



PLANT PEST
DIAGNOSTICS
BRANCH

ANNUAL REPORT
2002

Plant Pest Diagnostics Branch Annual Report 2002

Dennis E. Mayhew, Branch Chief

Mission

The primary mission of the Plant Pest Diagnostics Branch (PPDB) is to provide timely and accurate diagnostics in support of the California Department of Food and Agriculture (CDFA) pest prevention programs. The branch also serves as a scientific resource for a number of clients in addition to CDFA, including the United States Department of Agriculture, other federal and state agencies, County Agricultural Commissioners, University of California Cooperative Extension, the agriculture industry, and the public. The scientific and technical staffs contribute to global scientific knowledge in plant pest diagnostics and biosystematics.

Workload

The number of diagnostic samples processed in 2002 at the Plant Pest Diagnostics Center include:

Nematology	5,042
Plant Pathology(inc. special projects)	88,402
Entomology	41,529
Seed Sciences	3,861
Botany	4,150
Total	142,984

New Staff

Three new staff members joined PPDB staff during 2002. These include scientists Dr. Jeffrey Skevington (Hemiptera, Diptera) and Dr. Charles Bellamy (Coleoptera), and Margie Barela (Office Assistant). In addition, we have a federal employee (Julia Scher) assigned to our seed lab to develop web based identification keys.

Facility Changes

Work continued on adapting existing laboratory space to accommodate new molecular technology. PPDB has made a major commitment to expand molecular biological techniques to all levels of diagnostics in the five laboratories in the branch. In addition, a major renovation to the library was completed adding new compactor storage for scientific literature. The plant pathology laboratory added a new large capacity autoclave and a third large environmental growth chamber.

Collections

Two new curators, Dr. Gaimari and Dr. Bellamy, were named to manage the ever-expanding California Collection of Arthropods. This collection of approximately 1.8 million labeled specimens has an estimated value of over \$10 million. Other collections in PPDB including the literature collection (approximately 60,000 volumes), the plant herbarium (40,000 specimens), and the seed collection (over 50,000 specimens) are also expanding with new additions.

Research

Part of the ongoing mission of the branch is the research and publication of scientific papers by the professionals in the five laboratories. In the past year the scientific staff published 15 peer reviewed papers, 9 book chapters, and 15 non-peer reviewed publications on topics ranging from nematode, plant, and insect systematics, to reports of new plant diseases, to basic biology. In addition, PPDB scientists participated in a number of professional scientific meetings and symposia. Initiation of new research grants, including a \$350,000 grant for fruit fly genomics research, will greatly expand the research function in the Branch.

Projects

The major new project begun in 2002 was the initiation of planning for the merger of the diagnostics functions of PPDB with the University of California, Davis, using the transfer of the veterinary diagnostics function as a model. Eventually, a larger, more comprehensive diagnostic capability will provide advanced scientific support for the CDFA pest prevention programs.

A second initiative, funded by USDA, was begun in 2002 as a response to the need for better homeland security for agriculture. The creation of the National Plant Pest and Disease Diagnostics Network resulted in UC Davis being selected as the western regional center. Because the University lacks a diagnostic capability, PPDB was asked to partner with UCD to provide the scientific support. PPDB will now provide advanced diagnostic support to nine western states and the Pacific protectorates.

For Official Use Only

Plant Pest Diagnostics Branch
California Dept. of Food and Agriculture
3294 Meadowview Road
Sacramento, CA 95832-1448

Terry N. Seeno : Director & Producer
Thomas D. Eichlin : Editor
Scott Kinnee : Technical Advisor

Cover: Conidiophores and spores of the *Botrytis* state of *Botryotinia sphaerosperma*. Original drawing by Diana Fogle, rendered by Dennis Mayhew using Adobe Photoshop 7.0.

Spore diameter 23-25 μm .

March 15, 2003

Table of Contents

ENTOMOLOGY

(pages 1-20)

Brochosomes—Unique Structures Useful in the Identification of Sharpshooter Egg Masses Scott Kinnee	1-2
Video-camera Diagnosis of Border Station Rush Samples by Internet John Sorensen	3
California State Collection of Arthropods: 2002 Progress Report Chuck Bellamy and Steve Gaimari	4
Research on Western Hemisphere Sesiidae Tom Eichlin and Scott Kinnee	5
Freshwater Snails in the Aquarium Trade in California Alan Hardy	6
A New Leucopine Genus with species Attacking <i>Ceroplastes</i> Wax Scales in South America Steve Gaimari	7-8
Use of Raman-Atomic Force Microscopy on the Compound Eyes of Flies Steve Gaimari	9-10
The Species of <i>Leucopis</i>, Subgenus <i>Leucopella</i> Malloch, from Northeastern Africa and Yemen Steve Gaimari	11-12
Diptera Systematics—Focus on Pipunculidae Jeff Skevington	13-17
Simplified Digital Photography for Museum Work Eric Fisher and Steve Gaimari	18
Catalog of Leaf Beetles of America North of Mexico Terry Seeno	19
American Beetles and Plant Pest Diagnostics Branch Chuck Bellamy, Ron Somerby and Fred Andrews	20

NEMATOLOGY

(pages 21-27)

Creation of a Non-indigenous Nematode Pest List . . . Ke Dong	21-22
Preliminary Studies on the Culturing and Feeding Activity of a Predatory Nematode . . . John Chitambar	22-25
Plant Pest Diagnostics Branch—Annual Report 2002	v

