infected ruminants (Wilesmith et al. 1988). In 1997, the United States banned the use of "most mammalian proteins" in the manufacture of feed for cattle and other ruminants (Federal Register 1997).

Detecting the presence of illicit feed additives is critical; risk assessors believe that if there were full compliance with the ban, a U.S. case of BSE would be self-limiting (HCRA 2003). However, the diagnosis of BSE in a third cow in Canada (Jan. 11, 2005) points to a weak link in the system: compliance. Investigation confirmed that the affected Canadian animal was fed contaminated feed that had been produced before the ban was established, illustrating both the consequences of noncompliance with the illicit additive ban (the rancher used this contaminated feed after the ban) (GAO 2005), and that contaminated feed may still exist in Canada (Skelton 2004). A $7 billion class-action suit representing 100,000 Canadian farmers accuses the Canadian government of negligently allowing mad cow disease to devastate their cattle industry (Makin 2005).

At present, the FDA accepts only one method of testing ruminant feed for banned substances, a microscopic examination for the presence of animal tissue such as hair and bone particles (GAO 2005). However, other tests are under development for FDA consideration. At present, the FDA and state regulatory agencies use the latter tests for initial screening but not confirmation. We performed a side-by-side comparison of two of these detection methods, evaluating their ability to detect ruminant contamination in cattle feeds. The tests were distinctly different: a real-time polymerase chain reaction (PCR)-based assay (developed by our laboratory) that detects ruminant-specific DNA, and a commercially available, antibody-based, lateral-

The indirect costs could be large if a false negative in the field was ultimately confirmed positive in the laboratory, even if the contaminated feed did not result in a case of BSE.

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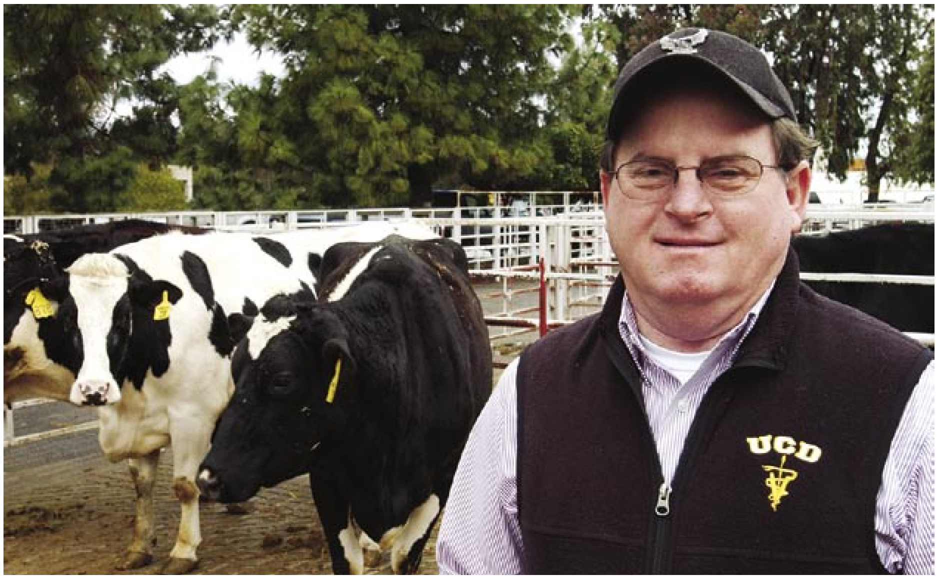


UC Davis veterinarian Jim Cullor heads the team that is developing tests for animal contamination in livestock feeds.

flow assay that detects ruminant muscle protein (Reveal, Neogen, Lansing, Mich., Product #8100, lot 16096B).

DNA and antibody-based assays

The need to detect DNA at the lowest possible levels led to the early application of PCR technology for bovine DNA (Tartaglia et al. 1998; Wang et al. 2000; Kremar and Rencova 2001). The PCR method we have developed relies on species-specific variation of mitochondrial DNA (mtDNA). MtDNA has two features that make it appealing as a species-specific marker. First, it is abundant, present at 1,000 times higher concentrations than most single-copy nuclear genes, allowing for an extremely low limit of detection. Second, because mtDNA evolves much more rapidly than nuclear genes, there is substantial species-specific variability upon which to design species-specific PCR reagents, those that bind to the mtDNA of a particular species. An additional advantage of using PCR assays to test cattle feeds is that DNA is heat-stable and may be detected even in rendered products, which have been subjected to high-heat processing (244°F to 289°F). In contrast, proteins can be substantially denatured by such high heat and made unrecognizable to antibody-based assays.

In current practice, feed is tested by both the FDA and state officials who have contracts with FDA to test and inspect animal feed. Samples of feed are taken for testing from manufacturing facilities, bulk feed sold to cattle feedlots and bags of feed sold at retail stores. No amount of any banned substance is allowed, regardless of how small the concentration. In the event that prohibited materials are found in the feed, the FDA can either issue a warning letter, ask firms to voluntarily issue a recall of the feed, or get a court order to seize the feed and feed ingredients (GAO 2005).

In the PCR analysis method we developed, a feed sample is first digested chemically to release nucleic acids. We then multiply (amplify) these nucleic acids, using PCR technology. Additional PCR reagents (probes) are used to detect the PCR products. This analysis takes advantage of several advanced technologies, including real-time fluorescent instruments that offer extremely fast real-time monitoring of PCR amplification reactions. In addition, we can achieve further specificity of the result via a melting-curve analysis in which the double-stranded PCR product is melted apart. Since this melting temperature value is directly related to the DNA sequence of the amplified product, it provides a fast, more reliable and less labor-intensive way to verify the identity of the amplified product in every sample assayed. The use of fluorescent resonance energy transfer (FRET) probes further increases the lower limit of detection, making the assay even more sensitive than conventional PCR methods. These innovations have greatly improved laboratory sensitivity for the detection of specified risk materials in feed (Rensen et al. 2005). While sensitivity levels vary, in our trials the PCR method detected the presence of contaminants when the other tests did not. However, while PCR technology is sensitive and accurate compared with other methods, the equipment to run such tests is expensive and requires laboratory support.

The commercial, antibody-based, lateral-flow kit — called Reveal — addresses the need for a field assay for mammalian proteins. This method uses antibodies on a test strip to detect the presence of ruminant proteins in the sample. When the antibodies bind to ruminant proteins, the test strip develops a colored band within 15 minutes. While typically not as sensitive as DNA-based assays, antibody-based assays are much simpler and easier to perform. Producers could use such field tests at their livestock facilities.

Testing different feed types

PCR test. To test the efficacy of these two assays under laboratory conditions, we spiked five representative types of cattle feed down to 0.1% w/w concentrations of either bovine meat-and-bone meal (BMBM) or bovine dried blood (BDB). These low concentrations may represent accidental contamination of feed during processing. Prior to real-time PCR analysis of the feed samples, DNA extraction was accomplished using modifications of a commercial kit, adapting the protocol to accommodate a larger sample size (0.22 gram) (Qiagen Plant Kit, Qiagen Inc, Valencia, Calif.). Detection and analysis were performed on each concentration of BMBM and BDB through fluorescent real-time PCR using the LightCycler (Roche Applied Sciences, Indianapolis, Ind.) (Rensen et al. 2005).

Before applying PCR analysis, each feed sample was ground to a fine powder and BMBM or BDB was added at the specified concentration. RNAse (DNA- and RNA-free) (Roche Applied Sciences, Indianapolis, Ind.) was added at a rate adjusted to the volume of the shredder column nucleic acid eluate (Sawyer 2004). After the final extraction, the concentrated DNA was aliquotted and subjected to real-time PCR analysis.

Antibody-based test. Using the antibody-based Reveal test kit analysis, the five cattle feeds were processed according to the kit instructions. The appropriate spiking amount of BMBM or BDB was added directly to the extraction vessel to attain a total of 10 grams of spiked feed. After swirling and then boiling for 10 minutes, the sample was removed and swirled again. An aliquot of the digested liquid was immediately transferred to a microcentrifuge tube. A test strip was inserted and evaluated after precisely 15 minutes. The test is considered positive when colored bands develop in both the control and target zones. Unspiked feeds were included as negative controls.

Feed types. The type of feed has been reported to be an important factor in how well these two tests detect ruminant protein or DNA (Sawyer et al. 2004). We tested five cattle feeds with a range of concentrate-to-roughage ratios: (1) finishing ration, 80-to-20, without molasses or bovine tallow; (2) finishing ration, 80-to-20; (3) starter calf ration, 40-to-60; (4) grower calf ration, 60-to-40; and (5) weaning calf ration, 70-to-30 (Trophy Maker Calf Maker, Alderman-Cave Milling and Grain Company of New Mexico, Roswell, N.M.). The first feed was the only type tested in which molasses and bovine tallow were originally excluded due to anticipated sample processing problems. All the other feeds tested included 0.01% to 0.04% molasses and 1.5% to 2.5% bovine tallow, as determined by the formulations.

Confounding factors

Roughage concentration. RNA and other inhibitors released from components of feed during sample digestion have been implicated in false-negative PCR results, where chemicals in the sample may interfere with the enzymes used in the PCR reaction. Treating the nucleic acid extract with RNAse (enzymes that break down RNA) prior to PCR results in a consistently lower DNA limit of detection (Sawyer et al. 2004). In a previous study, feeds containing the highest amounts of roughage were most frequently associated with false-negative PCR results. We were consistently unable to achieve the same lower limit of detection obtained with lower roughage feeds (nos. 1 and 2, 20% roughage) when analyzing higher roughage feeds (nos. 3 and 4, 60% and 40% roughage, respectively).

In the current study, the melting-curve analysis indicated that 0.01% BMBM in the two higher-roughage feeds was detected near the threshold limit of detection (fig. 1). With the antibody-based Reveal assay, the highest roughage feed (no. 3) produced inconclusive results at the same concentration of BMBM (table 1). This supports the theory that roughage may be an inhibitory factor in both the DNA- and antibody-based assays for detecting ruminant contamination of cattle feed.

Detecting blood. The antibody-based Reveal test was unable to detect blood at all since the antibody only binds to a muscle protein (troponin). As for the DNA-based assay, the bovine-specific mtDNA primers used can only detect nucleated cells. Most of the BDB cells are mature red blood cells, which are nonnucleated. White blood cells (which are nucleated) contribute only about 1% of the total cellular component of dried blood (Kramer 2000). In contrast, BMBM contains much greater numbers of nucleated cells than BDB and thus has a greater probability of being detected by the DNA-based assay. Both BMBM and BDB are commercially available rendered products. When testing for these combined products in cattle feed, the lowest limit of detection depends on the product or product combination, the type of ration being tested and their respective concentrations in the sample. BDB would require higher concentrations as it is the least detectable tissue.

Comparison of assays

Limits of detection. The DNA-based real-time PCR technology consistently detected BMBM in three replicate samples of all five feeds at the 1%, 0.1% and 0.01% limits of detection. BDB was detected at the 1% but not at the 0.1% limit of detection (table 1).

The antibody-based Reveal test, also performed in triplicate, detected BMBM at the 1% limit of detection in all feeds except no. 3, which was inconclusive. However, the Reveal assay did

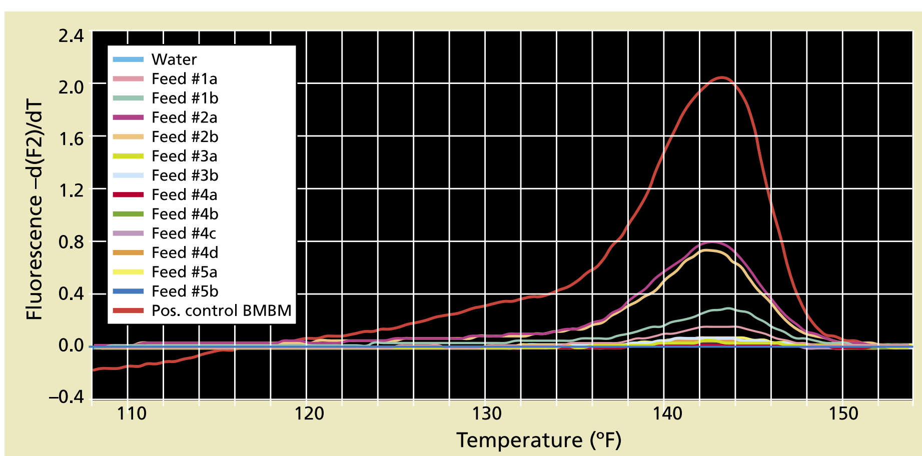


Fig. 1. A permanent record of results is provided by the printout of the melting-curve analysis of amplified PCR products. The five feeds spiked with 0.01% bovine meat-and-bone meal (BMBM) exhibit characteristic melting-temperature peaks of 143.6°F–145.4°F (62°C–63°C), similar to the positive control (tall, red peak). The negative PCR system control (blue, baseline peak) demonstrates the absence of any aberrant reagent contamination. The other five peaks identify product in each of the five feeds as BMBM.

TABLE 1. Comparison of two technologies detecting the presence of bovine dried blood (BDB) and bovine meat-and-bone meal (BMBM) in five representative cattle feeds*

% Spiking	PCR					Reveal antibody-based assay				
Feed no.	1	2	3	4	5	1	2	3	4	5
5% BDB	NP†	NP	NP	NP	NP	—	—	—	—	—
1% BDB	+++	+++	+++	+++	+++	NP*	NP*	NP*	NP*	NP*
0.1% BDB	—	—	—	—	—	NP*	NP*	NP*	NP*	NP*
1% BMBM	+++	+++	+++	+++	+++	+++	+++	+/- +/-	+++	+++
0.1% BMBM	+++	+++	+++	+++	+++	—	—	—	—	—
0.01% BMBM	+++	+++	+++	+++	+++	NP*	NP*	NP*	NP*	NP*

* Results of triplicate assays. All unspiked feeds were negative with both tests.
† NP = not performed; NP* = not performed, since the samples spiked at the next higher concentration were assay negative. (1% and 0.1% BDB and 0.01% BMBM were not performed with the Reveal kit.)

not detect BMBM in any of the feeds at the 0.1% limit of detection. While the presence of 5% BDB was readily observed visually in the digested feed sample fluids (see picture); as anticipated, the Reveal test strip did not detect BDB at this concentration since the specific antibody to troponin, the muscle protein, does not cross-react with blood cells. Failure to detect BMBM at the 1% level of spiking in some feed types or to detect BDB at any level of spiking appears to be a disadvantage of the Reveal assay.

Subjectivity. The LightCycler melting-curve analysis of results printout — provided by the PCR assay — provides a permanent record for litigation or enforcement documentation (fig. 1).

On the other hand, in these trials the results of the Reveal test at the lower (1%) limit of detection were subjective and ambiguous. In each case, a defined positive control line was apparent within 5 minutes, however most of the test samples required 15 minutes to develop a barely perceptible line. For some samples, the intensity of the test sample line increased and became more apparent with an additional 10 to 15 minutes, but in all cases never attained the intensity of the positive test line (see picture, page 216). The subjectivity in interpretation of these test-strip results and the fact that the test sample line became more apparent over time suggests that it may not be possible to use the stored test-strip as an accurate and permanent record.

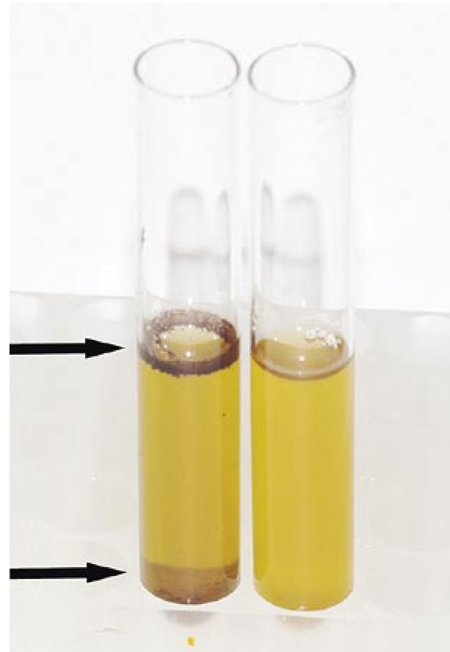
Time. The Reveal test was developed for field use. It offers the obvious advantages of on-site convenience, flexibility and time savings. In contrast, the PCR technology test requires laboratory support, augmented by a sophisticated analysis system that is dependent upon processing large numbers of samples in order to be cost-effective in an agricultural setting.

The real-time PCR process has three phases: 1.25 hours for DNA preparation and extraction (including sample grinding and weighing); 0.5-hour to set up the real-time PCR reaction; and 0.75 hour to 2.5 hours per sample for the real-time PCR analysis. This adds up to 2.5 to 4.25 hours per sample. However, our current real-time fluorescent PCR systems are able to assay from 32 to 96 samples simultaneously, which translates to 512 to 1,536 samples per 40-hour workweek.

In contrast, Neogen states that the total Reveal sample-processing time is less than 0.5 hour. With duplicate equipment, one person may be able to conveniently process five samples simultaneously in that time-span, which translates to 400 samples per 40-hour workweek. Taking advantage of batch processing, real-time PCR technology could process about 1.3 to 3.8 times as many samples as Reveal in a 40-hour workweek.

Cost. The direct costs per sample for both technologies, including supplies and technical help, are comparable ($8.87 to $9.47 per sample for real-time PCR, and $9.20 per sample for Reveal). (The initial cost of real-time PCR equipment, about $50,000, would be borne by a laboratory.)

Despite the obvious disadvantage of not being a field test, real-time PCR offers a considerably lower limit of detection and greater accuracy. The indirect costs of the antibody test could be large if the result was a false negative in the field that was ultimately confirmed positive in the laboratory (Wyatt 1992), even if the contaminated feed did not result in a case of BSE. Moreover, the loss of public confidence is less quantifiable but equally significant.



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Comparison of the visual appearance of tubes containing test fluid of feed sample no. 5, spiked to attain 5% bovine dried blood (BDB) (left) and an unspiked sample (right). The presence of BDB in fluid to be tested by the Reveal assay is readily identifiable on the left. Blood cells are floating at the interphase (arrow) and suspended in the fluid and sediment (arrow). Since the antibody incorporated in the Reveal test strips does not cross-react with blood cells, the same sample (5% BDB) tested negative. Samples of all five feeds spiked to attain only 1% BDB tested positive with the real-time PCR technology, which detects the bovine mitochondrial DNA in nucleated white blood cells.

Sampling errors. In contrast to the DNA-based assay, the antibody-based Reveal assay can accommodate a larger sample size and the sample does not have to be finely ground prior to processing. In general, a larger-size sample will increase the ability to detect contamination at lower concentrations. Grinding the feed sample to a powder, as required for the PCR test, does yield a denser, more consistent sample, and thorough mixing of the ground, spiked sample is essential for homogeneously dispersing the contaminating BMBM or BDB prior to weighing the aliquots for testing. As the instructions in the Reveal test kit point out, the particulate contaminant could easily sift out through the fibers of the feed and be missed when the sample is collected.

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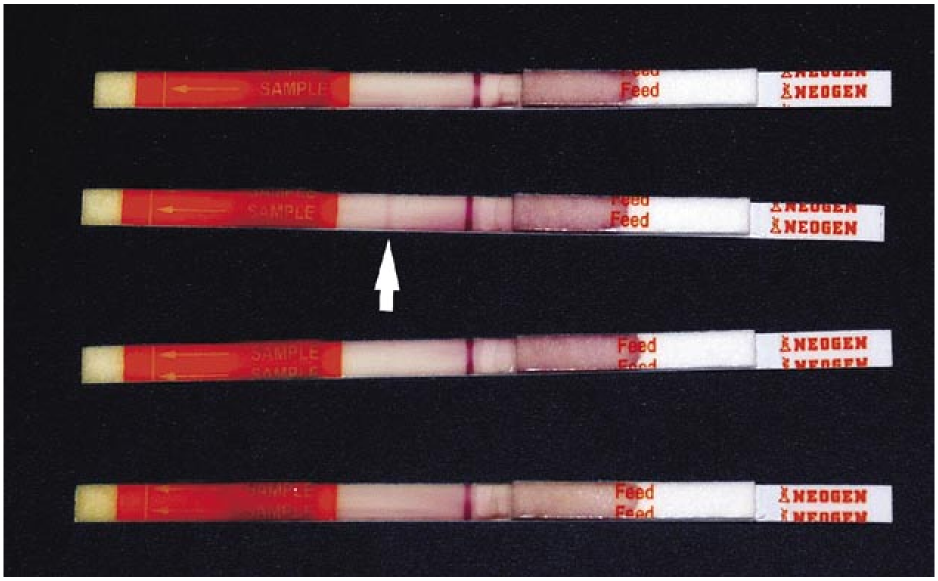

Reveal test strips performed on feed no. 5 are displayed, *top to bottom*: unspiked negative control; feed spiked to attain 1% bovine meat-and-bone meal (BMBM); 0.1% BMBM; and 5% bovine dried blood (BDB). All positive control bands are clearly seen as the red lines in the middle of the strip . The faint sample band line observed in the 1% BMBM sample (arrow) was considered positive at the development time of 10 minutes. A positive sample band failed to develop with the 0.1% BMBM or 5% BDB spiked samples.

Likewise, in practice the contaminant would tend to sift out through the feeds being tested. Because these contaminants are granular substances, great care must be taken at all stages of the process to obtain a sample that contains particulate material, regardless of the kind of assay chosen.

Advantages and disadvantages

The ability to prevent ruminant byproduct material from entering ruminant feed is essential to preventing BSE transmission. A method to detect these contaminating byproducts is necessary, not only to detect and deter the intentional illegal amending of feeds, but also to detect inadvertent contamination due to inadequate clean-out procedures, material mislabeling, or other production errors. Because the results of these tests for ruminant proteins could lead to legal action, it is important that they be objective, definitive and reproducible. Additionally, results should provide a permanent record.

Both assays offer advantages and disadvantages, and provide tools for producers, processors and regulators in particular settings. In these trials, consistent antibody-based Reveal results were obtained with four of the five feeds at a concentration of 1% BMBM; however, the inconclusive result with the 1% BMBM-spiked sample of feed no. 3 suggests that a negative Reveal test should not be considered reliable with certain feeds in the lower concentrations (1% and below) (table 1). The ability to detect 1% BDB via the DNA-based real-time PCR is of potential importance. At the time of this publication, blood had been removed from the FDA list of banned ruminant feeds due to the lack of evidence that it transmits BSE, but it is still considered a material of concern and may be added back to the list in the future. We conclude that despite the disadvantages of time, convenience and cost, the consistent detection of smaller amounts of contamination is more likely with the more sensitive, quantitative, real-time PCR analysis.