The 34th and 35th Annual California Nematology Workshops Bob Hackney	26
Regulatory Diagnostics of <i>Meloidogyne mayaguensis</i> Using Molecular Technology Bob Hackney	27

SEED SCIENCE
(pages 29-38)

Seed Technologist Training J. Effenberger, E. Harris, D. Merer, P. Peterson, E. Ramos, M. Stephenson, and C. Weiner	29-30
Customized Tests Provide Expanded Description of Planting Seed Quality Jim Effenberger and Marion Stephenson	31-32
Effect of Temperature on Germination of Blue Wildrye, <i>Elymus glaucus</i> Buckley Marian Stephenson, Evelyn Ramos and Jamie Sallee	33
Laboratory Germination of a Potentially Invasive Plant Pest, <i>Sesbania punicea</i> Jamie Sallee and Marian Stephenson	34
Comparison of Purity Testing Methods of Weeping Alkaligrass (<i>Puccinellia distans</i> (Jacq.) Parl.) Deborah Meyer and Jim Effenberger	35-37
USDA-CDFR Collaboration Julia Scher	38

PLANT PATHOLOGY
(pages 39-48)

Determination of a Potential Recombinant Potato Virus Y(PVY) Using serological and Serological and Nucleic Acid Based Analysis Tongyan Tian, Joyce Tuttle, and Terra Irving	39
Laboratory Diagnosis of <i>Phytophthora ramorum</i> from Field Samples Cheryl Blomquist and Tom Kubisiak	41-43
Nursery Testing of Prune Dwarf Virus, Prunus Necrotic Ring spot Virus, Grapevine Fanleaf Virus, and Grapevine Leafroll Associated Viruses YunPing Zhang and Umesh Kodira	44
The Epidemiology of Pierce's Disease Barry Hill and Jennifer Hashim	45-46
2002 Calendar Year Annual Report Tim Tidwell	47
Report for the Year 2002 Dan Opgenorth	48

Brochosomes – Unique Structures Useful in the Identification of Sharpshooter Egg Masses

Scott Kinnee

A method for differentiating egg masses of two closely related species of sharpshooters in the genus *Homalodisca* using a nucleic acid diagnostic was

described in the 2001 Plant Pest Diagnostics Branch Annual Report¹. The diagnostic, utilizing a mitochondrial DNA restriction fragment length polymorphism (mtDNA RFLP), was developed to separate the egg and nymph stages of *Homalodisca lacerta* (smoketree

sharpshooter), a native California species, from *H. coagulata*, (glassy winged sharpshooter), an introduced pest capable of transmitting Pierce's disease.

Although a very reliable test, the PCR based diagnostic could not be used on all egg mass samples submitted to the PPDB for analysis.

Egg masses that were completely hatched or parasitized did not contain sufficient DNA for amplification.

A conversation with a visiting scientist, Dr. Roman Rakitov, suggested a possible alternative method for telling the egg masses apart. Dr. Rakitov, a researcher at the Illinois Natural History Museum, has done extensive studies on unique objects called brochosomes. Brochosomes are durable, crystalline structures composed

of protein and lipids excreted from the malpighian tubules of leafhoppers (Cicadellidae). Although most, if not all, cicadellids produce brochosomes, it appears

that only female sharpshooters of the tribe Proconiini (Figs. 5 & 6), to which the *Homalodisca* belong, produce specific brochosomes; these are used to coat the egg masses at the time of oviposition. To accomplish this, the brochosomes are excreted by

the female sharpshooter and placed onto patches on the wings (Fig. 1). The material is then rubbed onto the newly laid egg mass (Fig. 2). There is video of this behavior on Dr. Rakitov's website². The true purpose of these structures is still in question.

Egg masses submitted to the laboratory

for identification were prepared for PCR amplification in the usual manner. In addition, the plant tissue covering the egg mass was coated with gold/palladium and viewed with a scanning electron microscope.

Brochosomes were also removed from

the wing patches of identified female sharpshooter museum specimens for comparison with SEM. Under high magnification (>2000 X), it became apparent that the brochosomes placed on the

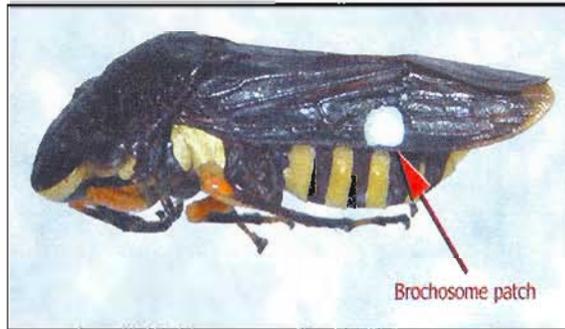


Figure 1. Female Sharpshooter with brochosomes



Figure 2. Sharpshooter egg mass with brochosomes

egg masses by the two species in question were quite different. The brochosomes produced by *H. coagulata* (Fig. 3) are approximately 10 micrometers long, with a shape resembling a bow tie. Those brochosomes placed on the egg mass surface by *H. lacerta* (Fig. 4) are much smaller, approximately 1 micron in size, with an irregular, punctate, round shape, much like a golf ball. These structures matched the comparison material from preserved adult female sharpshooters. PCR results correlated 100% with those identifications made by SEM.