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REVIEW ARTICLE

Managed grazing and seedling shelters enhance oak regeneration on rangelands

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Melvin R. George

Livestock grazing remains a common practice on California's hardwood rangelands. This can create problems for oak regeneration because grazing has been identified as one of the factors limiting the establishment of certain oak species. Previous research, as well as recent studies at the UC Sierra Foothill Research and Extension Center, suggests that cattle will damage both planted and/or naturally occurring oaks, but damage varies by season with less during the winter when deciduous oaks do not have leaves. Damage is also influenced by the density and distribution of cattle stocking. Oaks taller than 6.5 feet seem relatively resistant to cattle damage in lightly to moderately grazed pastures, but smaller seedlings need protection.

Most oak woodlands in California are privately owned and used primarily for grazing; however, livestock grazing is an important factor in the state's documented low rates of oak regeneration.

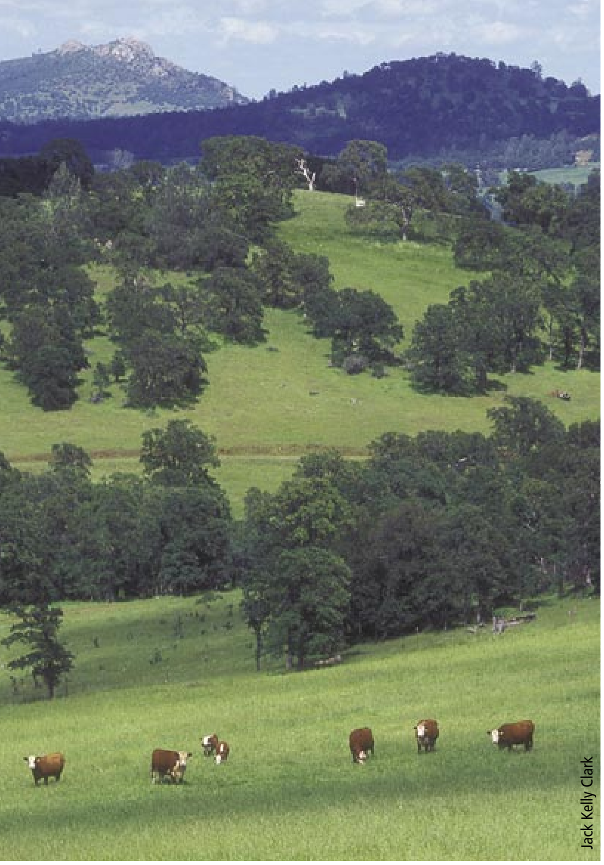
For nearly a century there has been concern that several of California's 20 native oak species are not regenerating adequately (Jepson 1910). Such concern was partially responsible for the 1986 establishment of the Integrated Hardwood Range Management Program (IHRMP), a cooperative effort to promote oak woodland conservation by UC, the California Department of Forestry and Fire Protection, and the California Department of Fish and Game (Standiford and Bartolome 1997). Evidence indicating that there is an oak regeneration problem in California is based largely on the observed paucity of young seedlings and saplings in the understories of existing oak stands (Bartolome et al. 1987).

Describing the foothill oak woodlands in the Carmel Valley, White (1966) stated that "a prevailing characteristic . . . is the lack of reproduction . . . with very few seedlings." A survey of 15 blue oak *(Quercus douglasii)* locations throughout the state showed that stands at 13 locations were losing oak density due to unreplaced mortality (Swiecki and Bernhardt 1998). The oak species having the most difficulty regenerating are members of the white oak subgenera of *Quercus*, including blue oak, valley oak *(Q. lobata)* and Engelmann oak *(Q. engelmannii)* (Muick and Bartolome 1987; Bolsinger 1988). Blue and valley oak are endemic to the state, while Engelmann oak extends into Baja California; however, the latter species actually has a far narrower distribution range than the other two (Griffin and Critchfield 1972).

During the last 2 decades, research has focused both on understanding the major factors contributing to oak regeneration failures and developing strategies to overcome these obstacles, including: how site and management factors affect oak regeneration; how acorns should be collected, stored and handled; how to propagate seedlings; and the best techniques for planting, protecting and maintaining seedlings in the field (McCreary 2001). The need to maintain and sustainably manage oak woodlands is important because these areas provide a wide range of societal benefits including aesthetics, recreational opportunities, watershed protection and wildlife habitat. Trees also provide shade for livestock and help enrich the soil through nutrient cycling. It is therefore not surprising that range managers are interested in obtaining information about raising livestock and oaks simultaneously.

The role of livestock grazing

Livestock grazing is a principal factor in poor oak regeneration in California. In the late 1980s, Lang (1988) surveyed hardwood-rangeland resource professionals and nearly 60% cited cattle herbivory as significantly limiting oak recruitment. One reason grazing and poor oak regeneration are believed to be connected is that the observed decline in regeneration roughly coincided with the widespread introduction and spread of livestock into the state during the Mission Period in the late 18th and early 19th centuries (Pavlik 1991). Both cattle and sheep eat oak seedlings, acorns and foliage, as evidenced by distinct browse lines on trees within grazed areas. While



The UC Sierra Foothill Research and Extension Center in Yuba County supports a large herd of research cattle, which has helped scientists to study methods for improving oak regeneration in the presence of grazing.

The question of how cattle and oaks can be raised together is important because more than 80% of California oak woodlands are privately owned, with much of this land in livestock production.

oak foliage may not be preferred browse and blue oak foliage has been rated as poor forage for cattle (Sampson and Jesperson 1963), cattle did browse oaks at the San Joaquin Experimental Range and seemed to prefer blue oak foliage to that of interior live oak *(Q. wislizenni)* (Duncan and Clawson 1980).

Cattle have also been linked to poor oak regeneration by comparing grazed and ungrazed plots. Blue oak saplings were eight to nine times more likely to occur in nongrazed than grazed plots (Swiecki et al. 1997). Heavy grazing — especially over many years — can also indirectly affect oak recruitment because it increases vegetative density and soil compaction, and reduces organic matter, all of which can make it more difficult for oak roots to penetrate downward (Welker and Menke 1987). This study also reported that more moisture was available to the oak seedlings in the ungrazed site, presumably because there was more litter as well as lower plant densities than in the grazed site. However, protection from grazing and fire at the San Joaquin Experimental Range did not result in an increase in oaks (Duncan and Clawson 1980).

Bartolome, McLaran et al. (2002) examined the impacts of grazing on oak-seedling establishment over 14 years and found that protected (ungrazed) seedlings grew significantly more than unprotected ones. They concluded that browsing pressures probably played an important role in suppressing height growth, although this likely would not have been sufficient to prevent regeneration at one of the two sites studied. These studies make it clear that even if oak seedlings are browsed, they can survive for years if not decades. Griffin (1971) observed oaks at the Hastings Reservation that remained stunted for at least 25 years before becoming large enough to escape deer browsing. However, in grazed settings some seedlings are obviously killed by livestock. Bernhardt and Swiecki (1997) reported extremely high mortality, especially for seedlings from acorns that were planted without protective cages. They also monitored 20 volunteer or natural valley-oak seedlings for 6 years and reported that two died, apparently due to grazing and trampling by cattle.

Other factors affect regeneration

Protecting small seedlings from cattle is one way to enhance regeneration. Protected seedlings may even do better in some grazed sites than in ungrazed sites. Bernhardt and Swiecki (1997) reported that at two of three Northern California sites evaluated, the survival and growth of seedlings in protective cages were significantly greater in grazed than in ungrazed pastures, apparently due to reduced competition from herbaceous vegetation.

In a statewide oak-regeneration assessment, Muick and Bartolome (1986) reported that the presence or absence of livestock was not sufficient to explain the pattern of oak regeneration. Moreover, Griffin (1973) stated that "experiences in nongrazing areas, such as the UC Hastings Natural History Reservation in Carmel Valley, suggest that even without cows, sapling valley oaks may be scarce." In the Hastings study, deer and gophers had significant impacts on oak regeneration, and Griffin reported that "a high deer population can devour most of the acorns and keep the few successful seedlings chewed down to nubbins."

Another factor that has been suggested as limiting natural oak regeneration in California is competition from introduced annual plants in the understory (Welker and Menke 1987). According to this theory, plants such as wild oats *(Avena fatua)*, ripgut brome *(Bromus diandrus)* and Italian rye *(Lolium multiflorum)* utilize moisture differently than perennials, making it more difficult for oaks to become established in the spring.

Fire may also play a role. Due to fire suppression activities for much of the 20th century, the frequency of fires has been decreased on many hardwood rangelands, and fuels have accumulated in the understory. This fuel buildup may have created conditions unfavorable for oak recruitment by forming a thick layer from which it is difficult for seedlings to grow (Mensing 1992). However, neither prescribed burning (Allen-Diaz and Bartolome 1992) nor wildfire (Swiecki and Bernhardt 2002) have been found to positively affect oak recruitment.

Clearly there is no simple explanation for what is causing poor oak regeneration statewide. Multiple factors are involved, and those limiting recruitment at one site may be different at another. Competition from ground vegetation, herbivory by a variety of animals, environmental conditions, past management history and even landscape characteristics (Carmel and Flather 2004) likely contribute to the oak regeneration patterns in California today.

Oak-cattle research at SFREC

The UC Sierra Foothill Research and Extension Center (SFREC) is a 5,700-acre field station in the low-elevation Sierra Nevada foothills of Yuba County, which supports a large research cattle herd. It also provides land and facilities for natural resources research, part of which has been aimed at developing practical,

Tree shelters have proved effective in preventing cattle from trampling and grazing on oak seedlings. By the time oaks reach about 6.5 feet, they are generally able to withstand cattle damage in little- to moderately grazed pastures.

low-cost procedures for restoring oaks. Several of these studies have been conducted in areas grazed by cattle, with the objective of identifying how oaks can be established in grazed pastures without removing these lands from livestock production. The question of how cattle and oaks can be raised together is important because more than 80% of California oak woodlands are privately owned (Ewing et al. 1988), with much of this land in livestock production.

Grazing season. In 1989, Lillian Hall, a UC Davis graduate student, initiated an experiment at SFREC to evaluate how planted oak seedlings fare in fields accessed by cattle (Hall et al. 1992). She planted 1-year-old blue oak seedlings in pastures grazed by cattle at different stock densities (animals per unit area), and included a control where cattle were excluded. Although this study was limited in that the grazing plots were small and the grazing treatments were only carried out for a single year, some findings warrant noting. Damage to seedlings was significantly less in the winter and fall, when they did not have foliage and were apparently less appetizing to the cattle. Cattle did not seem to seek out or prefer young oaks, but in the spring they browsed the oak seedlings while grazing. She observed heavy damage to oak seedlings in the summer at all cattle densities. This may be because the young oaks were often the only green vegetation in the grazed pastures, and therefore more attractive than the dry annual grasses. Within each season, total oak-seedling damage also increased with increasing stock density.

Riparian restoration. In 1994, a study was initiated at SFREC to evaluate alternative practices for restoring woody plants along a perennial stream cleared in the late 1960s. Initially, few trees or shrubs were adjacent to the stream, and the predominant vegetation included broadleaved cattail *(Typha latifolia)*, rushes (*Juncus* spp.) and sedges (*Carex* spp.). This study evaluated three methods for restoring woody plants along a 2,000-foot section: (1) fencing that excluded cattle but still gave deer access, (2) tree shelters to protect individual plants, and (3) a control consisting of planting but no protection. Tree shelters are rigid, translucent, double-walled plastic tubes placed over individual seedlings, protecting them from animals such as deer and cattle. The ones used in this study were 4 feet tall, and from about 3 inches to 5 inches in diameter (they are available in a variety of sizes). In grazed pastures, it is critical that these shelters be secured with heavy metal fence posts so that they do not bend over or break when cattle rub against them. These shelters also stimulate shoot growth of seedlings inside the tubes since they create a very favorable growing environment (McCreary and Tecklin 2001).

Each of the two protection treatments was replicated five times along 100-foot stretches of the stream, and in each replication 70 total seedlings and cuttings were planted, including Fremont cottonwood *(Populus fremontii)*, Arroyo willow *(Salix lasiolepis)*, narrow-leaved willow *(S. exigua)*, blue oak, valley oak and interior live oak. During each year of the study, cattle grazed the area where the plantings were located. Generally 30 to 60 head were placed for a 3- to 6-week period in the 130-acre pasture that surrounded and enclosed the study area. All plantings were evaluated annually for 4 years, and each plant was assessed for survival and year-end height.

The results of this study indicate that protecting individual seedlings with tree shelters was required for successful restoration of the oaks (McCreary 1999). After 4 years, average survival in tree shelters for all oak species combined was 58%, while seedlings in fenced plots had only 5% survival and unprotected seedlings in control plots had less than 1% survival. However, oak seedlings that did survive in tree shelters grew quite vigorously, with an average height of nearly 6.5 feet after 4 years.

Ungrazed and grazed plots. In 1997, a 4-acre blue oak planting that had been established at SFREC beginning in 1990 (Tecklin et al. 1997) was divided in two. Half of the six plots remained ungrazed while the other half were exposed to limited grazing for approximately 5 weeks per year (two cows for 2 to 3 weeks in both the fall and spring). The blue oak seedlings in the study area varied greatly in size because they had

The poor natural regeneration of oaks, *right,* can be partially offset by careful management of grazing in woodland areas, coupled with protection for oak seedlings.

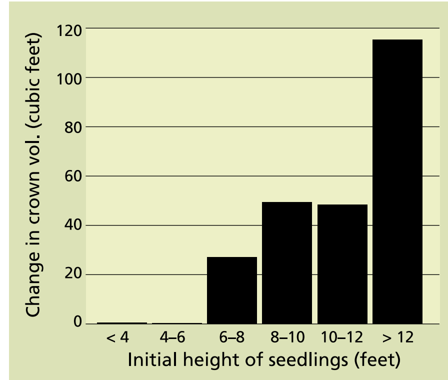


Fig. 1. Change in crown volume for seedlings of different heights after 1 year of grazing.

been established in different years and some were protected with tree shelters while others were not. As a result, the oaks ranged from a few inches tall — usually resprouts after seedlings had their bark stripped off near their bases by voles *(Microtus californicus)* — to healthy, robust saplings that had grown above the tops of the 4-foot tree shelters.

After 3 years, oaks inside the grazed plots were compared to those outside (Tecklin et al. 2002). Grazing did not result in any increased mortality, but there were differences in seedling condition. Unprotected seedlings in ungrazed plots had significantly more vole damage than those in grazed plots (52% vs. 0%). The ungrazed plots had a large increase in dead thatch, which is ideal habitat for voles and apparently resulted in higher populations and more bark stripping and girdling of oak seedlings.

For the oaks protected with tree shelters, however, the results were almost the opposite. There was evidence of far greater animal damage in the grazed plots — in this case by cattle — while there was virtually no animal damage to the oaks inside tree shelters in the ungrazed plots. In the grazed plots shoots above the tops of the 4-foot shelters were clipped, resulting in noticeably sparser crowns. Some of the shelters were also partially bent over from cattle rubbing (though all were secured with heavy metal fence posts), but no seedlings were killed. There were also differences in height and basal diameter growth between sheltered plants in the grazed and ungrazed plots, with those in grazed plots growing less. However, these differences were relatively small and browsed seedlings were not seriously damaged.

Oak size. A study to evaluate how cattle affect a range of sizes of oaks was initiated at SFREC in 2003. This study used a blue oak planting established between 1988 and 1990 by Ted Adams (Adams 1995), a UC Davis wildland specialist. Adams had established several hundred oaks inside a half-acre plot, fenced to exclude both deer and cattle. When our study began there were 144 living seedlings and saplings ranging from 17 inches to 14 feet high. This plot was within a 100-acre pasture that was subsequently grazed for 6 weeks each year by 50 cows and 49 calves as part of the Center's normal grazing operation. In 2003, half of Adams' plot was opened to cattle grazing. Prior to removing the fence around half of this plot, the researchers assessed each seedling in both halves for height, basal diameter, crown spread and crown height. From these latter two variables, crown volume was calculated (Karlik and McKay 2002).

After a full season of grazing, each oak was assessed for the same parameters. Seedlings and saplings that remained inside the fenced portion of the plot grew significantly taller than those exposed to cattle (an average of 8.7 inches vs. 3.1 inches for ungrazed and grazed, respectively). Although only one seedling was killed in the grazed portion, the cattle did severely damage a substantial number of the plants by browsing and rubbing.

However, damage from cattle varied greatly depending on the seedling's initial size. Oaks less than 6.5 feet tall were most likely to suffer damage. Of the 79 surviving oaks in the grazed plot, 11 lost more than 6 inches in height and these were all less than 6.5 feet tall when the study began. Furthermore, for the 46 oaks taller than 6.5 feet at the start, the average height gain during the 2003 growing season was 12 inches. In contrast, the 33 oaks less than 6.5 feet at the start of the study lost an average of 9 inches in height. The response was similar for basal diameter, with seedlings taller than 6.5 feet gaining an average of more than one-half inch in girth, and those shorter than 6.5 feet shrinking slightly (presumably because of clipping and rubbing by cattle). Crown volume also increased significantly for seedlings taller than 6.5 feet, while it diminished slightly for shorter seedlings (fig. 1).

Although this study has only been under way for the first of at least 3 planned years, initial results indicate that there is a threshold height — apparently near 6.5 feet — above which oaks may be large enough to withstand cattle damage in lightly to moderately grazed pastures and continue growing.

Seedling protection recommended

These trials, as well as other research, demonstrate that enhancing oak regeneration in areas grazed by livestock can be challenging since animals naturally browse seedlings. Without intervention, oak plantings in grazed areas often have little chance of significant growth or survival. However, the chances of success can be greatly increased by physically protecting seedlings and managing

— *Continued on page 222*

Grazing management guidelines

TO reduce the risk of livestock damage to oak seedlings, grazing managers can control the grazing season and frequency, stocking rate and density, and practices that affect cattle distribution (George et al. 1996). Grazing leases usually include in-and-out dates (season) and number of head grazed (stocking rate and density), and many leases also dictate distribution practices such as the placement of salt or feed supplements. Under most conditions, the following practices can help reduce the risk of damage to oak seedlings by grazing livestock.

Grazing season. Rest (that is, do not graze) pastures to minimize damage in the summer, when oak seedlings and saplings are attractive to livestock because they remain green and are surrounded by less-attractive dry grass.

Grazing intensity. Moderate grazing removes about half of the annual forage production, preventing thatch buildup associated with vole damage to oak seedlings and saplings. In California's hardwood rangelands, moderate grazing is achieved in normal rainfall years with stocking rates of about 10 to 20 acres per cow per year, depending upon site productivity. Light grazing and no grazing results in thatch accumulation, while heavy grazing will likely increase the risk of seedling damage by livestock. Light grazing results from a lower stocking rate than moderate grazing, often about 50% of moderate grazing (20 to 40 acres or more per cow per year). Heavy grazing results from a stocking rate that is often 50% to 100% greater than moderate stocking rates (less than 10 acres per cow per year) (Bartolome, Frost et al. 2002; George et al. 1996).

Stock density. Avoid high stock densities during restoration projects in oak woodlands. Seedlings and saplings are at a greatly increased risk of damage when pastures are subdivided into many paddocks for intensive grazing regimens. Stock densities of less than one cow per acre are preferable to higher stock densities.

Cattle distribution. Planting oaks more than 0.5 mile from stock water and on slopes greater than 20% can reduce the risk of grazing impacts on oak seedlings. This is because cattle do not like to walk great distances from water and they prefer to graze on flat-to-gentle slopes. The time livestock spend near oak regeneration sites can also be reduced by placing attractants such as salt, supplements, rubbing posts and water as far away from the oaks as practical. In addition, knowledge of cattle's preferred resting sites, feeding sites and trail corridors can help in the selection of oak regeneration sites that are less vulnerable to browsing or trampling. Cattle follow a predictable daily path of grazing and rest. At sunrise they begin grazing at their night resting location and generally graze toward water. After grazing for about 4 hours, they rest until moving to water sometime between late morning to midafternoon. They will then rest again, often near the water source, preferring shade in the summer months. In midafternoon to early evening, they will have another grazing bout of approximately 4 hours before reaching a night resting site (Harris et al. 2002).

Protecting planted seedlings. Areas with planted oaks should be fenced until seedlings are at least 6.5 feet tall, or individual seedlings should be protected until they attain this height. Excellent protection in moderately grazed pastures can be achieved by placing 4-foot-tall tree shelters around young seedlings; such shelters cost about $3 each and are difficult to reuse since they usually must be cut off. These devices not only protect seedlings from a variety of potentially damaging animals, including cattle, but also stimulate rapid aboveground growth.

Where livestock are present, shelters must be well secured to heavy metal fence posts to ensure that they remain upright and are not bent over from cattle rubbing. However, even this degree of protection may not be adequate in heavily grazed pastures since cattle in confined areas will often repeatedly rub against the shelters and posts, and can knock them over and damage the young seedlings. Oaks growing up and out of the tops of 4-foot tall shelters are also vulnerable to livestock clipping of the exposed shoots, but in lightly to moderately grazed pastures such damage appears to have limited long-term impact on seedling survival or growth. Where shelters are used, it is important to leave them in place for at least 2 years after the seedlings have grown up and out of the tops. If they are removed too soon the seedlings will be vulnerable to cattle damage since they will not be sturdy enough to withstand cattle rubbing and clipping.

Protecting natural seedlings. Another approach is to protect existing volunteer or natural seedlings. Such seedlings are often heavily browsed and have little chance of surviving without protection from cattle. Little research has been conducted on this, but Bernhardt and Swiecki (1997) reported that caged juvenile seedlings grew significantly more than uncaged controls in a grazed pasture. Tree shelters can therefore be used on naturally established seedlings to increase their chances of maturing into oak trees.

These practices can enhance the chances for regeneration success of native California oaks in areas grazed by livestock. But whatever steps are taken, it is important to monitor the results and alter practices as needed. We do not yet know what will and will not work in all situations, so it is vital to pay attention and modify procedures as needed.

For more information, go to: http://danr.ucop.edu/ihrmp.

— D.D. McCreary and M.R. George

the stock densities and grazing seasons. In addition, managing physical features such as salt blocks, supplements and water can influence cattle distribution and limit impacts to oak seedlings and saplings (see sidebar).

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Introduced parasitic wasps could control glassy-winged sharpshooter

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The glassy-winged sharpshooter (GWSS) is an introduced pest that spreads the bacterium* Xylella fastidiosa*, which causes a variety of diseases such as Pierce's disease in grapevines and leaf scorch in oleanders. GWSS has been established in Southern California since about 1990 and has also successfully invaded French Polynesia, Hawaii and Easter Island. Researchers from UC, the U.S. Department of Agriculture and the California Department of Food and Agriculture have introduced parasitic wasps for the biological control of GWSS. Four parasitoids from the southeastern United States have been released and appear to be establishing in Southern California. Parasitoids from Argentina are also being evaluated in quarantine but have not yet been released.

The glassy-winged sharpshooter, *above*, a native sharpshooter pest in the southeastern United States and northeastern Mexico, is now well established in California, Hawaii, Tahiti and Easter Island. *Left*, Pierce's disease, which is transmitted by bacterium vectored by the sharpshooter, is having an economic impact on California grape growers.

Photos: Jack Kelly Clark

The glassy-winged sharpshooter (GWSS) is a native insect of the southeastern United States that has become extremely pestiferous in Southern California, where it became established around 1990 (Sorensen and Gill 1996), possibly imported as eggs on nursery or private plant material. GWSS (*Homalodisca coagulata* Say [Homoptera: Cicadellidae]) is now widely established in several Southern California counties (CDFA 2003) (fig. 1, page 224), and successfully invaded Tahiti in 1999 (Cheou 2002), Hawaii in 2004 (Hoover 2004) and Easter Island in 2005.* The insect's population growth in California has been extraordinary, facilitated by a lack of coevolved natural enemies, coupled with the irrigation of agricultural and urban areas in desert habitats that would otherwise be too dry to support GWSS populations (Hoddle 2004a).

The GWSS feeds exclusively on xylem fluids, and its ability to spread the xylem-dwelling plant pathogenic bacterium *Xylella fastidiosa* is at the core of its classification as a pest in California. In California, the GWSS-*X. fastidiosa* combination causes Pierce's disease in grapes, almond leaf scorch, alfalfa dwarf disease and oleander leaf scorch. In addition, the number and type of plant maladies caused by GWSS-vectored *X. fastidiosa* is likely to increase. Already the bacterium has been found to cause two previously unrecognized diseases in olive trees and liquidambar, where it causes scorchlike symptoms. The GWSS-*Xylella* combination has devastated or has the potential to devastate many agricultural crops, native vegetation, and urban ornamental and landscape plants. The potential effect of GWSS vectoring *X. fastidiosa* into native California vegetation that previously had no association with the bacterium may lead to new epidemics, and so is particularly worrisome. Consequently, the establishment of GWSS in California has irrevocably changed the ecology of *X. fastidiosa* in

*GWSS was first detected on Easter Island in August 2005. There appear to be no natural enemies attacking GWSS and it has been observed feeding on citrus, mangoes, guava and Bougainvillea (Sandra Ide, personal communication, October 2005).

GWSS has shown strong invasive potential, having become established in a variety of places outside its native range, including California, French Polynesia, Easter Island and Hawaii.

California's wilderness, agricultural and urban landscapes.

The presence of Xylella in Tahiti and Hawaii is currently unknown. It is possible that the bacterium was introduced to these South Pacific islands via the importation of ornamental plants from areas in the Americas where Xylella is native. These ornamental plants could be silent Xylella reservoirs that harbor the bacteria without expressing disease symptoms. However, once a vector such as GWSS arrives, they could transmit the bacteria to susceptible host plants.

Economic impact of GWSS

The economic costs to California attributed to GWSS are immense. For example, oleander leaf scorch has caused damage in excess of an estimated $52 million on 2,000 miles of freeway median plantings (Costa et al. 2000). In 1998 and 1999 grape growers in Riverside and San Diego counties accrued estimated losses of $37.9 million due to Pierce's disease (Siebert 2001). In 2000, researchers in cooperation with affected grape and citrus growers spent $6.9 million to apply spray applications of Admire and Assail (acetamiprid) directly on GWSS habitat in Temecula and Bakersfield, in an effort to manage populations migrating into vineyards. In 2002, primary producers incurred additional economic costs resulting from GWSS containment activities such as inspections of export nursery stock and shipments of bulk grapes and citrus from GWSS-infested counties (CDFA 2003). There are currently more than 70 state and federal research programs studying GWSS or *X. fastidiosa*.

Traditional pesticide use and cultural practices to assist in reducing the populations of GWSS may be augmented by a long-term and cost-effective use of biological control, an area heavily researched in California with a history of success.

Biological control strategies

Classical biological-control strategies to reduce GWSS populations in California are being pursued by researchers at UC Riverside, the U.S. Department of Agriculture's Agricultural Research Service (USDA-ARS) and the California Department of Food and Agriculture (CDFA). Biological control involves identifying natural enemies of the target pest. After the control agent is deemed safe and cleared from secure quarantine facilities, they are released into the environment, where they utilize the pest as food and thereby regulate its population growth and subsequent abundance.

In the southeastern United States and northeastern Mexico, GWSS eggs are parasitized by several species of mymarid and trichogrammatid parasitic wasps. The most common natural enemies associated with GWSS eggs in the southeastern United States are all mymarid wasps: *Gonatocerus ashmeadi* Girault, *G. triguttatus* Girault, *G. morrilli* Howard and *G. fasciatus* Girault (Triapitsyn and Phillips 2000). In the late 1990s, in an effort to use natural enemies to control GWSS populations in Southern California, UC and CDFA imported these four parasitoids from southeastern states, cleared them through quarantine and introduced them into California urban and agricultural areas.

G. triguttatus was first released in fall 2000 in Riverside, Ventura and Kern counties. *G. ashmeadi* and *G. morrilli* releases started in 2001, and *G. fasciatus* in 2002. *Anagrus epos* (from Minnesota) releases started in 2005. Over 1.2 million parasitoids have now been released in 13 California counties where GWSS populations have been reported. More than 1,900 releases have been made at 373 release sites covering agricultural, riparian and urban environments (see CDFA [2005] for release locations).

Two of these species (*G. morrilli* and *G. ashmeadi*) are already established in California. *G. morrilli* is native to California and *G. ashmeadi* is self-introduced from the southeastern United States (with no direct assistance from human intervention). While *G. ashmeadi* may have established on early GWSS populations, it more likely established on the native smoke-tree sharpshooter (*Homalodisca liturata* Ball) (Vickerman et al. 2004).

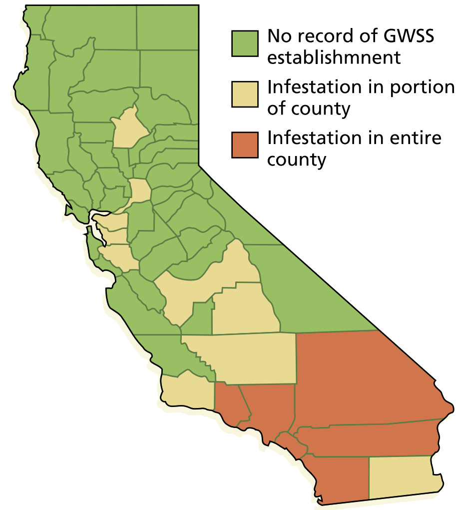
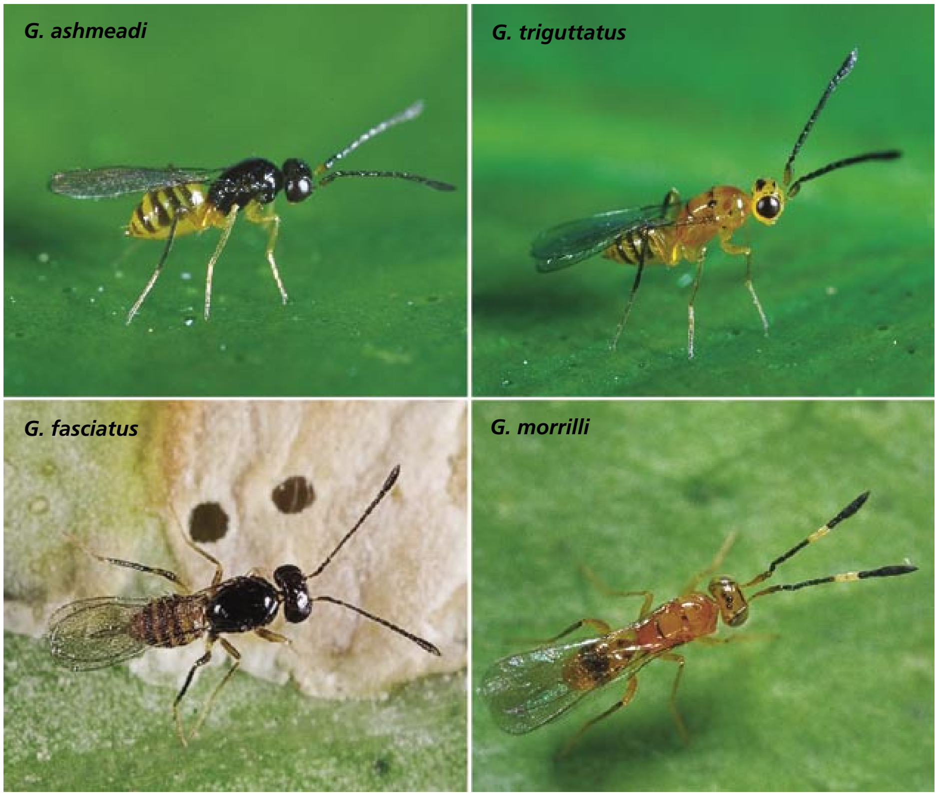


Fig. 1. GWSS establishment in California counties and infested parts of counties.

While these two species were already established, additional introductions of the same species may be more effective. For example, the introduced southeastern parasitoid populations may exhibit a greater ability to tolerate cool winter temperatures and dry conditions, or be more aggressive reproductively. These characteristics could help the parasitoids become established and make them more effective as control agents. The new parasitoid stock will likely increase the genetic variability of the already established *G. morrilli* and *G. ashmeadi* populations, which could lead to improved biological control.

CDFA has also made more than 90 separate recoveries of egg masses parasitized by *G. triguttatus* or *G. fasciatus* at 23 sites in seven Californian counties, suggesting that these control agents are becoming established.

Biological control is a long-term strategy for the suppression of GWSS in areas where it has already become established. To this end, CDFA has established two facilities based in Riverside and Kern counties to mass produce, release and monitor introduced biological control agents.



Photos: Jack Kelly Clark

◀ **Parasitoids can aid in control of the glassy-winged sharpshooter. *Clockwise, from upper left: Gonatocerus ashmeadi* is widespread in California and was most likely self-introduced from the southeastern United States and northeastern Mexico; *G. triguttatus* is a subtropical and tropical species introduced into California from Mexico and southeastern Texas; *G. morrilli*, native to Southern California, is having its genetic pool augmented with conspecifics collected from other U.S. regions; *G. fasciatus* is native to the southeast and Midwest and has gregarious reproductive habits.**

USDA-ARS and CDFA have also been evaluating the safety of mymarid parasitoids imported from Argentina for possible use against GWSS in California. Quarantine work at UC Riverside indicates that Argentinean parasitoids will readily attack GWSS even though they did not evolve in the native range of GWSS. These evaluations are ongoing and no releases have been made.

Nontarget impact of parasitoids

Biological control has come under increased scrutiny because there is some evidence that under certain circumstances natural enemies released for the control of a pest species may attack nontarget species and adversely affect the populations of these organisms (Hoddle 2004b). To minimize such unwanted environmental effects, UC Riverside scientists have studied native sharpshooters to see if they are vulnerable to attack by parasitoids native to the southeastern United States, northeastern Mexico and Argentina. While none of these native sharpshooters is beneficial in the sense that they predate or parasitize insect pests, their demise would likely trigger a cascade of nutritional effects that could adversely affect other native species and the food web on which they all depend.

UC and CDFA scientists screened all introduced GWSS biological control agents for their ability to parasitize closely related nontarget species of Homoptera found in the area of introduction. These include the southeastern species *H. insolita* (Walker) and the southwestern species *H. liturata* (both Proconiine sharpshooters); three sharpshooters of the Cicadellini tribe, *Colladonus montanus* (Van Duzee) (Cherry mountain leafhopper), *Graphocephala atropunctata* (Signoret) (blue-green sharpshooter) and *Xyphon fulgida* (Nottingham) (red-headed sharpshooter); and other species of leafhoppers from a different subfamily, *Euscelidius variegatus* (Kirschbaum), and *Macrosteles fascifrons* (Stål) (Aster leafhopper). To date, the only nontarget species susceptible to the introduced agents, as determined by UC Riverside scientists, is *H. liturata*, a species that is implicated in *X. fastidiosa* transmission in agriculture in California and the southwestern United States, but that exists primarily in desert habitats where parasitism by introduced mymarid wasps is often low. The susceptible *H. liturata* is from the same tribe (Proconiini) and genus *(Homalodisca)* as GWSS, and is similar to the GWSS in its egg-laying and generalist plant-feeding habits. As such, it is expected to be utilized by introduced *Gonatocerus* spp.

Scientists at UC Riverside are investigating three other common native sharpshooters (Cicadellinae). Preliminary observations have revealed that the GWSS parasitoids may affect their populations. However, the habitats occupied by these common native sharpshooters have less overlap with the GWSS, and they are from different tribes. The habitats of the Cicadelline sharpshooters *(X. fulgida)* and the green sharpshooter (*Draeculocephala minerva* Ball) both consist of grasses such as Bermuda and Johnson grass (*Cynodon dactylon* [L.] and *Sorghum halapense* [L.] respectively), making them improbable hosts for *Gonatocerus* spp. The blue-green sharpshooter *(G. atropunctata)*, also of the Cicadellini tribe, prefers humid, partially shaded and densely vegetated habitats. This sharpshooter is often found in coastal or riparian habitats consisting of trees, vines and succulent shrubs. These unlikely foraging areas, combined with the differing tribal origins of the sharpshooter and the absence of any records indicating *Gonatocerus* emergence from any egg masses may make this sharpshooter an improbable alternate host for the three *Gonatocerus* parasitoids.

Additionally, sticky-card traps in the Southern California habitats occupied by *G. atropunctata* have yielded no capture of the widespread and established parasitoid *G. ashmeadi* (Boyd, unpublished data). Native sharpshooter eggs are approximately one-half the size

Parasitoid exit holes on a citrus leaf indicate that adults successfully chewed through the egg casing and leaf epidermis to emerge.

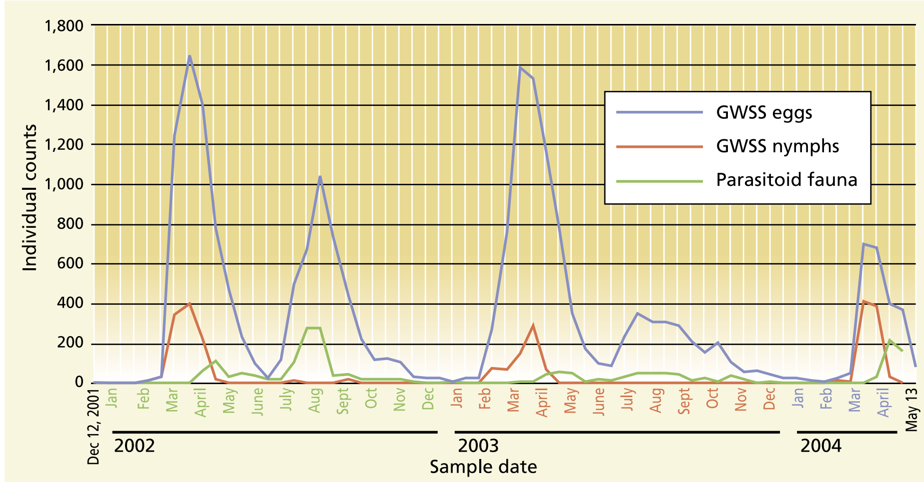


Fig. 2. Population data on GWSS nymphs, adults and parasitoids gathered from citrus orchards at UC Riverside. This information is invaluable in assisting our understanding of pest and natural enemy phenology and population abundance over time.

of GWSS eggs, are laid singly, and are embedded into the stem material rather than in groups just below the epidermal layer as is characteristic of GWSS egg masses (Boyd, unpublished data). These characteristics, coupled with the different taxonomic placement, make the blue-green sharpshooter an unlikely host for any of the GWSS parasitoids.

GWSS and natural enemies

UC Riverside scientists working in Southern California and USDA scientists working in eastern Texas collected phenological data on GWSS and *G. ashmeadi* populations for 2 years. (Phenological data is information on populations with relation to the climate and time of year.) In Southern California, GWSS exhibits two distinct population peaks (fig. 2), the first in the spring when an average of 12% of GWSS eggs were parasitized, and the second in the summer when an average of 19% of eggs were parasitized. This summer figure contrasts with reported parasitism rates of up to 100% in some regions, such as Florida (Triapitsyn and Phillips 2000). A possible explanation for this discrepancy in parasitism is that data being collected are “snapshots” from any given season. For example, the data collected from citrus grown at UC Riverside Agricultural Operations often reflects nearly 100% parasitism rates in individual sampling events, but this level of oviposition is generally associated with low GWSS egg numbers and is not reflected in the overall mean parasitism rates for any one season. Of the sharpshooter egg masses surveyed in Riverside, 17% had at least one egg parasitized by *Gonatocerus* spp. in the spring, compared to 30% in the summer.

In Weslaco, Texas, USDA researchers observed summer parasitism rates ranging from 38% to 100% of discovered GWSS egg masses with at least one egg parasitized (*G. triguttatus* is the key natural enemy in this area). The Texas data demonstrates that GWSS populations might be successfully suppressed if efficacious egg parasitoids are successfully established in California. However, given that GWSS is such an effective vector of the *X. fastidiosa* pathogen, even highly successful suppression of GWSS may lead to populations that remain above acceptable levels for vineyard managers. Regional lowering of GWSS populations will, however, assist greatly in all control efforts and management programs to curtail its spread in agriculture and the urban environment.

Parasitoid biology

Mymarid wasp parasitoids that attack GWSS eggs are small, approximately 0.02 to 0.06 inches (0.5 to 1.5 millimeters). Parasitoid larvae pupate within GWSS eggs and then chew circular holes, through which they emerge in search of mates and new host eggs to attack. *G. ashmeadi*, *G. morrilli* and *G. triguttatus* are solitary endoparasitoids that lay one egg per GWSS egg within an egg mass, and the developing larva feeds within this host egg thereby killing it. In contrast and possibly due to its relatively smaller size, *G. fasciatus* is gregarious, and individual females deposit more than one egg per GWSS egg, yielding multiple parasitoids per host egg (Triapitsyn et al. 2003).

Researchers at UC Riverside have shown that the density of female parasitoids searching for hosts has a significant effect on the sex ratio of progeny produced (Irvin and Hoddle 2005b). When female *Gonatocerus* parasitoids fail to encounter other ovipositing females of the same species on a GWSS egg mass, progeny output is strongly female-biased. Laboratory experiments indicate that when the female does not encounter a competitor while ovipositing, the ratio of males to females produced is approximately 1 to 8, 1 to 14 and 1 to 9 for *G. ashmeadi*, *G. triguttatus* and *G. fasciatus*, respectively. However, increasing the number of ovipositing females of the same species from one to two per egg mass reduces the female offspring produced by up to 15% for all three *Gonatocerus* species. These results suggest that local mate competition affects progeny production, and more males are produced when females encounter other females of the same species that are producing daughters with whom their sons might mate.

Laboratory studies where each parasitoid was presented with eggs of

Almond leaf scorch, *left* and *center*, is a relatively slow-spreading disease that may take many years to establish in cropping systems. *Right,* oleander leaf scorch is causing millions of dollars of damage to California's freeway median strips and other ornamental plantings.

a single age showed that progeny production was greatest from GWSS eggs 3, 4 and 2 days old for *G. ashmeadi*, *G. triguttatus* and *G. fasciatus*, respectively. Furthermore, each parasitoid species was able to utilize a range of egg ages around their most preferred age: 1 to 4, 3 to 6, and 1 to 3 days old for *G. ashmeadi*, *G. triguttatus* and *G. fasciatus*, respectively. GWSS eggs that were parasitized at 8 to 10 days of age produced few parasitoid progeny and those that did emerge had been oviposited into sterile or dead host eggs lacking a GWSS embryo (Irvin and Hoddle 2005a). This suggests that the low production of parasitoid progeny in older GWSS eggs was most likely due to the advanced stage of development of the GWSS embryos.

No oviposition preferences were observed when GWSS eggs 1, 3 and 5 days old were presented simultaneously to *G. ashmeadi* and *G. triguttatus*. This suggests that these two parasitoids will attack host eggs without preference as long as eggs are of a suitable age for oviposition. These choice studies indicated that *G. fasciatus* preferred GWSS eggs 1 and 3 days old, while eggs 5 days old were not utilized. The small size of *G. fasciatus* in comparison to *G. ashmeadi* and *G. fasciatus* possibly limits the range of GWSS egg ages available for parasitism. This may occur because the smaller ovipositor of *G. fasciatus* may be unable to pierce the chorion of older eggs as they harden during maturation.

Field survival and parasitism

Most agricultural environments are unfavorable habitats for natural enemies. Herbicides can remove shelter and floral resources that biological control agents depend on, and pesticide residues can kill biological control agents (Gurr et al. 2003). UC Riverside scientists have demonstrated that compared to plain water, providing honey-water and flowers of buckwheat (*Fagopyrum esculentum* Moench) significantly increased the longevity of both male and female *G. ashmeadi*, *G. triguttatus* and *G. fasciatus* up to 94.6%, 92.4% and 93.1%, respectively. These results indicate that resource procurement may be extremely important for enhancing parasitoid survival in agroecosystems. However, this assertion is speculative until extensive field experimentation is undertaken. Since resource procurement increases the longevity of female parasitoids, this may enhance biological control of the GWSS because females that live longer may encounter more GWSS egg masses and consequently parasitize more eggs.