The advantages of using brochosomes for identification for these two species are: 1) it is faster; 2) it is less expensive; 3) the presence of other DNA is irrelevant and 4) brochosomes are persistent on leaf surfaces even after egg eclosion.

References

1. California Plant Pest and Disease Report. Volume 21, Nos. 1-3, pp. 20-21. January-June 2002.
2. <http://www.inhs.uiuc.edu/staff/cbd/rakitov.html>

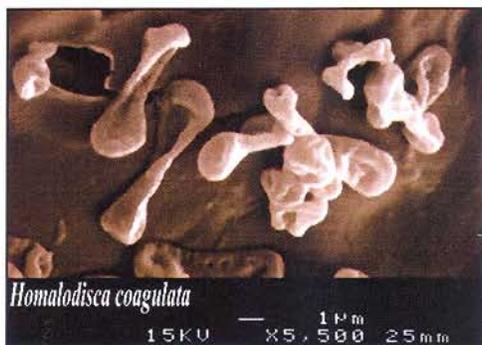


Figure 3. Brochosomes of *H. coagulata*

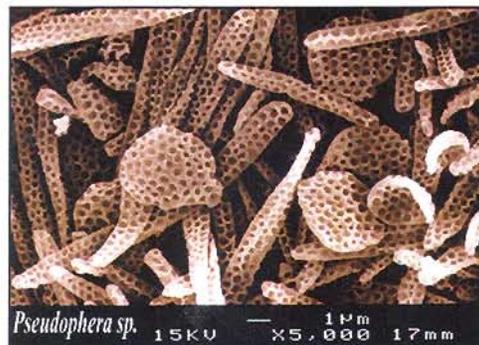


Figure 5. Brochosomes of *Pseudophera* sp.



Figure 4. Brochosomes of *H. lacerta*

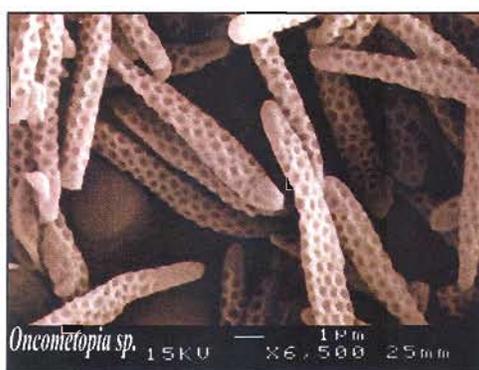


Figure 6. Brochosomes of *Oncometopia* sp.

Video-camera Diagnosis of Border Station Rush Samples by Internet

John Sorensen

The Pest Exclusion branch runs several Agricultural Border Stations at key road and Interstate highway access points into California. It is the mission of these stations to intercept and exclude plant pests as they enter the state at these points. Because this important function lessens the chance of any introduction and establishment of a rated insect pest, which may then require a costly eradication program, these stations and their program have long been considered vital to the preemptive protection of the agricultural industry of the state.

At the stations, interstate trucking shipments are stopped and screened for pests. When a pest is found, it is sent as a "rush" sample to the Plant Pest Diagnostic Branch, in Sacramento, for identification before action can be taken. Until the identification can be made in Sacramento, trucks must frequently wait at the station. Alternatively, the truck may be rejected, and then required to exit the state, or it may be sealed and sent to its destination county, where the county waits for the Sacramento identification before allowing the truck seal to be broken and the truck to be unloaded.

Prior to 2002, at the Blythe and Needles stations, where the bulk of interstate trucking passes, shipment of such "rush" samples to Sacramento could take two days. Because of workweek business hours and necessary transit time, if a truck entered after Thursday morning, often the sample was not received at Sacramento before Monday noon. This imposed a weekend layover for some trucks at the stations. This previously necessary imposition precluded post-Thursday morning trucking from proceeding to their California destinations for delivery and reloading with out-of-state shipments before Friday afternoon – an efficiency and cost goal of most truckers.

In February 2002, to decrease the station-to-lab shipment time, the Blythe and Needles border stations and the Sacramento diagnostics lab implemented a new protocol for "rush" samples. These stations were equipped with video camera equipped microscopes

and Internet access (now satellite-based). Station supervisors were trained in the protocol for dissection and preparation of specimens, capturing a video image of the insect on the microscope camera, and the Internet e-mail shipment to the Sacramento lab, where an Insect Biosystematist can make diagnosis and rating.

In February and March 2002, this protocol was tested successfully for Red Imported Fire Ant interception on incoming honeybee hives used for spring pollination of orchards. By May, the protocol had been expanded to all "rush" ant samples coming through the Blythe and Needles stations. Later in the summer, "rush" samples from the Vidal station were driven to Blythe for processing to Sacramento.



While it is not possible to employ this technology for all insect pests because of the nature of their diagnostic characters, the protocol has since been used for some fly and moth larvae, beetles and scales. The protocol is particularly useful in ants, which account for 85 to 90+ percent of "rush" border station samples.

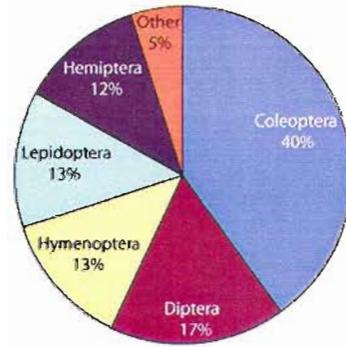
Because CDFA's computer network servers can be remotely accessed by telephone-dialup connection, it has been possible for stations to notify the ant specialist between 6 A.M. and 11 P.M., including weekends and holidays, which allows immediate remote diagnosis and rating of the sample. Typical response time to notifications is less than an hour, once the pest photo has been sent to Sacramento, allowing a considerable decrease in trucking down time. Thus, this successful protocol increases the efficiency of both interstate commerce and the Diagnostic lab's response time, and maintains the protection of the California agriculture.