UC Riverside scientists have also shown that the longevity of *G. ashmeadi*, *G. triguttatus* and *G. fasciatus* feeding on citrus flowers and GWSS excrement was equivalent to that on water. This indicates that these field resources may not supply parasitoids with adequate nutrition to maximize survival. One potential way to enhance parasitoid populations, and consequently increase the efficacy of pest control by natural enemies in agricultural systems, is understory management or the deliberate management of flowering plants beneath orchards and vineyards (Landis et al. 2000). Sowing flowering plants (such as buckwheat, dill [*Anethum graveolens* L.] or alyssum [*Lobularia maritima* L.]) as an understory could potentially provide a food source to *Gonatocerus* spp. and increase GWSS biological control. The UC Riverside researchers found that survival times for both sexes of *G. ashmeadi*, *G. triguttatus* and *G. fasciatus* increased by up to 85.2% with softscale (*Coccus hesperidum* L.) excrement than with citrus foliage alone, suggesting that in citrus orchards nondamaging levels of softscale may also be beneficial for enhancing parasitoid survival and could enhance biological control of GWSS.

Invasive potential of GWSS

GWSS has shown strong invasive potential, having become established in a variety of places outside its native range, including California, French Polynesia and Hawaii. Modeling that combines regional climate data and relevant biological information indicates that California's premier wine-growing areas of Napa, Sonoma and Mendocino counties are vulnerable to invasion by GWSS (Hoddle 2004a). In contrast, states north of California, which also have substantial grape industries, may be too cold to harbor permanent populations. Other climates conducive to GWSS invasion — should it be accidentally introduced — include the major wine-growing regions of New Zealand, Australia, the Bordeaux region of France, most areas of Spain, and central and southern Italy (Hoddle 2004a).

Data on GWSS in Tahiti is sobering. Populations of this pest grew exponentially because there is an abundance of suitable native and exotic host plants; the mild climate permits year-round breeding (in contrast to California, where there are just two generations, spring and summer, each year); and natural enemies are lacking, while no obvious competitors exist in urban or natural settings. Naturally occurring parasitism of GWSS eggs is very low on the French Polynesian island of Mo'orea. UC surveys indicated that less than 2% of total GWSS eggs were attacked by parasitoids (table 1).

TABLE 1. GWSS egg-mass survival, Sept. 3–9, 2003, Mo'orea, French Polynesia

GWSS eggs examined	GWSS eggs parasitized	GWSS eggs eaten	GWSS that died of natural causes*	GWSS eggs from which nymphs emerged
		no.		
2,586 (246 egg masses studied)	32	444	50	2,060
% of eggs	1.24	17.17	1.93	80

* Bacterial and/or fungal infection.

The wasp that attacked these GWSS egg masses is a platygasterid, a family that does not specialize on sharpshooters but will parasitize various species of leafhoppers. The data on parasitism in Tahiti indicates that there are no specialized parasitoids attacking GWSS. Only a few eggs in an egg mass are attacked, indicating inefficient and opportunistic exploitation, and only males were reared from GWSS eggs, suggesting poor host quality because females did not oviposit fertilized eggs that yield female offspring. This data clearly indicates that GWSS populations in French Polynesia are free of the pressures associated with natural enemies. A classical biological control initiative against GWSS has been launched and is a cooperative enterprise between UC Riverside, UC Berkeley and the French Polynesian government.

Natural enemies and IPM

GWSS is a major pest in California because of its ability to vector *Xylella*, and the invasive potential of this pest has been clearly demonstrated by its establishment in French Polynesia, Hawaii and Easter Island. In all invaded areas, biological control with host-specific mymarid egg parasitoids appears to be the only feasible control strategy for providing long-term, area-wide suppression of this pest. This is a challenging problem because, in agriculturally dependent areas such as California, even excellent biological control of GWSS may not be sufficient to reduce substantial crop losses caused by *Xylella* vectored by GWSS. In this instance, natural enemies must form the cornerstone of integrated pest management programs for GWSS.

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Imported parasitic wasp helps control red gum lerp psyllid

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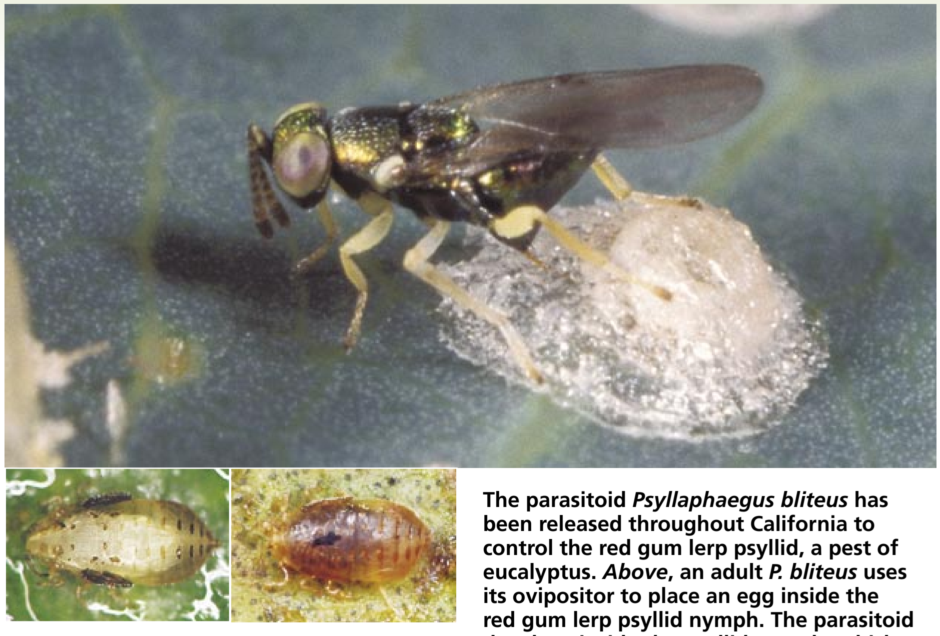

The parasitoid *Psyllaphaegus bliteus* has been released throughout California to control the red gum lerp psyllid, a pest of eucalyptus. *Above*, an adult *P. bliteus* uses its ovipositor to place an egg inside the red gum lerp psyllid nymph. The parasitoid develops inside the psyllid nymph, which typically does not show any signs of parasitism until the nymph reaches the fifth instar, when the parasitoid pupa — *far left*, white body, and *left*, dark body — can be seen through the mummified psyllid.

The red gum lerp psyllid is an insect native to Australia, where it feeds upon eucalyptus species. Since 1998 this psyllid has spread throughout California, resulting in millions of dollars in damage and control costs. To help suppress the red gum lerp psyllid, a biological control program was initiated and a psyllid-specific parasitic wasp was imported from Australia in 1999 and released in 2000. In most coastal regions this biological control agent has provided substantial control, but in some interior regions the psyllid still remains a problem. Researchers are continuing their investigations to determine if full statewide suppression will be realized eventually, or if further importation of new parasitoid species is needed.

Eucalyptus trees and shrubs, valued for their ability to flourish in arid regions and their varied horticultural uses, have been a familiar feature of California's urban and rural landscapes since they were first introduced from their native Australia more than 150 years ago. Until recently, eucalyptus trees in California were relatively free from damaging insect pests. Most of California's native insects cannot feed on eucalyptus, which is well protected from herbivores by chemicals such as distasteful essential oils (which are familiar to anyone who has smelled the strong odor of the leaves). The Australian insects that have adapted to feed on eucalyptus were not transported to California with earlier shipments of plant propagation material, usually in the form of seeds. This began to change in the early 1980s and at least 15 eucalyptus-feeding insect species from Australia were accidentally introduced and are now established in California (Paine and Millar 2002). While eucalyptus trees may be unwanted in some areas because they crowd out native vegetation, their extensive value in many other locations led to efforts to control the psyllid.

River red gum *(Eucalyptus camaldulensis)* is among the most commonly planted shade and windbreak trees in California and is also grown commercially for fuel wood and fiber (Cockerham 2004). The red gum lerp psyllid (*Glycaspsis brimblecombei* Moore; Hemiptera: Psylloidea) was first found on river red gum in June 1998 in Los Angeles County and had spread throughout the state by 2000, and throughout Mexico and parts of Florida by 2002. In Australia there are a number of eucalyptus species that the red gum lerp psyllid can feed on, but in California the only favored eucalyptus species present is the river red gum; the forest red gum *(E. tereticornis)* and flooded gum *(E. rudis)*, both also in California, are less-favored trees that the psyllid can feed on as well.

Red gum lerp psyllid nymphs build white conical shelters called lerps from excreted honeydew and waxes, and live underneath these structures. The nymphs feed by sucking plant sap from leaves. The accumulation of the sticky lerps and honeydew on leaves and under infested trees creates a nuisance, while heavy infestations lead to defoliation, branch dieback and occasionally tree death (Paine et al. 2000).

The first attempts to control red gum lerp psyllid focused on the use of

▼ ***Below*, life stages of the red gum lerp psyllid include, (left to right) large nymph, row of eggs, winged adult and small lerp (the protective covering produced by nymphs).**

◀ ***Clockwise from top left:* Karen Sime, UC Berkeley postdoctorate researcher, checks a caged eucalyptus leaf for evidence of *P. bliteus* activity and parasitism rates; the late Don Dahlsten (first author) was a leading UC researcher (1966–2003) in biological control of urban and forest pests (shown in 1974); small discs coated with a light oil were used to capture and sample populations of adult red gum lerp psyllid and *P. bliteus;* red gum lerp psyllids feed on eucalyptus leaves, building up to such high densities that the accumulation of psyllids and honeydew causes sooty molds, defoliation and even tree death; in the San Joaquin Valley (Tulare County), a dead red gum eucalyptus (left) near an undamaged and uninfested blue gum eucalyptus (right) demonstrates the psyllid's feeding preferences.**

systemic insecticides, mainly to target heavy infestations on particularly valuable trees. The proper timing of treatments was difficult to determine and control was not always achieved (Paine et al. 2000). The obvious impracticality of using insecticide treatments on trees throughout the state led us to investigate more sustainable options. We first investigated whether any predators already present in California could provide control. Lady beetles *(Hippodamia)*, green lacewings *(Chrysoperla)*, minute pirate bugs *(Orius)* and syrphid flies feed on adult and immature red gum lerp psyllids (Erbilgin et al. 2004). However, even when present in large numbers, these predators did not provide adequate control.

In Australia, red gum lerp psyllid populations are held in check in large part by species of parasitic wasps that specifically attack them and their close relatives. Other parasitoid species imported from Australia had successfully controlled earlier outbreaks of other introduced Australian psyllid species in California, including the blue gum psyllid (Dahlsten et al. 1998) and the eugenia psyllid (Dahlsten et al. 1995). Classical biological control appeared, therefore, to be the most promising approach for controlling the red gum lerp psyllid. We report here on a large collaborative effort between UC, the California Department of Food and Agriculture (CDFA) and research scientists in Australia.

Prerelease psyllid sampling

Before the biological control program began, we gathered detailed information about red gum lerp psyllid populations throughout California. Beginning in July 1999, sample sites were established in Alameda, Santa Clara, Monterey, Los Angeles and San Diego counties. By July 2002, we had established 32 sample sites, with at least one site located in every California county in which red gum lerp psyllid had been reported.

Sampling psyllid populations accurately can be difficult. The most accurate way of measuring densities and damage levels is to count psyllid nymphs on leaves. This method, however, is time-consuming and impractical for the large number of sample sites and frequent sampling dates needed to follow psyllid and natural-enemy population dynamics throughout the state. We therefore investigated whether red gum lerp psyllid populations could be tracked with sticky traps, which had been used for the blue gum and eugenia psyllid programs (Dahlsten et al. 1998). The traps consisted of transparent 4-inch (10-centimeter) plastic disks coated with a thin layer of motor oil additive and clipped over a yellow backing. At each site, 10 to 12 traps were hung in eucalyptus trees and changed weekly.

In 1999 and 2000, we tested trap accuracy at two sites, one in Northern California (Alameda County) and the other in Southern California (Los Angeles County). Near each trap at these sites, two 12-inch (30-centimeter) foliage samples were collected every 3 weeks (20 to 24 samples per site per sample date). We found a good correlation between the mean number of adult female psyllids per sticky trap and the mean number of psyllid eggs per leaf sample ($P < 0.01$, $r^2 = 0.82$; Paine et al. [2000]), indicating that the sticky-trap counts provided a good estimate of psyllid activity in eucalyptus. Thereafter, we relied exclusively on the

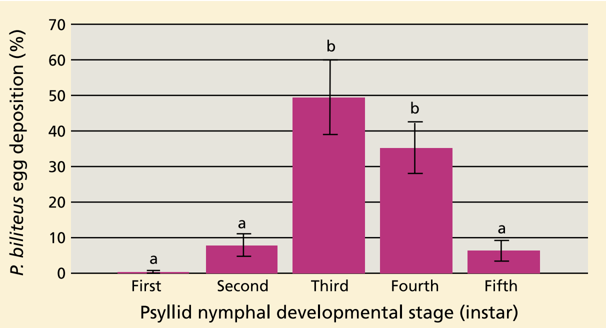

Fig. 1. *P. bliteus* oviposition success in different host developmental stages, as indicated by the percentage egg deposition (± SEM) of parasitized red gum lerp psyllids, was significantly different (F = 12.48, df = 4,25, *P* < 0.001). Above each bar, means followed by different letters are significantly different (Tukey's HSD test, *P* < 0.05). Source: Daane et al. (2005).

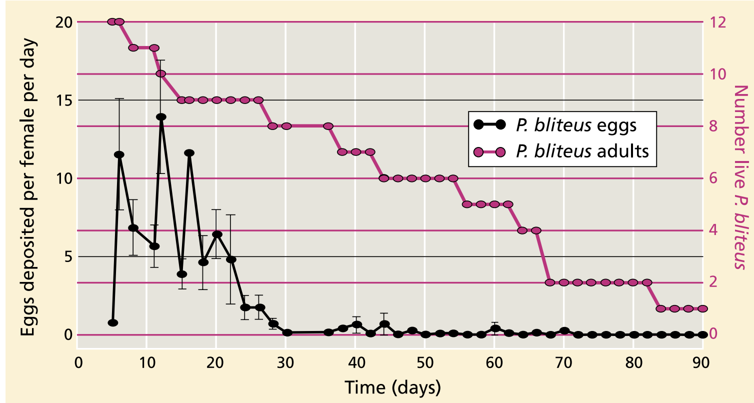

Fig. 2. *P. bliteus* lifetime fecundity under glasshouse conditions, as estimated by egg deposition with an overabundant host supply. Source: Daane et al. (2005).

sticky-trap technique to monitor the psyllids. In addition, we used the same traps to monitor parasitoid populations after we began releasing them.

Initially, we counted both male and female adult psyllids per trap by sample week. However, we noticed strong seasonal changes in the relative proportions of adult females and males. For example, female psyllids typically had the highest populations in the summer months and dropped significantly the rest of the year. Because their numbers are most closely associated with egg-laying (and thus nymphal activity and damage), we plotted only the adult female psyllids caught on the traps. At each monitoring site, we reported the averages of 10 to 12 traps.

Finding, importing parasitoids

To find promising parasitoids, mummified red gum lerp psyllids were field-collected and shipped from southern Australia to the UC Berkeley Quarantine Facility in August 1999 (the "mummified" psyllid is visibly dead, killed by the internal parasitoid that is nearing completion of its development). This region, near Adelaide, has a Mediterranean climate, with temperatures similar to California's coastal regions. From the field-collected psyllids, we reared eight species of parasitoids in the genus *Psyllaephagus* (Hymenoptera: Encyrtidae). Of these, two were hyperparasitoids (which attacked the beneficial "primary" parasitoids) that attacked the other *Psyllaephagus* species, and most others failed to propagate in the insectary. The remaining species (*Psyllaephagus bliteus* Riek) did well in culture and was selected for release after experiments showed that it specifically attacked the red gum lerp psyllid when tested against three other psyllid species (Eugenia, blue gum and melaleuca) (Paine et al. 2000).

Parasitoid biology. To facilitate insectary rearing and field release, we collected basic biological information on *P. bliteus* (Daane et al. 2005). First, we determined which stages of the host (red gum lerp psyllid) were preferred by the female parasitoids *(P. bliteus)* for oviposition. Potted eucalyptus trees were infested with 300 to 500 psyllid nymphs, with the population composed of all five nymphal stages in similar proportions, and isolated in organdy sleeve cages with 15 to 20 adult female *P. bliteus*. After 24 hours, all psyllids were collected and cleared in chloralphenol, which makes any *P. bliteus* eggs inside the psyllid body visible under a dissecting microscope. The number of eggs and the psyllid stages were recorded. We also used similar methods to investigate *P. bliteus* larval development.

We collected the psyllids every 3 to 4 days after exposure to female wasps, cleared them in chloralphenol, and then recorded both the presence of *P. bliteus* eggs or larvae and the developmental stages of parasitized psyllids. There were five host preference and four larval development replicates; the treatment means were separated using Tukey's HSD test. Our results showed that *P. bliteus* can oviposit into psyllid nymphs of any age, but that they usually parasitize third and fourth instars (fig. 1). In our studies, regardless of the stage of the host exposed to *P. bliteus* for oviposition, the parasite larvae did not fully develop until after the host reached the fifth (last) instar. In addition, adult female wasps also occasionally killed psyllid nymphs by host feeding, stabbing them with their ovipositors and drinking the body fluids leaking out from under the lerp. Usually younger nymphs are attacked but we observed this host feeding on all stages.

Longevity and fecundity. Adult *P. bliteus* longevity and fecundity were also determined. Newly emerged and mated female *P. bliteus* were individually isolated in clear plastic tubes that each enclosed a single infested leaf on a potted river red gum tree in the glasshouse (71.6 ± 3°F). Each leaf was infested with 10 to 30 psyllids, mostly third instars. Each female *P. bliteus* was transferred to a new leaf every 2 days throughout her lifetime. After each transfer, the parasitoid-exposed psyllids were cleared in chloralphenol, and the presence of *P. bliteus* eggs was recorded. Under these conditions, we found that average female *P. bliteus* longevity was 60.4 ± 6.4 days and average lifetime egg deposition was 125.7 ± 24.6 eggs per female (range 34 to 302). Most eggs (88.1%) were deposited during the initial 22 days, although one parasitoid deposited eggs up to 70 days after emergence (fig. 2).

These results have implications for insectary operations and release strategies in classical biological control programs. Although adults may survive for long periods, most egg deposition occurs early in the adult's lifetime. Insectary colonies should therefore be supplied with the needed number of

TABLE 1. *P. bliteus* releases by California county, 2000 to 2003, show the widespread collaborative effort to release and establish the imported red gum lerp psyllid parasitoid

County	Sites	Releases	Release period	Released
	no.	*no.*		*no.*
Alameda	2	5	June 2000–Aug 2001	571
Amador	1	2	Aug 2002	802
Butte	1	1	July 2002	735
Calaveras	1	1	June 2002	1,047
Colusa	1	1	July 2002	408
Contra Costa	1	1	May 2002	654
Fresno	1	2	Nov 2000, Aug 2001	473
Glenn	1	1	Sep 2001	569
Imperial	2	1	June 2002	998
Kern	1	1	July 2002	245
Kings	1	1	May 2002	522
Lake	1	1	Sep 2002	625
Los Angeles	8	15	June 2000–June 2002	4,016
Madera	1	1	June 2002	752
Marin	1	1	May 2002	571
Mariposa	2	2	Aug 2002, Jan 2003	728
Merced	1	1	Nov 2001	933
Monterey	2	2	Dec 2000, Sep 2001	518
Napa	1	1	Apr 2002	650
Orange	3	4	Nov 2000, Dec 2001	1,846
Placer	2	2	July, Sep 2002	1,514
Riverside	6	7	Nov 2001–Mar 2002	3,910
Sacramento	4	8	Oct 2000–June 2002	3,070
San Benito	1	1	Aug 2002	587
San Bernardino	5	5	Oct 2001–Mar 2002	3,775
San Diego	3	8	Sep 2000–May 2002	1,914
San Joaquin	2	2	Apr, Sep 2002	1,070
San Luis Obispo	1	3	Sep 2001–Jan 2002	2,863
Santa Barbara	2	2	Feb, July 2001	217
Santa Clara	2	1	Sep 2000	50
Santa Cruz	1	1	Aug 2002	610
Shasta	3	2	June 2002	2,002
Solano	1	2	Sep 2001, July 2002	1,405
Sonoma	1	2	Sep, Oct 2001	1,309
Stanislaus	1	1	May 2002	836
Sutter	2	2	May, June 2002	1,465
Tehama	2	2	Oct 2000, June 2002	1,048
Tuolumne	2	2	Aug, Oct 2002	875
Tulare	1	1	June 2002	800
Ventura	1	1	Sep 2000	71
Yolo	1	1	June 2002	573
Yuba	1	1	June 2002	955
Totals:	78	102		48,582

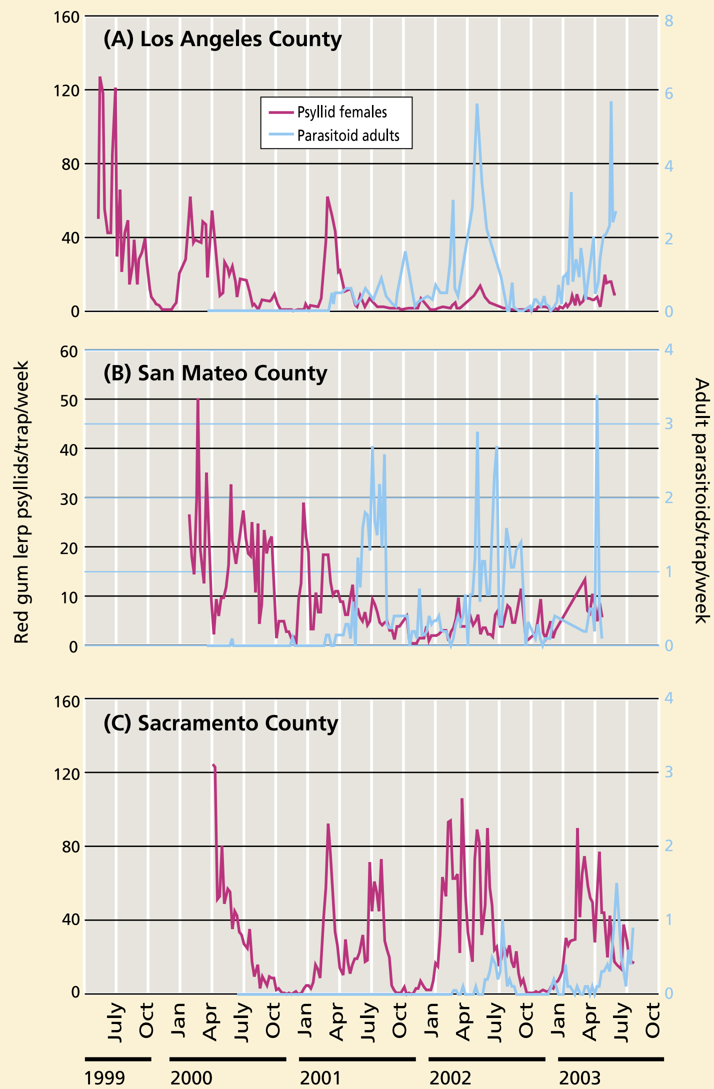

Fig. 3. Red gum lerp psyllid adult females and *P. bliteus* parasitoid adults (both mean per trap per week) and parasitoid release dates in a single site each in (A) eastern Los Angeles County, (B) San Mateo County (parasitoids originally released in nearby counties) and (C) southern Sacramento County.

third- or fourth-instar psyllids for an oviposition period of 2 to 3 weeks. In addition, first or second instars should also be provided because they are used for host feeding. Finally, the parasitic wasps can be released to the field shortly after emergence and mating, as high rates of egg deposition begin immediately.

Parasitoid release and impact

For field release, most parasitoids were reared at the CDFA Biological Control Facility (Sacramento), with smaller numbers reared at the UC Berkeley Insectary and Quarantine Facility. Release of *P. bliteus* began in June 2000 in Los Angeles and Alameda counties. Between September 2000 and January 2003, we released a total of 48,582 adults in 78 release sites located in 42 counties throughout the state (1,156 ± 154 per county, range 50 to 4,016) (table 1).

Parasitoids were recovered in sticky traps as early as 8 weeks after initial release. Recovery in traps occurred earliest in the Central Coast sites, followed by the Southern California, North Coast and Central Valley sites (table 2). Psyllid densities typically peaked between August and October, and these peak periods were used to compare annual changes at each site. Eastern Los Angeles County is an example of a site with a marked decrease in psyllids and an increase in parasitoids (fig. 3A). Peak psyllid counts dropped from more than 100 in 1999 (before the wasp release) to fewer than 20 females per trap per week in 2003 (after the second wasp release). During the same period, trap catches of *P. bliteus* increased for 3 years after the initial release in June 2000 and then showed steady seasonal cycles. A similar pattern of psyllid decrease and parasitoid increase was found farther north, in San Mateo County (parasitoids were originally released in nearby counties) (fig. 3B). In contrast, *P. bliteus* has to date had less effect on psyllid densi-

TABLE 2. Average number of days (± standard error) between initial *P. bliteus* release and recovery in traps in four California regions (see fig. 4)

Region	Sites	Days ± SE
Central Valley	2	623 ± 28.0
North Coast	5	302 ± 59.7
Central Coast	5	156 ± 56.6
Southern California	13	252 ± 32.9

ties in some interior sites. For example, 3 years after parasitoid releases at one Sacramento County location, parasitoid levels were relatively low and psyllid numbers remained high (fig. 3C).

To summarize the changes in psyllid density across all 32 monitored sites, we compared the average peak densities of psyllids per trap in 2003 to those in years before the parasitoid was established (1999 to 2001). The results of this comparison, grouped by region, indicate a postrelease drop in peak psyllid densities of 78.6%, 59.5% and 44.8% in the southern, central and northern coastal regions, respectively. There was no change in peak psyllid densities in the Central Valley sites. The postrelease rate of increased parasitoid activity was estimated by calculating the average time from the release to a 50% or more decrease in peak psyllid density on the monitoring traps. At most coastal sites (18 out of 23) the average time to a 50% decrease in psyllid density was 13.2 ± 1.2 months after the initial parasitoid release. By comparison, at the nine Central Valley sites monitored, there was little or no detectable effect of *P. bliteus* on the psyllid population densities during the 2001 to 2003 sampling period.

Coastal versus interior sites

The differences in parasitoid effectiveness between coastal and interior sites were most likely due to the great climatic differences between the two regions. The coastal region has relatively mild summer and winter temperatures, while the interior valleys have much cooler winters and warmer summers. To date, the Central Valley sites lag behind the coastal sites in two measures of parasitoid impact: the time to establishment of parasitoid populations and the overall impact of the parasitoids on psyllid populations. To account for these discrepancies, we compared the performance of the parasitoids at coastal and interior sites that were paired by latitude using three measures: (1) parasitism rates, (2) the ability of a single wasp to parasitize psyllids and (3) the longevity of adult female wasps outdoors.

In the first study, three pairs of coastal and interior monitoring sites were sampled in July 2003: (1) Sonoma and Sacramento counties, (2) Alameda and Fresno counties and (3) San Luis Obispo and Bakersfield counties. Two 11.8-inch (30-centimeter) branch tips were taken from each site and up to 50 nymphs from each sample were dissected to check for parasitism. We found that the average parasitism rate at coastal sites (Sonoma, Alameda and San Luis Obispo counties) (29.7% ± 6.6%) was significantly higher than the average parasitism rate (1.2 ± 0.9%) at the interior sites (Sacramento, Fresno and Kern counties) (*t*-test, $P < 0.05$, n = 8).

In a second study, three to four mesh sleeve cages were fixed to river red gums at two paired sites (Alameda and Sacramento counties) in July 2003. These sites represented the coastal and interior temperature regimes at the same latitude; mean average and high temperatures for the cities of Berkeley (Alameda County) and Sacramento (Sacramento County), where the study was conducted, are 63°F and 71°F, and 74°F and 91°F, respectively. Approximately 100 psyllid eggs were placed in each cage, and the resulting nymphs then settled on leaves and began feeding. When the psyllids had reached the third instar, a single female wasp was released into each cage. The cages were removed from the field 2 weeks later and all psyllids inside were dissected to check for parasitism. Average parasitism rates in the cages were far higher at the Berkeley site (34.1% ± 9.31%) than at the Sacramento site (1.0% ± 1.0%) (2-tailed *t*-test, df = 14, $t = 2.145$, $P = 0.003$).

In a third temperature study, we compared the longevity of adult female parasitoids held in glass vials at the sites in Berkeley and Sacramento during summer 2004. During a period in June when temperatures were cool in Berkeley but warm in Sacramento, the wasps lived significantly longer in Berkeley (14.9 ± 2.4 days versus 5.8 ± 0.7 days) (2-tailed *t*-test, df = 3, unpaired $t = 3.182$, $P = 0.037$). During the July and September test periods, temperatures were similar at the two sites (with unusually cool weather in Sacramento in that part of July) and the wasps' longevity was similar at both sites (averaging 12 to 15 days).

The wasps performed poorly in the Central Valley compared to coastal areas. Parasitism rates were lower overall in the field, and individual wasps laid fewer eggs. A possible explanation for these differences is the shorter lifespan of the wasps in the summer heat. In our experiments on the basic biology of the parasitoids, we found that they laid eggs for several weeks in the greenhouse, which has mild, cool conditions similar to the ambient conditions in Berkeley in the summer. However, in warmer conditions, the wasps did not live as long and therefore laid fewer eggs over their lifetimes. The relatively poor performance of the wasps in the summer in the Central Valley, when psyllid numbers build up to their seasonal peaks, helps explain why we have observed longer times for wasp establishment in the interior and, to date, less impact on psyllid populations.

The red gum lerp psyllid now appears to be under control in most coastal regions of California due to the introduction of a parasitic wasp.

Parasitoid impact

By 2003, *P. bliteus* had been recovered at 29 of the 32 sites monitored throughout the season with sticky traps. Field-produced parasitoids far outnumbered insectary production and, for this reason, managed releases were discontinued. To provide a geographically comprehensive summary of parasitoid establishment, we surveyed 55 former release sites throughout California from mid-August through October

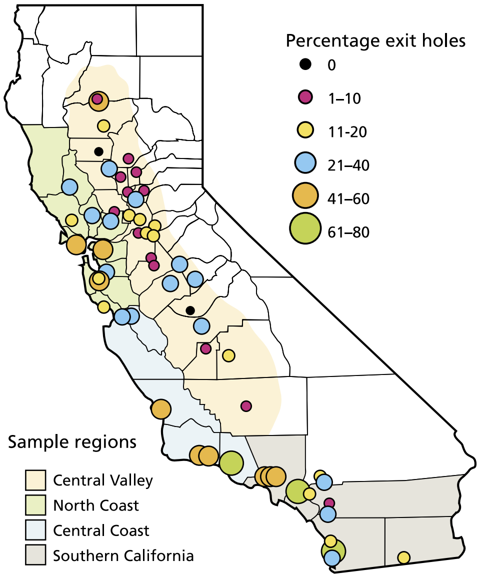

Fig. 4. Percentage of large lerps with parasitoid emergence exit holes during fall survey, 2003 (Roltsch et al. 2004). California county borders demark the four regional sampling zones (except for the Central Valley).

2003 (Roltsch et al. 2004). At each site, 15 branch terminals (11.8 to 15.7 inches long) were randomly collected from three or more trees. On each branch, 30 leaves were randomly selected and the numbers of exit holes (round holes that the adult parasitoid chews in the lerp when it exits), as well as healthy and visibly parasitized psyllids, were recorded by life stage.

Parasitoids were recovered at all but two of 55 locations (fig. 4). At several locations, levels of parasitoid activity, as indicated by the proportion of large lerps containing exit holes, were strikingly higher than those found in 2002, several months after the initial parasitoid releases, using similar survey techniques. For example, while *P. bliteus* was extremely rare at the Solano County site in 2002 (after two releases prior to this sampling), in October 2003 the parasitoid was common there. On average over all sites sampled, there were parasitoid exit holes in more than one-fifth (22%) of the large lerps. We note that while exit holes in the lerps provide an indication of parasitoid activity, this may not be as accurate as dissecting live psyllids to determine the percentage that are parasitized. Still, the survey confirms that the released *P. bliteus* have established in almost all regions and that parasitoid activity appears to be increasing annually.

Future biocontrol programs

The red gum lerp psyllid now appears to be under control in most coastal regions of California due to the introduction of *P. bliteus*. Suppression is best at coastal sites and lower in some parts of the Central Valley. Our field and laboratory studies suggest that the hot summer temperatures found in the interior regions may reduce parasitoid impact. Still, as psyllid numbers have dropped, the defoliation and death of eucalyptus trees due to the psyllid have been reduced. Of key importance for future control efforts is the observation that *P. bliteus* appears to be well established throughout California, including the interior locations. Therefore, although current *P. bliteus* densities and parasitism rates are low in the interior, their impact may continue to increase there, albeit more slowly than in coastal regions. We will continue to monitor the red gum lerp psyllid and the parasitoid populations to determine if it will be necessary to import either heat-adapted *P. bliteus* populations or additional *Psyllaephagus* species to improve biological control in California's interior.

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RESEARCH ARTICLE

Healthy Schools Act spurs integrated pest management in California public schools

Chris A. Geiger
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The Healthy Schools Act of 2000 established right-to-know procedures for pesticide use in California public schools, and mandated using least-toxic pest management methods as state policy. In a survey conducted 2 years after the law's passage, school districts that had integrated pest management (IPM) programs generally used more ecologically sound pest management tactics than districts that did not, and most of those said that IPM had improved their pest management effectiveness. The Healthy Schools Act requires that schools post warning signs, keep pest management records, provide notifications to parents and staff, and maintain a list of parents desiring further notifications. A majority of California's school districts have implemented at least three of these four requirements, with about half reporting full compliance.

Pressure from the local community is likely to be a strong factor contributing to a district's adoption of IPM.

Cyrus Harmon

A California law passed in 2000 requires that public schools inform parents about pesticide use and designate integrated pest management coordinators. Cole McCann-Phillips climbs on a play structure in Berkeley.

Before 2001, the kinds and amounts of pesticides used in public schools were mysteries to many Californians. For some parents, these mysteries were worrisome.

California lawmakers aimed to address these concerns by passing the Healthy Schools Act of 2000 (HSA), which established right-to-know requirements for pesticide use (see sidebar, page 236) in public schools and required all school districts to designate an integrated pest management (IPM) coordinator (HSA 2000). The law also directed the California Department of Pesticide Regulation (DPR) to collect certain pesticide-use information from schools, such as the amount of pesticides used in eating areas or in classrooms, and to support schools in their IPM efforts, emphasizing pest prevention instead of more hazardous pesticide spraying.

To track the progress of Healthy Schools Act implementation, DPR distributed surveys to all California public school districts in 2002. The results presented here reveal patterns of pest management practices used for two of the most common school pests, ants and weeds. The survey also examined the levels of adoption for various IPM-related policies (including those required by the Healthy Schools Act), and compared the practices of districts that have IPM programs with those that do not. (Private schools are not covered by Healthy Schools Act mandates.)

DPR based the 2002 survey on a previous survey distributed in 2001 (DPR 2001). The 2001 survey placed more emphasis on assessing school districts' resource needs; the 2002 survey was intended to profile schools' pest management activities and measure their progress in adoption of IPM. The 2002 survey also began measuring the long-term progress of DPR's School IPM Program, which is charged with organizing IPM trainings, creating an IPM guidebook, developing a Web site (www.schoolipm.info), and generally assisting school districts with implementing IPM (see page 201). The 2002 survey consisted of 21 questions; copies were mailed to IPM coordinators at all 988 California public school districts. The response rate was 42% (Geiger and Tootelian 2002).

To better understand the effects of geographic and demographic factors on survey responses, a subset of the survey results was linked to data from the California Department of Education public schools database (CDE 2002). This data was also used to check for systematic demographic and geographic differences between respondents and nonrespondents (nonrespondent error). Sampling error was calculated to be ± 5%, based on the question with the highest standard deviation of responses

The Healthy Schools Act requirements

The Healthy Schools Act (HSA 2000) requires that all public school districts must:

- **Provide annual written notification** to all school staff, parents and guardians listing all pesticide products (some products are exempt) expected to be applied by district staff or outside contractors in the upcoming year, and the Internet address to DPR's School IPM Program Web site (www.schoolipm.info).
- **Provide the opportunity for interested staff and parents to register** with the school district if they want to be notified of individual pesticide applications at the school before they occur.
- **Post warning signs** at each area of the school where pesticides will be applied, posted 24 hours in advance and until 72 hours after applications.
- **Maintain public records** of all pesticide use at the school for 4 years.
- **Designate an IPM coordinator** to carry out these requirements.

For more information about these HSA requirements, contact Dave Hawke, California Department of Education, at (916) 322-1459, or dhawke@cde.ca.gov.

The Act requires the California Department of Pesticide Regulation (DPR) to:

- **Prepare a school pesticide-use reporting form** to be used by licensed pest-control businesses when they apply any pesticides at a school.
- **Establish and maintain a school Web site** to provide specified information to the public on school IPM (see www.schoolipm.info).
- **Promote and facilitate the voluntary adoption of school IPM programs** through specified activities, and assist districts in complying with the law.

In addition, the Act requires that:

- **Licensed pest-control businesses must report pesticide applications** by school annually to the DPR director.

For information on these requirements, contact Nita Davidson, DPR, at (916) 324-4100 or school-ipm@cdpr.ca.gov.

Ants, such as the adult Argentine ant, *top,* were ranked by California school districts as their third-most-difficult pest to control (after weeds and gophers). Rather than spraying them with pesticides, ants can be controlled with baits, *middle row*, or excluded by caulking cracks, *left.*

(question 5: "Which [pest control method] do you use most frequently to manage ants inside school buildings?").

Measuring progress toward IPM

The Healthy Schools Act includes its own IPM definition (see sidebar, page 237) and other general definitions of IPM abound (Bajwa and Kogan 1998). However, there is no widely accepted operational definition; that is, a definition specific enough to serve as the basis for measuring IPM adoption. While attempts have been made to set up such standards, their success has been hampered by the diverse nature of pest management systems.

Therefore, we developed our own approach to defining and measuring IPM in schools. After discussions with school personnel and a review of the IPM literature (Benbrook 1996; Huffaker and Messenger 1976; Stern et al. 1959), we categorized four activities as central to a successful school IPM program: (1) monitoring pest populations, (2) emphasizing pest prevention, (3) keeping records and (4) using chemical pesticides only as a last resort. Referring to these categories, DPR staff classified certain pest-management practices as "compatible with" or "contrary to" IPM (see sidebar, page 237). We asked about these practices in the survey and summarized each participant's responses as an IPM index, which was calculated by awarding one point for each compatible practice and subtracting one point for each contrary practice. This index provides a measure of school IPM adoption over time, and also helps to reveal whether self-categorization as an IPM district actually translates into better practices.

To keep the survey as short as possible and maximize response rates, we focused the questions on two representative categories of pests: weeds and ants. We chose weeds and ants because they

TABLE 1. Frequency of community inquiries on pest management–related issues*

	Weekly	Monthly	< 1 per month	Respondents
	%	%	%	no.
Districts with IPM programs	2.5	9.5	88.0	284
Districts with no IPM program, or not sure	1.6	2.4	96.0	124
All respondents	2.2	7.8	90.1	413

* Chi-squared $P < 0.01$ with weekly and monthly columns pooled.

represent both landscape and structural pest-management issues, and because they were ranked the first and third most serious school pests, respectively, in the 2001 survey (gophers were number two).

Weed management

Managing weeds requires a lot of labor, especially at rural schools with extensive turf and landscape areas. School districts varied widely in their decision-making approaches for weed treatment. Nearly one-third (30%) of school districts reported using the approach that would be preferred in an IPM program, which is treating weeds "when the abundance exceeds a pre-established threshold" (see Geiger and Tootelian [2002] for survey details). About one-fifth (23%) of districts treat "when weeds are first noticed." The single largest group (35%) reported treating weeds at regular, predetermined intervals. However, this result is difficult to interpret. While regular weeding is part of a sound IPM strategy, calendar-based herbicide spraying generally is not (UC IPM 2001). One possibility is that some respondents might have misinterpreted *treating* at regular intervals to mean *weeding* at regular intervals, thus inflating the 35% figure. Another possibility is that Healthy Schools Act notification requirements may have inadvertently increased calendar-based herbicide spraying. In phone calls to DPR staff, some schools reported that they now designate certain days of the year for pesticide treatments, thereby enabling them to send out fewer special notifications to parents.

For the treatment of weed problems, respondents cited physical controls such as hand-pulling, cultivating and mowing most frequently (68%), followed by regular spot treatment of turf/landscaping with herbicides (61%), use of mulches (26%), regular broadcast treatment of turf and/or landscaping with herbicides (23%), irrigation management (17%) and flaming — the use of special propane weed torches (7%). The use of broadcast herbicide treatments, which is considered contrary to IPM in this analysis, may be due to the pressure to maintain aesthetically pleasing turf in athletic fields as well as the perception that other controls are too labor-intensive for such large areas.

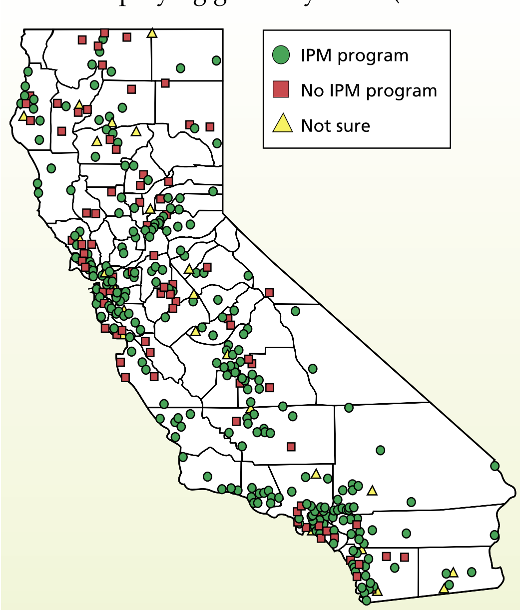

Fig. 1. Responding California school districts that reported having an IPM program in place in 2002.

Ant management

It only takes a few drops of soda or a few cookie crumbs in a child's desk to attract the familiar train of ants. For this reason, ants are the most universal indoor pest in California schools (DPR 2001) and prevention is a critical part of ant IPM. We would expect a district's ant management approach to reveal much about its overall pest-management philosophy, since ants are primarily an aesthetic pest that rarely justify emergency pesticide spraying (the red imported fire ant

HSA definition of IPM

Under the Healthy Schools Act, integrated pest management (IPM) is defined as: "a pest management strategy that focuses on long-term prevention or suppression of pest problems through a combination of techniques such as monitoring for pest presence and establishing treatment threshold levels, using nonchemical practices to make the habitat less conducive to pest development, improving sanitation, and employing mechanical and physical controls. Pesticides that pose the least possible hazard and are effective in a manner that minimizes risks to people, property and the environment, are used only after careful monitoring indicates they are needed according to pre-established guidelines and treatment thresholds."

For purposes of this survey, IPM was defined as including (or excluding) the following practices:

Compatible with IPM

- Keeping records of:
  - building inspections
  - pest sightings
  - results of pest monitoring
  - pest treatments used
- Treatment decisions based on pre-established thresholds for ants and weeds
- Ant baits
- Improving sanitation for ant control
- Caulking cracks for ant control
- Physical controls for weeds
- Irrigation management for weed control
- Mulches for weed control
- Flaming for weed control

Contrary to IPM

- Treatment at regular time intervals
- Insecticidal sprays from aerosol cans for ants
- Regular broadcast of herbicides for turf or landscape weeds

[Solenopsis invicta] is the exception).

The survey asked how school pest managers decide whether treatments for ants are necessary. Only 13% of districts treated for ants “when the number of ants exceed pre-established thresholds,” a strategy that is part of a sound ant IPM program. Another 16% of the districts treated for ants “at regular time intervals,” an approach that is not considered part of a sound IPM program, 31% do so “after a certain number of complaints are received” and 33% treat for ants when “first noticed.”

Improved sanitation and the use of ant baits were the most popular practices for controlling ants in school buildings. When asked to identify the “single method used most frequently” for ants in buildings, 32% of respondents identified ant baits, followed by improved sanitation at 22%. When asked to inventory all methods used to manage ants inside buildings, respondents most frequently reported the following IPM-compatible methods: improved sanitation (64%) ant baits (59%), soapy water (38%) and caulking (36%). Ant bait is considered an IPM-compatible ant management method because bait formulations kill the entire ant colony and children are not likely to be exposed to pesticides in baits (as opposed to sprays). However, many pest managers reported using baits as their sole technique, suggesting a lack of integration with preventive practices.