California State Collection of Arthropods: 2002 Progress Report

C. L. Bellamy, and S. Gaimari

The California State Collection of Arthropods (CSCA) is an international resource for research and identification of various groups of the Phylum Arthropoda, especially Insecta. The total number of prepared specimens exceeds 1.5 million, making this the 6th largest collection in California; the policy of retaining primary type specimens has only recently been instituted, so very few are included. This collection is maintained by the Entomology Lab of the Plant Pest Diagnostic Branch of the California Department of Food & Agriculture (CDFA) as an integral feature of the identification services provided to the citizens and business interests of the State and our peers and colleagues, both nationally and internationally; two curators directly supervise the care, use, growth and development of CSCA. We encourage the use of this collection for



of California and elsewhere in the country. Prominent visitors included Douglass Miller, USDA, Beltsville; Allen Samuelson, Bishop Museum, Honolulu; Brett Ratcliffe and Mary Liz Jameson, University of Nebraska, Lincoln. In addition, loans were made to several researchers working in Coleoptera, Diptera, Hymenoptera and Crustacea.

The CSCA is now registered with the U.S. Fish & Wildlife Service, the national management authority for the Convention on International Trade in Endangered Species of Wild Fauna and Flora and with Environment Australia, which will permit PPDB/CSCA scientists to legally borrow, collect or exchange with Australian institutional collections. In an effort to make this important resource better known in the entomological community, information was posted on PPDB web site:

<http://www.cdffa.ca.gov/phpps/ppd/Entomology/CSCA/CSCA.htm>

research on the taxonomy and systematic relationships of specific arthropod taxa.

Following the dedication of CSCA by Secretary Lyons on October 15, 2001, as reported in the 2001 PPDB annual report, Dr. Alan R. Hardy stepped down as curator, and we accepted the responsibility to serve as co-curators. During 2002, the collection was used by PPDB and CDFA entomologists, professional, student and amateur entomologists from various parts

This information includes a history of the collection, information on the loan of specimens and the proposal of a Research Associate program to encourage continued growth and utilization of the collection. An effort to database the entire collection and serve such a database on the web will move forward with the arrival of a computer for the collection room and the eventual appointment of a Collection Manager.

Research on Western Hemisphere Sesiidae

Thomas D. Eichlin and Scott A. Kinnee

Progress continued with ongoing research involving the clearwing moths (Lepidoptera: Sesiidae) of the Western Hemisphere. In order to prepare for the return of a specimen loan, certain Brazilian species were documented in the following publication: Eichlin & Kinnee, 2002, Brazilian Sesiidae in the collection of the Universität des Saarlandes, Saarbrücken, Germany (Lepidoptera), *Zootaxa* 108: 1-15. This international journal (our own Dr. Charles Bellamy serving as subject editor) produces both an on-line version and a hard copy version of each paper printed.

Tom and Scott are feverishly working to complete a monograph on the Mexican sesiids. Many species must be described, redescribed or at least diagnosed. The clearwing moth fauna of Mexico includes about 125 known species, of which approximately 25 were previously unknown. All salient features must be figured. This involves dissections and photography using various techniques to produce the best images possible for publication. All adults are being rendered in color, since this is critical for identification purposes.

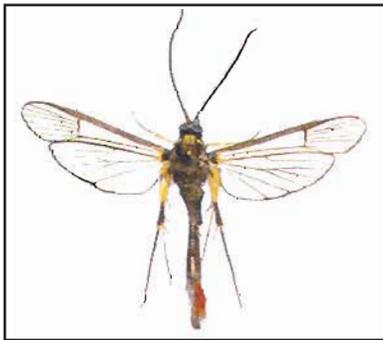


Figure 1. *Synanthedon hemigymna*
Zukowsky, female (holotype)

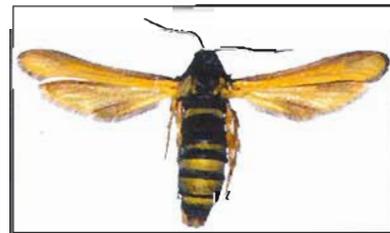


Figure 3. *Zenodoxus palmii*
(Neum.), female

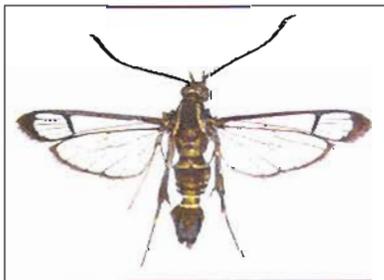


Figure 2. *Carmenta plaumanni*
Eichlin, male (holotype)

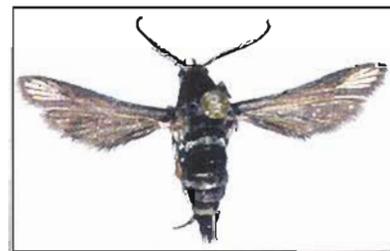


Figure 4. *Zenodoxus mexicanus*
Beut., male

Freshwater Snails in the Aquarium Trade in California

Alan Hardy

Much recent attention has been directed to the Channeled Applesnail, *Pomacea canaliculata*, a potentially major pest of rice, which has been sold under a number of common names, including Golden Snail and Mystery Snail. In addition to *P. canaliculata*, two other species of *Pomacea*, *P. haustrum* and *P. bridgesi*, have been intercepted at pet shops or their suppliers. As a result of this attention, other species of freshwater snails have been collected and submitted for identification.