Nearly one-tenth (9%) of responding districts most frequently used the ant control method that is least compatible with an IPM program, “insecticidal spray from an aerosol can.” In addition, 17% of districts reported using spray cans either alone or in conjunction with other methods.

How IPM stacks up

We asked whether school districts had adopted IPM programs, realizing that districts’ definitions of IPM were likely to vary widely, especially between agricultural areas (where the term is commonplace) and urban areas (where it is not). More than two-thirds (70%) of responding California school districts reported adopting an IPM program, and 87% reported that they are aware of DPR’s School IPM Program. Regions with the largest percentage of districts reporting IPM programs were the San Francisco Bay Area, Los Angeles Basin, southern coast, San Joaquin Valley and southeastern desert regions (fig. 1, page 237).

Pressure from the local community is likely to be a strong factor contributing to a district’s adoption of IPM. Local concern is a driving force because the Healthy Schools Act contains no enforcement provisions. As an indirect measure of community concern about pesticides, we asked districts how frequently they received community inquiries on pest management issues. We

TABLE 2. Inventory of IPM-compatible and non-IPM compatible pest management practices compared between districts with and without IPM programs (self-reported)*

IPM-compatible practices	IPM districts	Non-IPM districts	Z
	. . . . . %		
Keeping records of:			
Building inspections	**44**	28	*
Pest sightings	**27**	14	*
Results of pest monitoring	**20**	12	*
Pest treatments used	**89**	79	*
Treatment decisions based on pre-established thresholds for:			
Ants†	**16**	6	*
Weeds‡	**33**	22	*
Ant baits‡§	**62**	53	
Improved sanitation for ant control†	**67**	55	*
Caulking cracks for ant control†	**38**	30	
Soapy water for ant control†	**41**	28	
Physical controls for weeds‡	**71**	65	
Irrigation management for weed control‡	**21**	8	*
Mulches for weed control‡§	**30**	15	*
Flaming for weed control‡	8	7	
Non-IPM-compatible practices	**IPM districts**	**Non-IPM districts**	**Z**
	. . . . . %		
Treatment at regular time intervals for:			
Ants†	15	**19**	
Weeds‡§	**40**	33	
Insecticidal sprays from aerosol cans for ants†	16	**22**	
Regular broadcast of herbicides for turf or landscape weeds†	**23**	21	

* Districts reporting that they were not sure if they had an IPM program were pooled with non-IPM districts. When results differed by more than 5%, the higher number is shown in bold. Asterisks denote significant differences (1-tailed, Z-scores, $P \leq 5\%$).

† Percentage of total number of respondents who treat for ants.

‡ Percentage of total number of respondents who treat for weeds.

§ Not included in IPM index calculation; all other factors included.

Suzanne Paisley

Jack Kelly Clark

Managing weeds can be difficult and labor intensive for schools, especially those in suburban and rural areas with large turf and landscape areas. Physical controls such as mowing and hand-weeding, *above*, were reported by 68% of the survey respondents, while 61% regularly sprayed with herbicides, *top*.

Photos: Ingrid Carmean

Less-toxic practices to prevent school pests include: *left,* designing buildings to prevent roosting pigeons, which can carry diseases, and, *right,* installing mowing strips to prevent weed growth along fence lines.

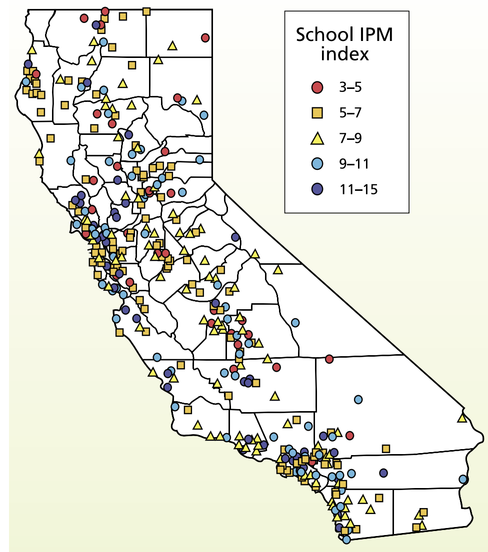

Fig. 2. IPM index scores of California school districts; higher scores indicate greater use of IPM-compatible practices for weeds and ants. IPM index calculation source: Geiger and Tootelian (2002).

would expect school districts with high levels of community concern to be more likely to adopt IPM programs.

In general, the results support this expectation. In table 1 (page 237), 8% more IPM districts than non-IPM districts received pest management inquiries either weekly or monthly, implying a correlation between community concern and the adoption of IPM. When the weekly and monthly columns are pooled (due to the skewed distribution of data), the IPM districts showed significantly more frequent community inquiries (chi-squared, $P < 0.01$). Very few districts (about 2%) receive one or more inquiries per week, while those that reported inquiries *at least* once per month were most prevalent in the southeastern region (19%), Bay Area (16%) and Los Angeles Basin (12%). Region was not, however, a significant predictor of IPM adoption.

Two other interrelated factors that could contribute to school IPM adoption are school size (enrollment) and type (such as rural, suburban or urban). We might expect that larger districts or those in urban areas, with more staff, centralization and specialization, would be better able to adopt new pest-management approaches and systems. For these analyses, we defined "urban" districts as those in medium or large cities or their urban fringes in the CDE database; all other categories were considered "rural."

The data showed that enrollment was a significant predictor of IPM adoption (t-test, $P < 0.05$). The average size of IPM districts was 8,455 students, compared to 4,686 for non-IPM districts (Los Angeles Unified School District, by far the largest in the state, was dropped from this analysis). There was also a weak but nonsignificant relationship between school type and IPM adoption. However, this relationship could not be separated from the enrollment effect because urban districts are also significantly larger than rural ones. Surprisingly, there was no relationship between district size and reported frequency of inquiries.

To find out what the reported use of IPM meant in terms of pest management tactics actually used, we summarized pest management practices deemed compatible with and contrary to IPM principles (table 2). Then we compared both the reported and actual use of these practices in districts that did and did not have IPM programs. We would expect a higher percentage of IPM districts to use IPM-compatible practices and a higher percentage of non-IPM districts to use IPM-contrary practices.

These expectations were generally confirmed, with two exceptions: a slightly higher percentage of IPM districts reported "treating at regular time intervals for weeds" and "use of regular broadcast herbicides for turf or landscape weeds," both of which might be considered contrary to good IPM practices. Another interesting result was that even IPM districts used insecticides from aerosol cans for ants, a method clearly contrary to sound IPM. In light of this finding, further educational efforts should be directed at keeping these cans out of the classroom.

We also examined differences between rural and urban districts' pest management practices. Rural residents are generally more familiar with agricultural pesticide use, and we might expect them to evaluate the risks of pesticide use differently than their urban counterparts, which could result in fewer inquiries to school districts and different pest-management philosophies. Indeed, rural districts did receive significantly fewer inquiries than urban districts. Only about 3% of rural districts reported receiving at least one inquiry per month, compared to 21% of districts in large cities and 13% of those in urban fringes of large cities.

To illustrate the geographical distribution of IPM-compatible practices, we constructed an unweighted IPM index using the practices listed in table 2 (fig. 2). Although it is difficult to discern a pattern, the highest-scoring districts appeared to be concentrated in coastal and metropolitan areas.

IPM costs and effectiveness

Pinning down a dollar figure for IPM costs and benefits can be a difficult task, especially with unknown benefits such as the long-term avoidance of new pest infestations and the reduction of human health risks. However, more than half (53%) of the responding districts

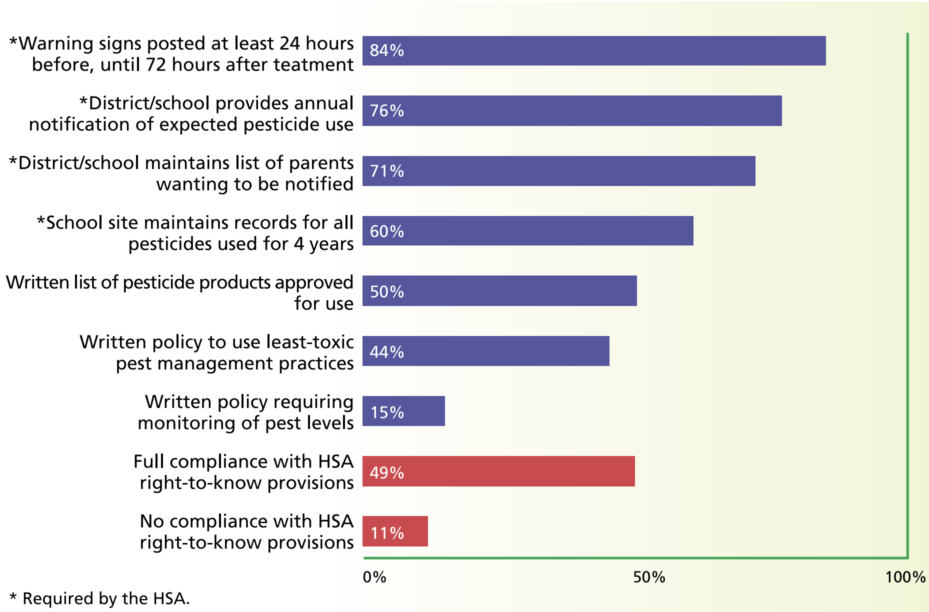

Fig. 3. IPM practices and policies reported as "officially adopted" by California school districts. Percentages shown may understate actual use of these practices.

TABLE 3. Comparison of 2001 and 2002 responses to survey questions regarding practices considered contrary to (red) and compatible with (green) IPM programs*

Responses	2001	2002	Z
	...... % *(no.)*		
Record-keeping:			
Pest sightings	16 (61)	23 (94)	
Results of pest monitoring	11 (44)	17 (73)	
Pest treatments used	79 (312)	86 (360)	
Decision to treat for ants inside buildings:†			
At regular time intervals	16 (48)	16 (49)	
When ants are first noticed	41 (119)	33 (101)	
When number of ants exceeds pre-established thresholds	10 (29)	13 (39)	
After certain number of complaints by constituents	30 (87)	31 (94)	
Other	3 (9)	6 (19)	
Practices used for managing ants inside buildings:†			
Insecticidal spray from aerosol can (2002 wording changed slightly)‡	32 (127)	17 (59)	
Insecticides sprayed using other application method	21 (81)	25 (86)	
Ant baits	37 (146)	59 (202)	*
Soapy water spray	14 (53)	38 (129)	*
Caulk in cracks	19 (75)	36 (123)	*
Improved sanitation (question added in 2002)‡	n.a.	64 (218)	
Other	13 (52)	22(76)	*
Decision to treat for weeds:§			
At regular time intervals	29 (104)	35 (123)	
When weeds are first noticed	28 (98)	23 (81)	
When weed abundance exceeds pre-established thresholds	34 (121)	30 (105)	
After certain number of complaints by constituents	4 (13)	2 (7)	
Other	6 (21)	10 (36)	
Practices used for managing weeds:§			
Regular broadcast treatments of turf/landscaping with herbicides	27 (107)	23 (84)	
Regular spot treatment of turf/landscaping with herbicides	62 (246)	62 (231)	
Use of mulches	23 (91)	26 (96)	
Physical controls (hand-pulling, cultivating, mowing)	56 (219)	69 (257)	*
Flaming	7 (29)	8 (28)	
Irrigation management (question added in 2002)‡	n.a.	17 (63)	
Other	9 (34)	10 (36)	

* Asterisks denote significant differences (2-tailed, Z-scores, $P \leq 5\%$).
† Percentages of total number of respondents who treat for ants.
‡ Due to wording changes, responses from 2002 and 2001 cannot be compared for this item.
§ Percentages of total number of respondents who treat for weeds.

reported that their IPM programs either reduced long-term costs (28%) or had no impact on those costs (25%). In contrast, more than a quarter (28%) reported that their IPM programs increased the long-term costs of pest management.

We also asked IPM districts to evaluate the overall effectiveness of their programs. Two-fifths (41%) reported that their program had resulted in more-effective pest management, one-fifth (19%) were uncertain of its effects, and one-fifth (20%) reported that their program resulted in less-effective pest management; the remaining districts did not respond.

IPM policies and HSA compliance

The survey listed a series of IPM-related practices and policies, and asked respondents which ones their district had officially adopted (fig. 3). The most common practices/policies reported were the four right-to-know provisions specifically required by the Healthy Schools Act: (1) posting warning signs, (2) providing annual notification of expected pesticide use, (3) maintaining a list of parents wanting to be notified and (4) maintaining a list of pesticides used during the previous 4 years. Nearly three-quarters (71%) of California school districts had officially adopted at least three of these four provisions. However, less than half (49%) of responding districts adopted all four provisions, which means that by this measure about half (51%) are still not in full compliance.

We also asked all respondents to rate their satisfaction in the previous year with six factors that we considered important to the success of school IPM programs (fig. 4). Significantly more IPM districts rated their performance as "good" for all factors except "contracting procedure," suggesting that they are more satisfied than non-IPM districts with their pest control efforts.

Schools' progress toward IPM

Although some questions in the 2001 and 2002 DPR surveys were not designed for direct comparison, a comparison of the two surveys shows that California schools are making progress toward adopting more-accountable,

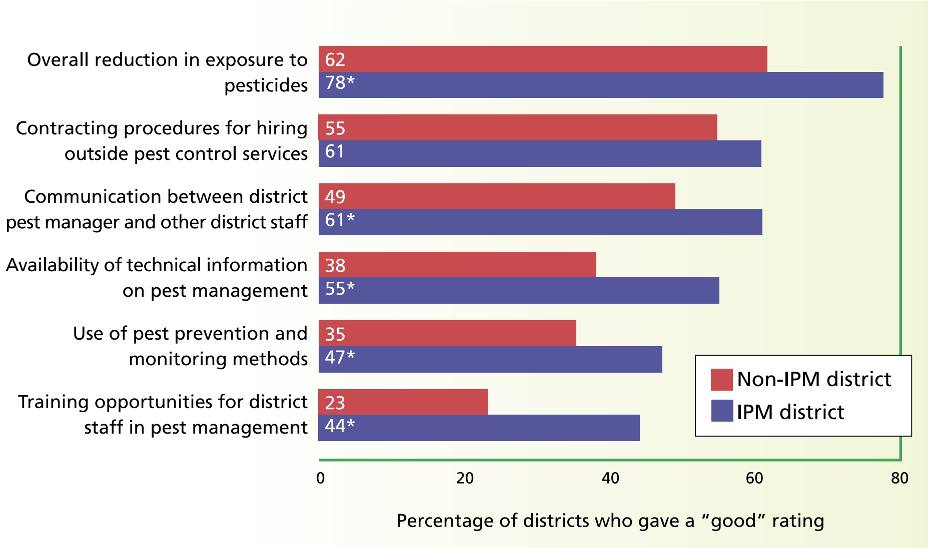

Fig. 4. Satisfaction (rated as "good," "fair" and "poor") with factors considered important to the success of IPM programs, compared between California school districts with and without IPM programs (self-reported). Respondents were asked to rate their satisfaction over the previous year. Significant differences ($P < 0.05$, logistic regression) are shown by asterisks (*).

California schools are making progress in adopting less-hazardous pest management strategies. *Above*, effective methods are available to control wasps, which pose a serious hazard to students allergic to their venom.

less-hazardous pest-management practices, in accordance with the goals of the Health Schools Act. For example, surveyed school districts kept better records in 2002 than in 2001. The percentage of districts recording pest sightings, pest monitoring data and pest control treatments all increased (table 3). Since good record-keeping is a core tenet of IPM, this appears to be a positive trend.

Comparing the 2001 and 2002 surveys also suggests a movement toward reduced-risk methods for managing ants. The use of baits, soapy water sprays and caulking in cracks to prevent entry all increased significantly between 2001 and 2002 (table 3). However, insecticidal spray use during the 2 years could not be compared directly, due to refinements in question wording. Schools' approaches to deciding when ant treatments are necessary did not change significantly between years.

The trends in weed management were more mixed. On the negative side, the IPM-contrary practice of treating weeds at regular intervals rose in 2002, while the generally desirable practices of treating "when weeds are first noticed" or "when the abundance of weeds exceeds pre-established thresholds" both declined somewhat. On the positive side, the percentage of districts using physical controls (such as hand-pulling, cultivating and mowing) increased significantly, and the percentage using mulches grew slightly. Also, the percentage of respondents using the IPM-contrary practice of broadcasting herbicides on a regular schedule declined slightly.

In summary, California's public schools appear to be making some progress toward an IPM approach in their ant management, monitoring and record-keeping practices, although Healthy Schools Act record-keeping requirements remain a challenge for many districts. The survey's picture of weed management practices is less clear; improving weed IPM and avoiding calendar treatment schedules may require additional attention. Increased training in methods such as weed flaming, and wider use of weed barrier technologies such as cloth or mulches, could improve some schools' weed management success, as could the trend toward artificial turf in athletic fields. Generally speaking, larger, urban schools seem to be performing better than smaller, rural schools; this could be function of inadequate training or merely a lack of resources in small districts. These surveys will be invaluable in monitoring progress of school IPM programs in future years.

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Almond growers rely on pest control advisers for integrated pest management

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A comprehensive survey of full-time almond growers in the three primary almond-producing regions of California showed that growers rely substantially on pest control advisers (PCAs) for pest management decision-making. Independent PCAs communicated more frequently with growers than PCAs who are employed by agricultural product suppliers. Growers who use independent PCAs tend to feel more knowledgeable about integrated pest management (IPM) and report the use of more complex pest-monitoring techniques and control practices. The use of insecticide sprays, however, is independent of the type of PCA employed, and the percentage of growers using them has declined substantially since a 1985 survey.

Jack Kelly Clark

UC IPM entomologist and co-author Walt Bentley demonstrates the use of a hand lens to monitor for arthropod pests of almonds. Almonds cover about 540,000 acres in California, making them a significant focus of UC integrated pest management (IPM) outreach and extension. A survey found that pest control advisers (PCAs) also play an important role in providing information to almond growers about IPM.

The goals of the UC Statewide Integrated Pest Management Program include increasing the adoption of integrated pest management (IPM) practices to improve pest control, and reducing growers' need for broad-spectrum pesticides. With more than 6,000 almond farms covering approximately 540,000 acres statewide, almond growers and their consultants are a major focus of UC research and extension (Zalom et al. 2005). The almond industry has worked closely with UC for more than 25 years to implement new IPM practices, most recently utilizing the partnership framework of the Almond Pest Management Alliance (Looker 2005).

Many complex factors affect pest management decisions, including the decision-maker's knowledge about and attitudes toward practices that are continually changing. Furthermore, the practices chosen must interact with multiple biophysical and economic variables. In California, state-licensed pest control advisers (PCAs) play a substantial role in helping growers work through these management decisions and are among the most important clientele for UC educational efforts. How these PCAs influence the adoption of IPM practices is a much-debated topic among academics and government agencies.

In particular, some PCAs are affiliated with agricultural product suppliers and so appear to have a conflict of interest. While these supplier-affiliated PCAs provide pest monitoring and consulting services for free, their employers stay in business by selling pest control products. Independent PCAs, on the other hand, are not on the payroll of a supply company and charge a per-acre fee for their services. Whether supplier-affiliated or independent, a PCA's reputation depends on his or her ability to help growers produce quality crops in the most cost-effective manner.

IPM mail survey

In 2000, the UC Statewide IPM Program and the Almond Pest Management Alliance conducted a comprehensive mail survey of almond growers intended to measure their use of specific pest-management practices and to learn more about factors that influence their decisions. We present a portion of the survey results, highlighting declining trends in the use of broad-spectrum insecticides, examining how growers' interactions with PCAs may be affecting these trends and exploring the impact of PCA affiliation on the adoption of IPM tactics.

Our sampling was based on the three major almond-production regions in California: the central and southern San Joaquin Valley (Fresno, Kern, Tulare and Madera counties), the northern San Joaquin Valley (Merced, San Joaquin and Stanislaus counties) and the Sacramento Valley (Butte, Glenn and Colusa counties). In order to focus on full-time growers, we sampled those with more than 20 bearing almond acres. Samples were drawn from lists of growers obtained from the agricultural commissioner's offices in eight counties, and from Cooperative Extension mailing lists in the remaining two counties.

The survey included five main sample groups. In each of the three growing regions, we systematically drew approximately equal-sized samples. For each region, we started with a random grower on the list and then drew every nth grower, defining "n" as the total number of growers on the list divided by the final desired sample size. Then, to also include growers with smaller almond farms, we similarly drew a fourth sample from almond growers with 20 or fewer acres across the three regions. Finally, we mailed surveys to a fifth group of almond growers, who were from the same almond-production regions and represented all farm sizes, and had participated in an earlier telephone survey phase of this project (table 1).

The survey was mailed in spring 2000. In order to encourage responses, mailings were personalized as much as possible, used first-class postage and included a postage-paid return envelope, and there were three follow-up mailings. Due to length considerations, half of those surveyed in each sample group received the insect and mite management version of the questionnaire while the other half received the disease and weed management version. Both versions included a set of identical questions pertaining to information sources, attitudes toward IPM and general decision-making factors (including reliance on PCAs for the control of insects/mites, weeds, diseases and nematodes).

A completion rate of 39% resulted in a final response set of 453 growers (table 1). Three hundred and twenty-two of the responding growers had more than 20 acres of bearing almonds in 1999, and 168 of these completed the insect and mite management version of the questionnaire. The results discussed in this article are based either on the larger set of 322 growers with more than 20 acres or on the subset of 168 growers who completed the insect and mite questionnaire.

We used nonparametric statistical tests for two reasons: first, in some instances the groups being compared had different variances; and second, in many cases the variables being tested were categorical (such as yes/no/don't know responses to questions about the use of a practice). We used the Wilcoxon 2-sample test to assess differences in a continuous variable (such as farm acreage) between two groups with unequal variances. Similarly, we used the Kruskal-Wallis test to assess differences in a continuous variable among more than two groups. We used the chi-square statistic to assess differences between two or more groups when categorical variables were involved. Finally, we used Fisher's exact test in cases when sample sizes were too small to allow the appropriate use of chi-square.

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Air blast sprayers are used for ground applications of pesticides in orchards. Almond growers reported applying dormant season, May and hullsplit insecticide sprays less frequently in the current survey than in a 1985 study.