Another species in the family Ampullariidae, known as the Goldenhorn Marissa or the Giant Ramshorn Snail, *Marissa* sp., prob. *cornuaurietus*, has been submitted. The economic potential of this South American native (with an introduced population in Florida) is uncertain, but USDA specialists recommend action against all species in the family Ampullariidae, except *P. bridgesi*.

Most snails encountered are species of *Physa* or *Physella*, which are easily recognized by their left-handed spiral. These have been in the trade for many

years and don't appear to be of concern. Other common aquarium snails are the ramshorn snails in the genera *Planorba* and *Planorbella*. These too seem benign. One easily recognized species is *Planorbella scalaris*, which is occasionally encountered in potting soil. This species is known as the Mesa Ramshorn, for its flattened top.

The increased interest in freshwater snails, most of which seem to be imported from Florida, have uncovered a variety of genera, which are not commonly seen in commerce. These may be mere contaminants on freshwater plants, rather than intentional imports. Included are the freshwater limpets in the family Ancyliidae (a number of genera), the turret snails in the genus *Elimia*, and Siltsnails (several genera).

The best reference for identification of Florida freshwater snails, and really most snails in the aquarium trade, (*Freshwater Snails of Florida—a Manual for Identification*, Fred Thompson, 1984) has been available from the University Presses of Florida, 15 NW 15th Street, Gainesville, FL 32603.

A New Leucopine Genus (Diptera: Chamaemyiidae) with Species Attacking *Ceroplastes* Wax Scales in South America.

Gaimari, S.D., & V.N. Tanasijtshuk. 2001.
Systematic Entomology 26: 311-328.

A new genus, *Echinoleucopis*, was proposed within the fly family Chamaemyiidae (Diptera). Within *Echinoleucopis*, five new species (*bennetti*, *grioti*, *iota*, *macula*, and *nigrolinea*) were described, in addition to a redescription for the only previously described species, *Leucopina ceroplastophaga*. In addition, evolutionary relationships among the included taxa are hypothesized. All known species of the genus are from South America and are predators on eggs within an ovisac of wax scales in the genus *Ceroplastes* Gray (Hemiptera: Coccidae).

Chamaemyiidae, also known as silver flies, represents a group of larval predators attacking aphids, scales and mealybugs. There are currently 27 described genera with nearly 250 described species. The vast majority of species-level work has been undertaken in the Palearctic Region; whereas, very little of the South American fauna is known. Although the species of some genera are quite general in their feeding habits, many genera are restricted to a particular host type. For example, species of *Leucopomyia* are predators on eggs within an ovisac of scales in the genus *Pulvinaria* (Coccidae); species of *Neoleucopis* attack woolly aphids (Adelgidae) on gymnosperms (evergreens); and species of *Chamaemyia* attack mealybugs (Pseudococcidae) in leaf sheaths of grasses. The genus *Leucopis* is generally known for having species with wide host range, with some feeding on more than 100 host species, whereas, others are restricted to a single host species.

Echinoleucopis Gaimari & Tanasijtshuk

Biology. All known members of the genus are predators of eggs within an ovisac of wax scales in the genus *Ceroplastes*.

Distribution. Most species of this genus are found in areas in the northern part of the Southern Cone region of South America, whereas, few are known to occur north of the Tropic of Capricorn. One species is found in the dry parts of Chile south of the Atacama Desert, whereas, others are found from the Brazilian Highlands to Patagonia. Note that all references herein to "Provinces" are biogeographical regions, not political provinces.

1. *Echinoleucopis ceroplastophaga* (Blanchard)

Biology. This species was described, in part, from a series collected as a predator of *Ceroplastes*

bruneri on guava, *Psidium guayava* (Myrtaceae). Various studies also added species to the list of scales attacked (*Ceroplastes grandis*, *Ceroplastes leonardianus*), noting that the egg stage of the scale was the target of predation. *Ceroplastes bruneri* is found on the following host plants: *Acacia bonariensis*, *Acacia furcatispina*, *Acacia retinodes*, *Acacia riparia*, *Ceratonia siliqua*, *Manganaroa furcata*, *Manganaroa platensis*, *Parkinsonia aculeata*, (Fabaceae), and *Eugenia edulis* (Myrtaceae); *Ceroplastes leonardianus* is found on the following host plants: *Eupatorium buniifolium*, *Tessaria absinthioides* (Asteraceae), *Heliocarpus* spp. (Tiliaceae), *Larrea divaricata* and other *Larrea* spp. (Zygophyllaceae); and *Ceroplastes grandis* has been recorded from at least 29 different species within 24 different genera.

Prior studies reported some very interesting observations on feeding behavior of larvae, noting that over 30% of *Ceroplastes grandis* obtained in December had at least one larval *Echinoleucopis ceroplastophaga*, and 2.7% had two larvae. A small portion of *Ceroplastes grandis* eggs were always left unconsumed by the feeding larva. According to collection information, adults are active in November and December.