Role of PCAs

Grower use of PCAs. Nearly all (97%) of the survey respondents used PCAs for pest management advice,

TABLE 1. Sample groups for 2000 mail survey* of California almond growers

Sample group	Eligible respondents sampled	Completed surveys
	number	
Central/South San Joaquin Valley (> 20 acres)	185	75
North San Joaquin Valley (> 20 acres)	193	71
Sacramento Valley (> 20 acres)	234	80
Small growers across three regions (≤ 20 acres)	185	55
Growers across regions and farm size who participated in earlier telephone survey	354	172
Total sample	1,151	453

*Survey completion rate = 39%.

showing that this is a nearly universal practice. About 73% used only one PCA, 21% used two PCAs and 3% used three or more. The degree of PCA influence on decision-making, however, varied with different kinds of pests. For example, 80% of growers reported following their primary PCA's recommendations for insect pest-management actions at least 80% of the time, and 78% of growers followed recommendations for disease management at least 80% of the time. In contrast, only 60% of growers followed their primary PCA's recommendations for weed management at least 80% of the time. Moreover, more than one-quarter (28%) of growers followed their primary PCA's recommendations for weed management only half of the time at most.

These differences in reliance on PCAs are likely due to the fact that for weeds, almond growers tend to follow a set pattern of management practices from year to year. In addition, weeds may not have as direct an impact as other pests on yield and quality, especially in mature orchards. On the other hand, insect/mite and disease management in almonds typically involves more complex monitoring techniques, treatment thresholds (especially for insects and mites) and timings, as well as the consideration of variable weather factors, which facilitates strategic decision-making. Expert input to such decisions can substantially affect pest control efficacy and cost. In addition, insects, mites and diseases directly influence crop quality and tree longevity, and therefore directly affect returns to the grower.

Independent vs. supplier-affiliated. Of all responding growers who used a PCA, nearly two-thirds (64%) worked primarily with a PCA affiliated with an agricultural products supplier, while almost a third (31%) worked primarily with an independent PCA. An additional 5% reported having an in-house or employee PCA.

Statistical tests show that growers with smaller acreage were less likely to use independent PCAs than those with larger acreage. Growers with supplier-affiliated PCAs managed a mean of 233 almond-bearing acres, while those who primarily used independent PCAs managed a mean of 307 almond-bearing acres (Wilcoxon 2-sample, $P < 0.001$). This difference may be due to the economies of scale afforded to PCAs by larger orchards. The practice of compensating independent PCAs on a per-acre basis provides a disincentive for the PCAs to accept contracts on small farms, where the compensation is smaller relative to fixed costs associated with traveling to and from the orchard regularly.

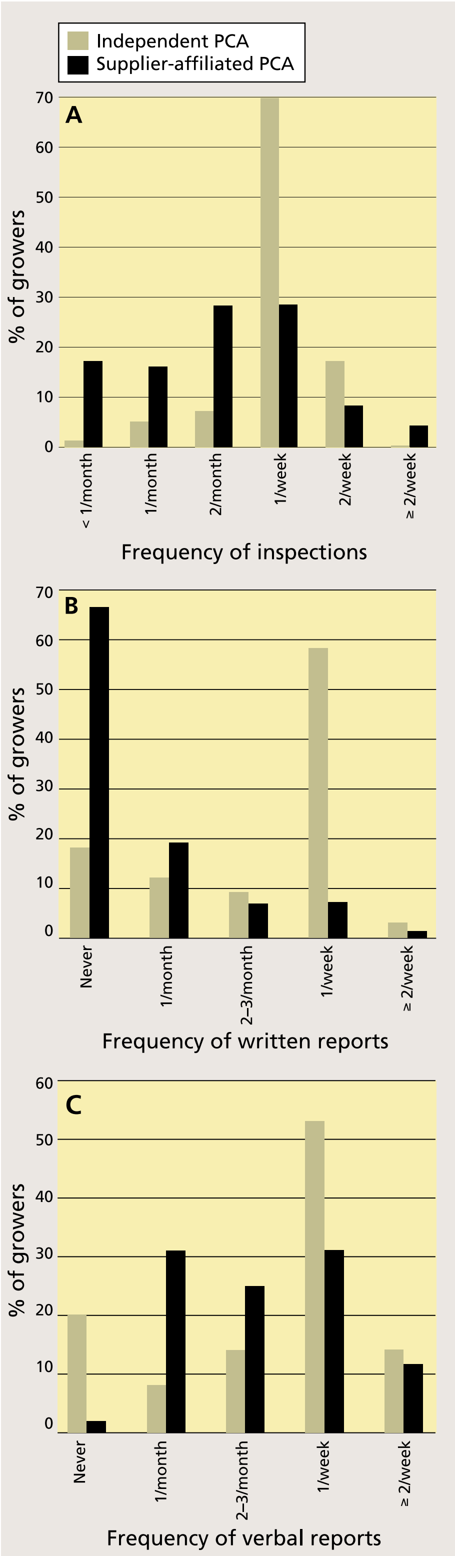

Fig. 1. Frequencies of (A) orchard inspections, (B) written reports and (C) verbal reports by primary PCA during peak season, as reported by surveyed almond growers.

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Strip weed control is used by many almond growers to manage orchard floors. Among the benefits of this approach is reduced pesticide runoff.

TABLE 2. Significance of differences in frequency of PCA orchard inspection and reports for growers with independent versus supplier-affiliated PCAs, by acreage

	Frequency of		
Acreage quartile	Inspection	Verbal reports	Written reports
	Fisher's exact test P *value (n)*		
21–45 acres	NS (53)	NS (34)	0.003 (12)
46–96 acres	0.024 (60)	NS (41)	NS (23)
97–250 acres	< 0.001 (71)	NS (53)	< 0.001 (27)
251–9,000 acres	0.003 (69)	NS (58)	0.005 (42)

Growers who reported primarily consulting an independent PCA also had a significantly greater tendency to follow their recommendations for insect/mite and disease management (Wilcoxon 2-sample, $P = 0.001$ for insect/mite and $P = 0.033$ for disease) than those who primarily used a supplier-affiliated PCA. The growers with independent PCAs also received more frequent orchard visits (chi-square, $P < 0.001$) (fig. 1A) and written status reports (figs. 1B and 1C) than growers using supplier-affiliated PCAs. About three-fifths (61%) of the growers employing independent PCAs indicated receiving written reports as often as once per week or more, a significantly higher percentage than the 8% of growers with supplier-affiliated PCAs (chi-square, $P < 0.001$). Furthermore, most growers (66%) using supplier-affiliated PCAs indicated receiving no written reports at all (fig. 1B). IPM is information intensive, so frequent written reports facilitate the grower's ability to implement least-toxic pest control approaches.

In contrast to independent PCAs, supplier-affiliated PCAs favored verbal reports and most (87%) gave these from once per week to once per month (fig. 1C). Even so, significantly more growers with independent PCAs received verbal reports once per week than growers with supplier-affiliated PCAs — more than half versus less than a third (chi-square, $P = 0.001$). We can only hypothesize the reasons that supplier-affiliated PCAs favor verbal over written reports. Written reports may take longer to complete, and supplier-affiliated PCAs may be more reluctant to take the extra time due to different compensation structures. Verbal interaction with the grower may also allow more opportunities for supplier-affiliated PCAs to promote the company's products.

Acreage. Since growers using independent PCAs also tended to have larger orchards, we performed the above tests on smaller subcategories of growers to determine whether total acreage affected the frequency of PCA orchard visits as well as of verbal and written reports. The four subcategories were selected by taking quartiles of the acreage variable: the first quarter of the sample had 21 to 45 acres, the second had 46 to 96 acres, the third had 97 to 250 acres, and the fourth had 251 to 9,000 acres. In all 12 cases, growers who used independent PCAs tended to report both more inspections and more frequent PCA reports than growers who used supplier-affiliated PCAs (table 2). Six out of the 12 tests resulted in significant P values ($P \leq 0.05$), suggesting that farm size may not be a substantial factor in determining the frequency of some PCA activities, while PCA type is an important factor.

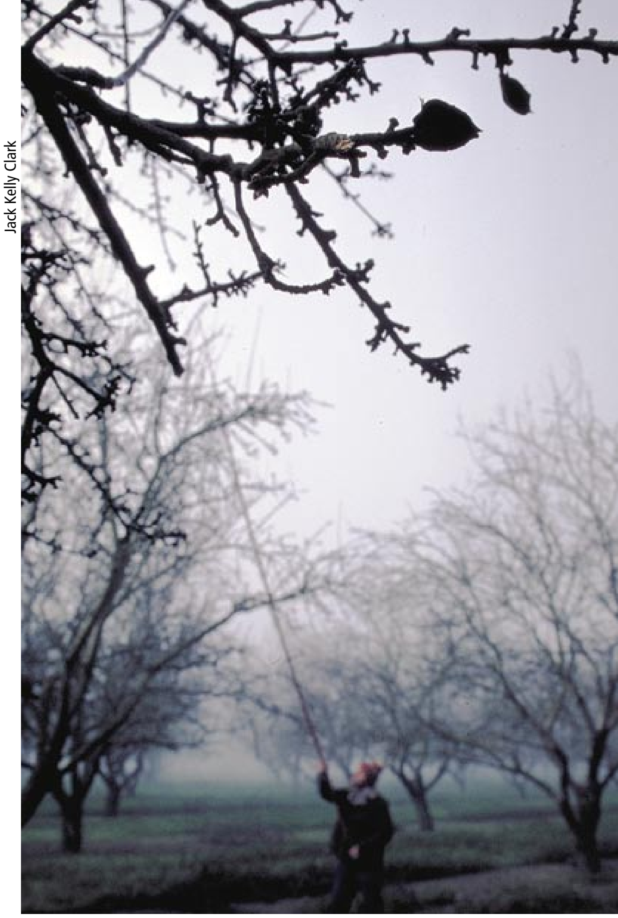
The higher frequencies of communication and field visits provided by independent PCAs may be partly responsible for the fact that they reportedly had more influence over grower decisions. Growers may also follow independent PCA recommendations more closely because they pay for them directly. In addition, the reports of PCA activity in this study were based on growers' perceptions rather than empirical measurements. It is possible that growers who pay their PCAs for services also pay more attention to them and therefore are more likely to remember what they did than growers receiving unpaid services from supplier-affiliated PCAs.

Trends in pest control practices

Pesticide use. In debates about the significance of PCA affiliation, an issue that is often raised is whether supplier-affiliated PCAs promote more chemical use. It is often assumed that independent PCAs are more likely than supplier-affiliated PCAs to recommend fewer sprays and to promote IPM. We tested this assumption by examining growers' responses about applying insecticides during the three most common insecticide-treatment timings for almonds: the dormant season, in December and January for almond growers; in May, when susceptible stages of navel orangeworm, peach twig borer, oriental fruit moth and San Jose scale may be present; or at hull-split, which typically occurs in early July.

Two-thirds (66%) of responding growers reported spraying insecticides during the 1998 to 1999 dormant season (n = 154), about one-fifth (22%) applied a May spray (n = 156), and more than half (59%) applied a hull-split spray (n = 158); for each practice, the percentage who answered "don't know" was less than 2%.

We found that the affiliation of PCAs did not have a significant effect on responding almond growers' use of common chemical controls for insect and



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Winter mummy-nut removal is critical to managing navel orangeworm. Growers with independent (non-supplier-affiliated) PCAs were more likely to perform winter-sanitation measures such as poling (shown), which helps to prevent overwintering of the pest's larvae.

Trends in pest control for almonds

Photos: Jack Kelly Clark

Between 1985 and 1999, there was a large increase in survey respondents who perceived mites and ants as control problems in almond orchards. *Left, Tetranychus* spider mites produce webs; *right,* the southern fire ant feeds on almond nut meats.

California's almond IPM program was chosen as a case study for the 1985 USDA National Evaluation of Extension IPM Programs (Klonsky et al. 1990). The 1985 mail survey was conducted by UC Davis agricultural economist Karen Klonsky and UC IPM director Frank Zalom in collaboration with the Almond Board of California, which provided mailing lists of growers affiliated with both the Blue Diamond Growers Cooperative and independent handlers from which names of growers were drawn at random.

Although less comprehensive than the 1999 survey, several questions — including the perceived importance of different pests, use of various IPM practices, and use of specific seasonal spray timings — were asked in both surveys. Comparing the 1999 mail survey of IPM use with the 1985 survey shows that almond growers continue to perceive the navel orangeworm and peach twig borer to be key insect pest problems, while the relative importance of mites and ants increased during this time (table 3). The navel orangeworm is a target of two spray timings (May and hull-split), while the peach twig borer is a target of all three spray timings (dormant season, May and hull-split).

Despite the continuing importance of key insect-pest problems in growers' perceptions, the reported use of insecticide sprays declined substantially during all three timings. From 1985 to 1999, May sprays declined from 78% to 22%, dormant-season sprays declined from 93% to 61%, and hull-split sprays declined from 82% to 59%. The reduction in dormant sprays — especially organophosphates — during the 1990s has been documented by other researchers analyzing pesticide-use reports submitted by almond growers statewide, as required by the California Department of Pesticide Regulation (Epstein and Bassein 2003; Zhang et al. 2004).

The decline in use of dormant and in-season sprays reflects to some extent the history of UC's almond pest management guidelines. In the 1970s and 1980s, UC guidelines preferred the use of dormant-season insecticide sprays to control peach twig borer, San Jose scale and the eggs of both brown almond mite and European red mite. Spraying during the dormant season reduces overwintering populations of these pests while minimizing insecticide exposure of biological control agents, nontarget organisms, and workers in orchards during the growing season.

TABLE 3. Grower perception of pests as problems requiring management in their orchards, 1985 and 1999

Pest	Growers who perceived pest as a problem 1985*	1999
	. . . . %	
Navel orangeworm	70	61
Peach twig borer	50	62
Mites	27	65
Ants	13	57
San Jose scale†	—	2
Oriental fruit moth	4	19

* 1985 sample includes all farm sizes; 1999 sample includes only farm sizes > 20 acres.

† Questions about San Jose scale were not included in the 1985 survey.

During the 1990s, however, the UC guidelines were revised to reflect the availability of new commercial products that control target pest species, new research findings on alternative pest-control practices and increasing environmental concerns. The new UC guidelines more strongly emphasize monitoring for the appropriate pests before applying sprays during any of the three timings, and also suggest alternative controls and treatment timings (Zalom et al. 2005). For example, monitoring for peach twig borer and navel orangeworm was recommended as a prerequisite to using in-season sprays, and the May spray was only suggested if warranted by monitoring and if a dormant spray and winter mummy-nut removal had not been performed.

While our study does not support the notion that supplier-affiliated PCAs encourage more chemical insecticide use, it does point toward possible increases in knowledge and use of IPM practices by growers employing independent PCAs.

mite pests. Whether or not they used an independent or supplier-affiliated PCA, growers were statistically as likely to use insecticide sprays during the 1998 to 1999 dormant season (62% and 70%, respectively, chi-square, NS), in May (19% and 27%, chi-square, NS) and at hull-split (56% and 65%, chi-square, NS) to control peach twig borer, San Jose scale or navel orangeworm.

There was also no difference in the use of two IPM practices without insecticides, dormant oil (33% and 27% respectively, chi-square, NS) and summer oil alone without insecticides (16% and 10%, chi-square, NS) to control scale, spider mites or leafhoppers. The reported use of biopesticides — those toxins derived from microbial or botanical sources, such as *Bacillus thuringiensis* (Bt) and spinosad — was also similar by growers using either independent or supplier-affiliated PCAs (50% and 46%, respectively, chi-square, NS). "Don't know" responses for all of these practices were 7% or fewer.

Grower knowledge. Almond growers using independent PCAs reported feeling more knowledgeable about IPM than those using supplier-affiliated PCAs (chi-square, $P = 0.009$). While the majority of growers in both groups reported feeling either somewhat or moderately knowledgeable about IPM (74% of those with independent PCAs and 83% of those with supplier-affiliated PCAs), considerably more growers with independent PCAs reported feeling very knowledgeable than did those with supplier-affiliated PCAs (19% versus 5%, respectively). It is possible, however, that almond acreage influences these results. We conducted the same statistical tests for differences within the four subcategories of growers as defined by acreage quartiles, and the results were nonsignificant in all four cases ($P > 0.05$).

In any case, even a discernible difference between growers using different types of PCAs does not mean that consulting with independent PCAs is in itself responsible for growers' greater confidence in their IPM knowledge. Rather, such results may only indicate that growers who are more knowledgeable and perhaps more interested in IPM also have a higher tendency to use independent PCAs. On the other hand, half of the surveyed growers with independent PCAs reported that they first heard about IPM from a private consultant or PCA, as opposed to only a fifth of the growers with supplier-affiliated PCAs. These results suggest that independent PCAs might be somewhat more likely to introduce growers to IPM.

Use of IPM. Growers using different types of PCAs varied significantly in the use of several cultural controls and monitoring techniques (chi-square, $P < 0.05$). For example, responding growers with independent PCAs were more likely than growers with supplier-affiliated PCAs (90% versus 65%, respectively) to perform winter sanitation by knocking mummies from trees by hand with poles or by shaking mummy nuts — the overwintering site of navel orangeworm larvae — from the trees with mechanical shakers. Winter sanitation is one of the most important means for controlling navel orangeworm and can reduce the need to apply insecticide sprays during spring and summer. Similarly, growers with independent PCAs were more likely to determine the effectiveness of sanitation by counting mummy nuts than growers using supplier-affiliated PCAs (78% versus 40%, respectively). However, both winter sanitation and counting mummy nuts also varied significantly by acreage (chi-square, $P < 0.001$ and $P = 0.032$). Growers with larger acreage were more likely to perform winter sanitation and count mummies than those with smaller acreage, suggesting that the role of farm size should be examined more critically.

Almond growers with independent and supplier-affiliated PCAs also reported significant differences in the use of IPM monitoring practices (chi-square, $P < 0.06$) (table 4). Notably, growers with independent PCAs were also more likely to respond "don't know" to monitoring questions than growers with supplier-affiliated PCAs ("don't know" responses ranged from 5% to 24% for the former,

TABLE 4. Differences in monitoring practices between surveyed growers using independent and supplier-affiliated PCAs

	Responding growers with	
Monitoring practice*	Independent PCAs	Supplier-affiliated PCAs
	. . . . %	
Monitor emergence of peach twig borer at overwintering hibernaculae	71	49
Sample blossom and shoot strikes to determine if sprays necessary for peach twig borer	70	62
Place pheromone traps for peach twig borer†	81	51
Use degree days with monitoring†	67	43
Place double-sided sticky tape to monitor San Jose scale crawler	36	9
Place pheromone sticky traps for San Jose scale males	24	8
Sample dormant spurs for San Jose scale†	55	30
Sample dormant spurs for mite eggs†	55	35
Use presence/absence spider mite monitoring	71	59
Brush or count mites per leaf	71	54
Place navel orangeworm egg traps†	76	36
Monitor navel orangeworm eggs or larvae on mummy nuts or hull-split nuts†	80	51
Count number of ant hills per orchard area	45	28
Monitor for predatory mites and six-spotted thrips†	88	66
Monitor sticky traps for San Jose scale parasites	32	10

* All practices chi-square $P < 0.06$.
† Performance of these practices varies significantly (Fisher's exact test $P < 0.03$) by acreage quartiles.

◀ **Almond growers with independent PCAs appeared to be more knowledgeable about IPM practices. *Left,* almond bloom is a preferred timing for some alternatives to organophosphate insecticides, to control peach twig borer. *Inset,* feeding by peach twig borer larvae on almond nutmeats causes shallow channels and surface grooves on the kernels.**

compared to 1% to 14% for the latter), suggesting that growers using independent PCAs rely more heavily on them to carry out monitoring activities and that the growers may not understand the PCA's specific methodology.

Linking growers to IPM extension

This survey shows that PCAs are important to almond growers as sources of information on IPM practices, especially for insect and mite pests and diseases. Furthermore, some of the findings suggest that greater contact between growers and PCAs, in person and through written reports, might help growers become better informed about IPM practices in general and more specifically about pest problems on their own farms.

Our study found a high degree of self-reported grower reliance on PCAs for assistance in pest management decision-making, supporting the assertion that PCAs can make a substantial difference in grower understanding and approaches to pest management. Moreover, the influence of PCA affiliation on grower knowledge and the use of different practices should be reconsidered and studied further. While our study does not support the notion that supplier-affiliated PCAs encourage more chemical insecticide use, it does point toward possible increases in knowledge and use of IPM practices by growers employing independent PCAs. However, this study does not show whether this association occurs due to PCA influence on growers or because growers who hire independent PCAs are already predisposed toward IPM.

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English walnut rootstocks help avoid blackline disease, but produce less than 'Paradox' hybrid

Joseph A. Grant
Gale H. McGranahan

While 'Paradox' hybrid seedlings are often the rootstocks of choice for California walnut orchards, there is a resurgence of interest in using English walnut seedlings because walnut blackline disease, which is endemic in many California walnut production districts, does not affect them. We compared the growth and productivity of walnuts on English rootstocks from a variety of sources to those on Paradox rootstock. The growth and productivity of 'Chandler' walnut trees were similar among trees on seedling English rootstocks in one trial, but trees on English rootstocks were smaller and had lower production than Paradox hybrid–rooted trees in the other.

John M. Mircetich

Blackline-infected trees on, *above left,* black walnut, and, *above right,* 'Paradox' rootstocks are slowly girdled by the death of rootstock tissue at the graft union. *Top,* over time, walnut trees with blackline decline in vigor, leading to dieback of branches and, ultimately, death of the tree. Trees on English rootstocks develop no graft union symptoms and escape the debilitating effects of blackline infection.

California's first walnut trees and orchards were planted during the Spanish mission period (around 1800), using seedlings of early varieties of Persian or English walnut *(Juglans regia)* chosen for their superior growth and nut quality. Since the 1890s, walnut trees in California have been propagated by grafting or budding desired cultivars onto rootstocks chosen for their adaptation to different physical, chemical or biological soil conditions at individual orchard sites. From the early to mid-20th century, seedlings of Northern California black walnut *(Juglans hindsii)* were used as rootstocks because they grew vigorously and were more tolerant of saline and saturated soil and more resistant to soil-borne pests than English walnut seedlings.