Distribution. This species is known from the northern part of Argentina, from the drier southern part of Espinal Province into Chaqueña Province, and farther south in Patagonian Steppes Province. Most of the localities are at lower elevations, 200-500 m, although the specimens from Tucumán are from the foothills of the Andes, at elevations above 500 m. This distribution is consistent with known hosts, *Ceroplastes bruneri*, *Ceroplastes grandis* and *Ceroplastes leonardianus*, which also occur in areas between these provinces.

2. *Echinoleucopis bennetti* Gaimari & Tanasijtshuk

Biology. This species is known to attack *Ceroplastes* spp. on *Baccharis dracunculifolia* and *Baccharis spicata* (Asteraceae). Several species of *Ceroplastes* are known to feed on the former plant (including *Ceroplastes iheringi*, *Ceroplastes longiseta*, *Ceroplastes lucidus* and *Ceroplastes novaesi*), while none are known to feed on the latter.

Distribution. Known from Brazil, with the following specific localities: São Paulo (São Paulo); Pelotas and Porto Alegre (Rio Grande de Sul). There

is a moderate distance between the known localities, which are along the eastern coast of Brazil. Pelotas and Porto Alegre are in the coastal northeastern corner of Pampeana Province; whereas, São Paulo is in the low elevation, coastal Brazilian Highlands of Atlantica Province.

3. *Echinoleucopis grioti* Gaimari & Tanasijtshuk

Biology. This species has been reared as a predator of *Ceroplastes grandis* and an undetermined *Ceroplastes* species.

Distribution. Like *Echinoleucopis iota*, the known localities for this species are widely disjunct, from the foothills of the Andes in the northwestern temperate part of Argentina's Chaqueña Province to Pampeana Province of eastern Argentina and Patagonian Steppe Province. This distribution is consistent with the known distribution for the host species *Ceroplastes grandis*, and it is expected that *Echinoleucopis grioti* is more widespread but undercollected.

4. *Echinoleucopis iota* Gaimari & Tanasijtshuk

Biology. This species has been reared as a predator of *Ceroplastes* spp.

Distribution. This species is known from Metán, in the state of Salta, Argentina, and from Montevideo, Uruguay. These localities are widely disjunct, from the foothills of the Andes in the northwestern temperate part of Argentina's Chaqueña Province to Espinal Province along the southern coast of Uruguay.

5. *Echinoleucopis macula* Gaimari & Tanasijtshuk

Biology. This species is, at least in part, an egg predator attacking *Ceroplastes* spp. on guava,

Psidium guajava, and *Ceroplastes cirripediformis* on an unknown plant, and an undetermined *Ceroplastes* species on *Celtis tala* (Ulmaceae). There are at least nine species of *Ceroplastes* that feed on guava in this part of the world (including *Ceroplastes actiniformis*, *Ceroplastes campinensis*, *Ceroplastes cirripediformis*, *Ceroplastes floridensis*, *Ceroplastes grandis*, *Ceroplastes janeirensis*, *Ceroplastes psidii*, *Ceroplastes rusci*, and *Ceroplastes sinensis*), *Ceroplastes cirripediformis* is known from well over 100 host plants worldwide, and the only *Ceroplastes* species known to feed on *Celtis tala* is *Ceroplastes confluens*.

Distribution. This species is known from Brazil, with the following specific localities: Analandia, São Paulo; Brasília, Distrito Federal; and Paraná, Entré Rios. These areas are in the Brazilian Highlands of the northern part of Parane Province and the southern part of Cerrado Province. Also known from Las Breñas, Argentina, in Chaqueña Province. This area is consistent with the known host species *Ceroplastes cirripediformis* Comstock.

6. *Echinoleucopis nigrolinea* Gaimari & Tanasijtshuk

Biology. This species is known to attack *Ceroplastes ceriferus* on *Baccharis rosmarinifolia*.

Distribution. Known only from Chile, with the following specific localities: La Cruz, Valparaiso; Quebrada de la Plata, Santiago; and near Vicuna, Coquimbo. The inclusive area is in the northern part of Cordillera de la Costa, south of the Atacama Desert, specifically in Central Andean Cordillera Province. The known host species, *Ceroplastes ceriferus*, is also known from Chile and Brazil but not Argentina.

Collaborative Research on the Use of Raman-Atomic Force Microscopy on the Compound Eyes of Flies.

Anderson, M.A., and S.D. Gaimari.
Journal of Structural Biology (in press)

The lens surfaces of the compound eyes in several species of flies and related groups were analyzed using a recently developed Raman-Atomic Force Microscope (RAFM). This was the first demonstration of this kind of spectroscopy on an intact biological surface. For the snipe fly *Chrysopilus testaceipes* (Rhagionidae), this reveals unique cerebral cortex-like surface ridges with regular variation in height and surface chemistry. Most other higher flies displayed the same morphology, while other taxa displayed various other characteristics, such as a nodule-like or coalescing nodule-like morphology, a smooth morphology with distinct pits and grooves, or an entirely smooth surface. The variation in microstructure and surface chemistry provides a new information source for studying evolution of flies and other insects.

Species within the insect order Diptera (true flies), with their prominent compound eyes, are one of the most micro-photographed subjects. Several years ago we acquired the first Atomic Force Microscope (AFM) scans of the compound eyes of several species, including the house fly (*Musca domestica*), the blue bottle fly (*Phormia regina*), and a snipe fly (*Symphoromyia* sp.), noting that the individual lenses or facets (ommatidia) have unusual ridges in a cerebral cortex-like pattern over the entire surface. We followed up our initial observations with an AFM analysis of several additional fly groups and also applied a newly developed RAFM to examine these surfaces. The resulting paper in press discusses our methods and results for one species of snipe fly, *Chrysopilus testaceipes*, primarily for the purpose of describing and discussing RAFM techniques for use with biomolecular surfaces. This work contributes to ongoing efforts in fly research, adding a new source of information that has been largely neglected (surface chemistry and microscale morphology) to understand evolution.

RAFM, a member of the scanning probe microscope family of instruments, can acquire sub-micron (less than 1/1000 of a mm) scale topography and spectro-chemistry of surfaces. The RAFM works in two modes, conventional and enhanced. In the conventional mode it acquires a micro-focused chemical signal and topography from the same location. In the enhanced mode, the RAFM uses a special gold or silver coated

AFM tip to locally enhance the chemical signal. This allows very high resolution (50 nanometers or better (one nanometer is less than 1/1,000,000 of a mm)) spectroscopy to be acquired from the sample surface.

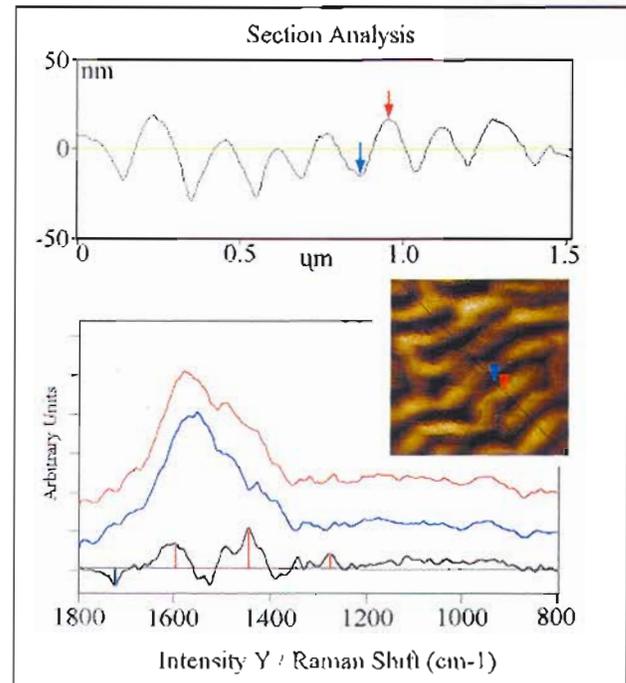


Figure 1. RAFM analysis of *Chrysopilus testaceipes*. Upper graph is the cross section of the eye facet. The corresponding AFM image (offset) shows the cerebral cortex-like ridges. Lower graph shows the ridge top chemistry (red, top spectrum) and compared to the bottom trough (blue, bottom spectrum), revealing variation in the fatty acids and protein groups on and off the ridges, with the top ridge showing more protein and water repelling character. Lower graph (black line) is subtraction of trough from ridge spectrum.

The ommatidium of *Chrysopilus testaceipes* has a cerebral cortex-like ridge structure that is revealed by conventional AFM imaging (Fig. 1). The height variation is regular in the cross section with ~190 nanometers between ridges, and a depth of about 20 nanometers from ridge to trough. There is also a corresponding variation in the surface composition (Fig. 1). The top of the ridges shows more protein character and water repelling character and the troughs show more fatty acids. This periodic structure and overall morphology could serve to prevent the eyes from

conspicuously reflecting light and may serve to control surface wetting; the chemistry could further modify and extend these and other functions.

The conventional AFM imaging of other taxa examined (Dipteran and non-Dipteran) shows significant morphological variation, as follows.

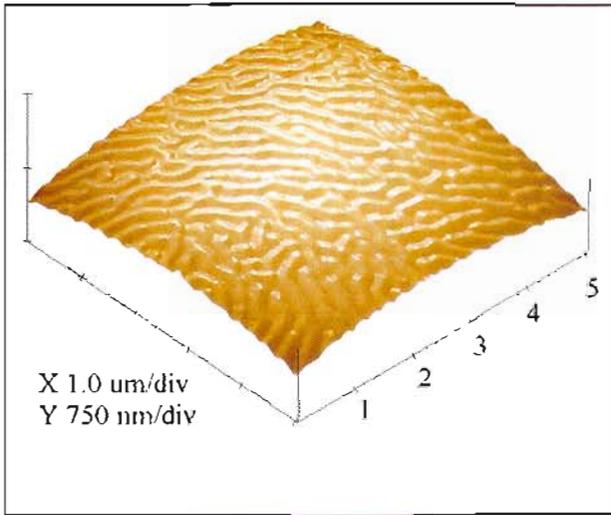


Figure 2. AFM image of an ommatidium of *Sylvicola fenestralis* showing the cerebral cortex-like pattern of ridges.

1) *Bittacus chlorostigma* (Mecoptera: Bittacidae), representing the closely related non-fly group, has a smooth eye surface with little or no notable surface topography.

2) *Tipula (Triplicitipula)* sp. (Tipulidae – crane flies) displayed a nodule-like morphology, with a uniform series of bumps over the entire surface. Similar morphology is found in other insects, referred to as “corneal nipples,” although these may not represent the same structures in other insects.