Since the 1950s, 'Paradox' hybrid seedlings have supplanted black and English walnut as rootstocks of choice for California orchards, though black walnut (*J. hindsii* and others) is still used occasionally. Paradox seedlings are hybrids of black and English walnuts, and the rootstocks are grown by nurseries using seed nuts collected from black walnut trees, particularly *J. hindsii* (Potter et al. 2002) pollinated by English walnut pollen. Paradox-rooted trees grow more vigorously than those on black or English walnut rootstocks, are more resistant to Phytophthora root and crown rot disease, and are more tolerant of lesion nematode *(Pratylenchus vulnus)*, both of which are widely distributed and problematic in California orchards (Browne et al. 1977; McGranahan and Catlin 1987;

TABLE 1. English seedling rootstocks used in the study came from a variety of commercial nursery sources

Seedling rootstock	Source
Site 1	
Eureka	Visalia, Calif.
Manregian	Davis, Calif.
Spanish	Tarragona, Spain
Ronde de Montignac	France
Corne	France
Site 2	
Paradox	Modesto, Calif.
Carpathian	Loomis, Wash.
Russian	Loomis, Wash.
Waterloo	Modesto, Calif.
Sunland	Modesto, Calif.
Eureka	Gridley, Calif.
Chandler	Modesto, Calif.

Fig. 1. Average trunk diameter (top) and nut production (bottom) of experimental trees at site 1 (left) and site 2 (right). Columns headed by common letters indicate cumulative yields that are not significantly different (Fisher's protected LSD, $P \leq 0.05$).

Serr and Rizzi 1964). Because of their generally poor performance, both in controlled experiments and limited commercial orchard use, English seedlings are now used only occasionally as walnut rootstocks in California.

The recent resurgence of interest in using English walnut seedlings as rootstocks in California is a result of the discovery in the 1970s that walnut blackline disease, caused by cherry leafroll virus (CLRV), was endemic in many California walnut production districts. CLRV infection kills tissue at the graft union of trees grown on black walnut and Paradox rootstocks, but not those on English walnut rootstock because it is naturally tolerant of CLRV. This reaction gradually girdles and kills black walnut– and Paradox-rooted trees (Mircetich et al. 1980; Mircetich and Rowhani 1984). There is some evidence from Europe of adverse impacts due to systemic CLRV infection on the growth of English-rooted trees (Mircetich et al. 1998). But these effects are not as well documented as other disadvantages of this rootstock and, thus, are not considered detrimental enough to preclude its use where otherwise indicated.

Seedlings of 'Manregian' and 'Eureka' English walnuts have been available to California growers for many years. In the 1980s, commercial nurseries began offering seedlings from a variety of other English walnut sources in response to renewed interest stimulated by the discovery and prevalence of blackline disease. We undertook this study to compare the orchard performance of seedlings from some of these English walnut sources as rootstocks for walnuts in California. The identification of a source of English walnut with superior vigor, productivity and pest tolerance could provide walnut growers in areas of high CLRV incidence with an acceptable alternative to the hypersensitive rootstocks currently in use.

Rootstock trials

Two trials were established in a commercial walnut orchard near Linden in San Joaquin County. Soil at both test sites was Archerdale clay loam. The trials were in 12-feet-by-24-feet high-density "hedgerow" plantings, which were sprinkler-irrigated. Formerly planted to walnuts, both sites were fumigated with methyl bromide prior to planting.

Site 1 was planted in 1989 and consisted of three rows of 'Chandler' trees that had been nursery-grafted on English walnut seedlings from Manregian and Eureka from California, 'Ronde de Montignac' and 'Corne' from France, and a source collected from Tarragona, Spain. Experimental plots were within pollenizer rows planted every eighth row in a 'Vina' orchard (table 1).

Site 2 was planted in 1994 and included seedlings from Eureka, 'Waterloo', Chandler and 'Sunland' provided by a California nursery, two English walnut sources named 'Russian' and 'Carpathian' by their respective suppliers (McGranahan and Leslie 1990), and Paradox hybrid seedlings from a California nursery. Experimental plots were in five alternate rows in a solid Chandler planting.

The trials at both sites were configured as randomized complete block designs, with three 5-tree plots at site 1 and five 8-tree plots at site 2. Trees for both sites were nursery-propagated and planted as grafted 2-year-old trees. Nursery seedlings for both sites were tested for CLRV, and infected trees were discarded to prevent the introduction of blackline virus disease to the test orchard.

Tree growth was evaluated at both sites by annual trunk-diameter measurements made 12 inches above the graft union. Yield (in-shell, 8% wet basis moisture) was measured at site 1 in 1993, 1995, 1997 and 2003, and at site 2 in annually from 1997 through 2003. Growth and yield data from the experimental plots were analyzed using two-way analysis of variance and Fisher's protected LSD for mean separation.

Tree growth and productivity

At site 1, there were no significant differences among English-rooted trees in annual trunk diameter or nut production (fig. 1). Similarly, at site 2, English-rooted trees from all sources were similar in trunk diameter during most of the study years (1995 through 1998, and 2001 through 2003) (fig. 1). The only significant difference in trunk diameter recorded among English-rooted trees was in 1999 and 2000 at site 2, when trees on Chandler seedlings were smaller than those on Waterloo seedlings. In contrast, trees on Paradox seedlings had significantly larger trunk diameters than those on any of the English rootstocks from 1996 through 2003.

Individual year yields at site 2 were not significantly different among rootstocks in 1997, 1999 or 2003. Paradox-rooted trees had significantly greater yields than trees on all English rootstock sources in 2000 and 2001, and all but those on Carpathian seedlings in 1998 and Russian and Carpathian seedlings in 2002. Individual year yields were similar among English sources except for in 2000 and 2001, when Chandler-rooted trees had significantly lower yields than Carpathian-rooted trees, and in 2002, when Chandler-rooted trees yielded less than Russian-rooted trees.

Cumulative yield (1997 through 2003) at site 2 was greater for Paradox-rooted trees than for those rooted on any English seedling source. The cumulative yield of Chandler-rooted trees was less than that of all other English sources except Eureka. We attribute the generally greater yields of Paradox-rooted trees and lower yields of Chandler-rooted trees to differences in tree growth and size on these rootstocks, since yield efficiency was similar among rootstocks between 1998 and 2003 (data not shown).

Planning a new orchard

Our results show that most English rootstock seedling sources produced trees with similar growth and nut production. The exception was Chandler, which produced generally smaller and less-productive trees than the other sources at site 2. Walnut growers wishing to plant orchards on English rootstocks should avoid the use of Chandler seedlings.

No English seedling source tested at site 2 produced trees with growth and productivity as high as those of Paradox hybrids. Therefore, walnut growers needing blackline tolerance will likely incur some loss in early orchard growth and nut production as a result of planting English seedling rootstocks.

However, it remains to be determined — in this trial as well as in commercial practice — whether the growth and yield advantages of Paradox-rooted trees will be outweighed by the potentially longer life of English-rooted trees, since blackline incidence increases over the life of the orchard. In addition, because English-rooted trees are still considered more susceptible to Phytophthora root and crown rot, and to damage by lesion nematode, growers must carefully evaluate all the ramifications of their rootstock choice when planning a new orchard.

Walnut blackline disease, caused by cherry leafroll virus, is endemic in many California walnut-producing districts. This has spurred renewed interest in using English walnut seedlings as rootstocks.

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Careful rootstock selection can help to prevent blackline infection. *Above,* healthy walnut trees on English rootstock (variety ‘Chandler’) in an orchard near Linden.

RESEARCH ARTICLE

Covering hay in the irrigated Sonoran Desert decreases heat damage

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Alfalfa hay is a key ingredient in dairy cattle feed. Exposure to sun and heat can degrade the nutritional quality of hay and reduce its price.

Hay stored for prolonged periods of time decreases in value for feeding livestock. The irrigated Sonoran Desert of southeastern California and western Arizona is the hottest inhabited part of the United States, with summer temperatures routinely exceeding 100°F from May through October. We evaluated the effects of three methods of hay storage there during the summer: uncovered, under a roof and under a tarp. After 21 weeks, hay that was protected from summer solar radiation, either by the use of barn storage or plastic tarps, had more digestible content.

With temperatures routinely exceeding 100°F from May through October, the irrigated Sonoran Desert of southeastern California and western Arizona is the hottest inhabited part of the United States. In the Imperial Valley (part of this desert) mean annual rainfall is just 2.85 inches. Due to the scarcity of rainfall, baled hay is commonly stored unprotected along roadsides. If stored for prolonged periods, it can become extremely dry and increase in both neutral detergent fiber (NDF) and acid detergent fiber (ADF) (Rotz and Muck 1994). With less than 10% moisture, hay becomes brittle and unpalatable to livestock. As NDF and ADF increase, hay quality decreases — and consequently so do hay prices.

In addition, hay stored at temperatures greater than 100°F for prolonged periods of time may form Maillard products (Pitt 1990), condensates formed from nonenzymatic reactions of sugars and amino acids. Maillard products possess many of the chemical properties of lignin, which is highly indigestible. The formation of Maillard products in hay is indicative of decreased protein and dry matter (DM) digestibility (Thomas et al. 1982) and consequently reduced livestock performance. Maillard products may be quantified in forages by measuring nitrogen in the ADF fraction, also known as acid detergent insoluble nitrogen (ADIN) (Goering et al. 1972). Maillard products may be part of the ADF fraction.

Alfalfa hay stored during the Sonoran Desert summer loses DM and has increased NDF, ADF and ADIN (Guerrero and Winans 1999). The objective of our study was to quantify the digestibility attributes of summer-stored alfalfa hay in the Sonoran Desert under three storage treatments. In collaboration with the Autonomous University of Baja California (UABC) in nearby Mexicali, Mexico, at the end of the 21-week storage period we fed the treated hay to fistulated Holstein steers at the UABC agriculture school.

Hay storage and digestibility

Alfalfa hay was baled on June 2, 1998, at the UC Desert Research and Extension Center (DREC) in Holtville in the Sonoran Desert. Individual bales were randomly allocated to three storage treatments: (1) uncovered, (2) under a roof and (3) covered with a plastic tarp. We allocated about 2 tons of baled alfalfa hay per treatment, and the hay was stored for 21 weeks. Daily climatological data was recorded from the DREC weather station. Biweekly (at 3 p.m.) during the 21-week storage period, we took four hay samples of about 30 grams per treatment using a hay-coring device and recorded bale temperature and moisture. Hay samples were ground using a 1-millimeter screen, and were composited and placed in airtight plastic bags that were kept at a constant 72°F. At the end of the 21-week storage period, the ground alfalfa hay samples were evaluated for DM, NDF, ADF and ADIN. All composited hay samples were analyzed in triplicate and mean values reported.

The experimental hay was then transported about 30 miles southeast to the UABC agriculture school. To evaluate its digestibility attributes, we used

TABLE 1. Mean chemical attributes of alfalfa hay stored from June to November 1998, irrigated Sonoran Desert

Chemical attributes	Initial conditions*	Aug. 25, 1998 Uncovered†	Roofed	Tarped	SE
	.... %				
DM	86.0a‡	94.0b	92.0b	93.0b	0.76
ADIN, DM	0.10a	0.51b	0.51b	0.56b	0.02
N, DM	3.90	3.95	3.76	3.72	0.54
ADIN/N	2.59c	12.79b	13.56ab	15.56a	0.64
NDF	33.98ab	32.68b	36.89a	34.50ab	1.73
ADF	24.00a	19.63b	26.74a	23.81a	2.54
Bale temperature§	79.70c	107.96a	98.42b	107.06a	2.69

* Hay baled June 2, 1998; 10 randomly selected bales prior to treatment allocation.

† Uncovered = stored outside uncovered; roofed = stored outside under a roof and protected from sunlight; tarped = stored outside covered with plastic tarp.

‡ Means in a row with different letters differ ($P < 0.05$), paired *t*-test.

§ At 3 p.m.

Glossary

ADF = acid detergent fiber; indicator of forage indigestibility

ADIN = acid detergent insoluble nitrogen (N); measure of N in ADF fraction; indicates formation of Maillard products in forages

ADIN/N = N content of ADF as a fraction of total N

CP = crude protein

DM = dry matter

NDF = neutral detergent fiber; indicator of forage consumption

three fistulated (rumen, ileum and duodenum) 330-pound Holstein steers. (A fistula is a passage, made by a veterinarian, from the internal organs to the exterior of the body. The rumen, ileum and duodenum are parts of the digestive tract.) The fistulated steers were offered unlimited access to experimental hay and fed twice a day (7 a.m. and 5 p.m.) over three rotational 14-day periods: a 10-day adaptation period and a 4-day sampling period. Steers consumed only experimental hay during the 4-day sampling periods. To determine DM digestibility, 15 grams of chromium oxide per day were administered to the steers in ground alfalfa. Since chromium oxide is indigestible, it acts as a digestibility marker. By placing a known amount in the feed and then measuring the concentration in the feces, indigestability can be calculated.

During the 4 sampling days, ruminal, ileal and duodenal digestive fluids (digesta) were placed into 500-milliliter (ml) plastic containers. Sampling times were 7:30 a.m. and 1:30 p.m. on day 11, 9 a.m. and 3 p.m. on day 12, 10:30 a.m. and 5:30 p.m. on day 13, and 12 p.m. and 7 p.m. on day 14. Digesta samples were lyophilized, ground and stored. Fecal samples were dried in a forced-air oven at 122°F for 72 hours, ground and stored. On day 14, 500 ml of rumen liquor (the liquid fraction of ruminal contents) was collected from each animal and the samples were centrifuged to isolate rumen bacteria. Using the rumen liquor, we measured purine content to calculate bacterial protein; bacterial protein content could then be discounted from the alfalfa protein digestibility determinations. Digesta and fecal samples were subjected to the following chemical evaluations: DM, crude protein, NDF, ADF and chromium (by atomic absorption spectroscopy). The chemical attributes of the stored alfalfa hay were compared with paired *t*-tests. The chemical attributes of digesta and fecal samples were compared using a 3 × 3 Latin square design.

Sample moisture and temperature

The summer of 1998 was cool by Sonoran Desert standards, with temperatures at DREC consistently lower than long-term average temperatures (fig. 1, page 255). Our September and October hay samples were discarded because they became moldy due to an electrical malfunction in the storage area. Consequently, we report the results of 11 weeks of hay storage during the Sonoran Desert summer.

We recommend that hay bales be protected from excessive heat during summer in the irrigated Sonoran Desert.

When the experimental hay was baled on June 2, 1998, its moisture was 14%. By late August, dry matter (DM) for the uncovered (T1), under a roof (T2) and covered with a plastic tarp (T3) samples had increased 6%, 8% and 7%, respectively (table 1). Guerrero and Winans (1999) reported similar bale moisture for alfalfa hay treated similarly. Extremely dry hay is brittle and, when ground and added to feedlot or dairy cattle diets, often turns into the consistency of flour. These "fines" are detrimental to cattle health and to the overall digestibility of the diet.

At baling, mean bale temperatures were 80°F. After 11 weeks of storage at DREC, bale temperatures for the uncovered, roofed and plastic-tarped hays were about 108°F, 98°F and 107°F, respectively (table 1). Guerrero and Winans (1999) reported similar temperatures for alfalfa hay treated similarly. Hay stored at temperatures greater than 95°F for prolonged periods decreases in nutritive value (Goering et al. 1973; Yu and Veira 1977; Thomas et al. 1982; Pitt 1990).

Heat affects hay quality

The initial NDF and ADF contents of our experimental hays were 33.98% and 24.00%, respectively (table 1). The ADF content of the uncovered hay decreased significantly after 11 weeks, but it increased in the roofed and plastic-tarped samples; for NDF content, the uncovered hay basically remained the same but increased significantly in the roofed and plastic-tarped samples. Rotz and Muck (1994) reported that after prolonged storage, hay desiccation and loss of soluble carbohydrates result in NDF and ADF increases. In a similar experiment, Guerrero and Winans (1999) reported increases ($P < 0.05$) of both NDF and ADF after 11 weeks of storage. However, after 11 weeks of storage, summer temperatures during 1998 did not affect ($P > 0.10$) NDF or ADF in our current experiment as adversely as in our previous experiment (Guerrero and Winans 1999).

Goering et al. (1972) suggested that forages with greater than 14% ADIN/N

Top, **alfalfa hay is normally stored unprotected along ditch banks in the Sonoran Desert;** ***middle,*** **hay can be stored outside but protected from rain and sunshine;** ***bottom,*** **tarped hay.**

(nitrogen content of ADF as a fraction of total N) may be considered heat-damaged. In our experiment, ADIN/N increased significantly ($P < 0.05$) under all treatments, suggesting the formation of Maillard products (table 1). After 11 weeks of hay storage in the irrigated Sonoran Desert, Guerrero and Winans (1999) had ADIN/N levels of 13%, 17% and 17% for similar storage treatments. The previous baled-hay storage trial was done during the summer of 1993, which was warmer than during our 1998 trial, with mean monthly maximum temperatures from June through October of 114°F, 113°F, 118°F, 112°F and 105°F, respectively.

We regressed climatological data on the amount of heat damage in the stored hays (table 2). Initially, by means of correlation analyses, we evaluated the relationships between cumulative degree-hours greater than 95°F, bale moisture, ambient relative humidity, bale temperature, solar radiation, NDF, ADF, ADIN, ADIN/N and lignin. Variables that were highly correlated (> 0.80) were not selected for the regression analyses. We used a backward elimination procedure ($\alpha < 0.10$) to eliminate insignificant regressors.

Summer heat in the Sonoran Desert affected hay quality for the uncovered and plastic-tarped hays; these storage treatments had warmer bale temperatures than those under a roof (table 1). For the uncovered hay, bale temperatures (X_1) accounted for 62% of the total variability in ADIN/N percentage. For the plastic-tarped hay, cumulative degree-hours greater than 95°F (X_3) accounted for 70% of the total variability in ADIN/N percentage. Uncovered bales were exposed to direct sunlight while plastic-tarped bales were in full sunlight but protected from direct solar radiation. The independent variables in table 2 are both different temperature measures: X_1 is bale temperature and

X_3 is a measure of cumulative ambient temperature. Comparing the uncovered and plastic-tarped β_1 values shows that the effect of direct solar radiation on bale temperatures was 55 times greater on ADIN/N (a measure of total protein unavailability). Evidently the interaction of both temperature and solar radiation in the Sonoran Desert summer affected hay quality.

TABLE 2. Regression analyses of environmental factors affecting heat damage of alfalfa hay stored during summer, irrigated Sonoran Desert

Dep. variable (ADIN/N%*)	β_o	β_1	β_1 SE	β_1 $P > F$	r^2
Treatment†		Indep. variable			
Uncovered	–11.69	0.55 X_1‡	0.16	< 0.01	0.62
Roofed	–8.39	0.001 X_2	0.003	< 0.01	0.57
Tarped	5.71	0.010 X_3	0.002	< 0.01	0.70

* N in ADF fraction as a percentage of total nitrogen, a measure of heat damage or formation of Maillard products.
† Uncovered = stored outside uncovered; roofed = stored outside under a roof and protected from sunlight; tarped = stored outside covered with plastic tarp.
‡ X_1 bale temperatures taken at 3 p.m.; X_2 ambient relative humidity, %; X_3 ambient cumulative degree-hours > 95°F.

Reduced digestibility

Thomas et al. (1982) suggested that heat-damaged forages have both increased DM and decreased protein digestibility. In our experiment with the fistulated cattle, the total gastrointestinal-tract digestibility of crude protein and DM was greater ($P < 0.05$) for the roofed and plastic-tarped hays than for the uncovered hay (table 3). While both were subject to high ambient temperatures, the roofed bales were not exposed to the radiant energy of direct sunlight while the plastic-tarped bales were. The tarps used in this study were blue while those used by Guerrero and Winans (1999) were grey. The effects of tarp color on the quality of hay stored under extreme heat and radiant energy are not known.

Based on previous research and our current experiments, we recommend that hay bales be protected from excessive heat during summer in the irrigated Sonoran Desert, because unprotected alfalfa hay bales become heat-damaged and thereby decreased in overall digestibility.

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TABLE 3. Mean digestibility of nutrients by 330 lb. Holstein steers consuming alfalfa hay stored from June to November 1998, irrigated Sonoran Desert

	Treatment* Uncovered	Roofed	Tarped	SE
Consumption, kg/day				
DM	4.07	4.11	3.60	0.14
NDF%	1.30	1.42	1.25	0.08
ADF%	0.92b†	1.08ab	0.88b	0.11
CP	0.89a	0.81b	0.84ab	0.03
Ruminal digestion, % DM consumption				
DM	49.5b	59.2a	53.4ab	2.1
NDF%	40.7ab	48.0a	32.4b	6.0
ADF%	35.7ab	45.8a	25.5b	7.0
CP	46.9b	58.0a	55.0a	2.6
Total digestion, % of consumption				
DM	59.5c	66.2a	64.7b	0.7
NDF%	39.2b	53.2a	47.2ab	4.2
ADF%	33.0c	47.5a	37.9b	2.0
CP	76.7b	81.2a	80.6a	0.3

* Uncovered = stored outside uncovered; roofed = stored outside under a roof and protected from sunlight; tarped = stored outside covered with plastic tarp.
† Means in row with different letters differ, ($P < 0.05$) paired *t*-test.

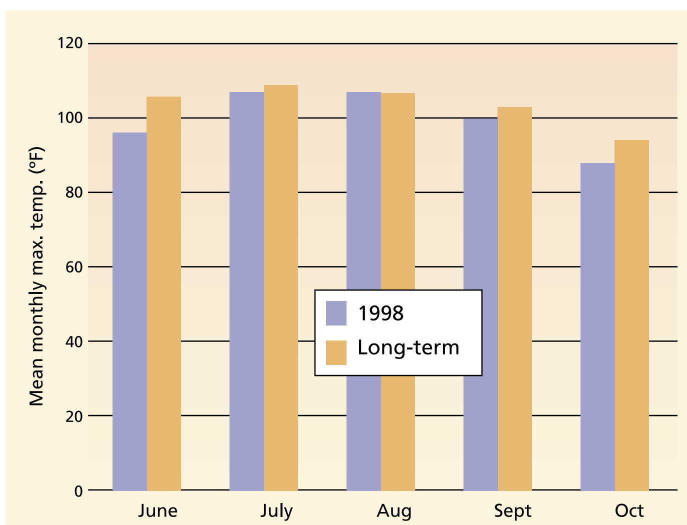

Fig. 1. Mean monthly maximum temperatures at UC Desert Research and Extension Center. The long-term mean was calculated over a span of 80 years.

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