3) *Dilophus orbatus* (Bibionidae – march flies) displayed a more or less flattened morphology, with distinct pits and grooves throughout. Another march fly, *Bibio marci* (Linnaeus), was noted to lack “corneal nipples.”

4) *Tabanus punctifer* (Tabanidae – horse flies) displayed a pseudo-nodular morphology, with adjacent sets of bumps coalesced to form short ridges. Interestingly, the structure for several species of horse flies has been referred to as “corneal nipple” arrays, although the structure is quite different from that found in other insects with this supposed morphology. The morphology seen here may be a transitional form from nodule-like structure to bifurcating ridges.

5) *Sylvicola fenestralis* (Anisopodidae) and most higher flies sampled (with the exception of #4 above) displayed the series of clearly anastomosing and bifurcating ridges in a cerebral cortex-like pattern over the entire eye facet surface, as is found in *Chrysopilus testaceipes*, and several other groups of flies, including various snipe flies, hover flies, house flies, and blow flies.

The Species of *Leucopis*, Subgenus *Leucopella* Malloch (Diptera: Chamaemyiidae) from Northeastern Africa and Yemen.

Gaimari, S.D., & A. Raspi. 2002.
African Entomology 10: 241-264.

The genus *Leucopis* contains slightly less than half of the described species in the family. Most species of the subgenus *Leucopis* are predators of aphids on a variety of plants, although some attack scales and mealybugs as well. The subgenus *Leucopella* was originally described from Kenya with the single species *Leucopis (Leucopella) africana*, which was recognized as a potentially important predator of the coffee pest, *Planococcus citri* (Hemiptera: Pseudococcidae). Later, in the description of *Pseudococcus concavocerarii*, immature stages of *Leucopella africana* were found among specimens of this mealybug on foliage and green stems of coffee in Kenya. As far as was known previously, members of this subgenus were larval predators attacking only mealybugs (Hemiptera: Pseudococcidae), but our evidence suggests they attack a wider variety of scales as well. The northeastern African fauna of this subgenus, found along various parts of the Great Rift Valley and across the Red Sea on the Arabian Peninsula, appears distinct from that of southern Africa.

The current study is focused on the species from northeastern Africa, including Eritrea, Ethiopia, Kenya, the eastern part of the Democratic Republic of the Congo, and Uganda; species surely occur in the other countries of northeastern Africa, but no specimens are currently known. A greater diversity of species occurs in South Africa, but the northeastern fauna is interesting from the standpoint that it appears restricted to the Great Rift Valley and its bordering foothills and highlands, appears completely different from the southern fauna, and one species occurs across the Red Sea on the border of the Ramlat al-sab'atayn desert region of Yemen, which is the first record for the subgenus on the Arabian Peninsula.

Leucopis (Leucopella) Malloch

Biology. All known members of the genus are predators of various scales and mealybugs, from eggs within a scale ovisac to free-living mealybugs.

Distribution. Species of this subgenus are found in Africa (Great Rift Valley and southern Africa), southwestern Arabian Peninsula (Yemen), and in South America.

1. *Leucopis (Leucopella) africana* Malloch

Biology. This species has been collected in all months except for March, July, September, November, and December. This is known as a predator of *Planococcus citri*, *Planococcus kenyae*, and *Pseudococcus concavocerarii* on coffee. The specimens in the current study also indicate that this species attacks other scales on additional hosts. Among these, they are known to attack a species of *Spilococcus* (Pseudococcidae) on leaves of *Cassia siamea* (Caesalpinaceae), and *Icerya purchasi* (Margarodidae) on pigeon pea, *Cajanus cajan* (Fabaceae). Additionally, this species attacks the following prey species, without reference to host plant: *Planococcus trispinosus* (Pseudococcidae), and *Ceronema africana* (Coccidae).

Distribution. This is the most widely distributed species within this subgenus, being known from Eritrea and Ethiopia through Kenya, and perhaps as far south as Tanzania. This distribution appears to encompass the subtropical desert/semidesert ecoregion of the northern and eastern Great Rift Valley.

2. *Leucopis (Leucopella) ardis* Gaimari & Raspi

Biology. This species has been collected in September and January, and attacks scales on several plants. It is known to attack a possible species of *Icerya* on coffee, and a sheet forming mealybug on *Albizia stipulata* (Fabaceae, subf. Mimosoideae). The only mealybug known from this plant in Africa is *Maconellicoccus ugandae* (Pseudococcidae), although 4 additional pseudococcids known from northeastern Africa, *Ferrisia virgata*, *Maconellicoccus hirsutus*, *Nipaecoccus viridis*, and *Rastrococcus iceryoides*, are known from other species of *Albizia*. This species is also known from an unknown host on *Gliricidia maculata* (Fabaceae), a legume introduced into Africa from Central America, and a common shade plant in coffee plantations. No pseudococcids are known from *Gliricidia maculata* in northeastern African coffee plantations, but two species of scales, *Vitrococcus conchiformis* and *Coccus longulus*, are known to attack this plant. Another northeastern African mealybug, *Dysmicoccus brevipes*, is known to attack other species of *Gliricidia*.

Distribution. This species is distributed in Uganda and the Democratic Republic of the Congo. This

appears to represent a tropical dry forest and savannah ecoregion distribution in the western Great Rift Valley, which may extend as far south as Mozambique.

3. *Leucopis (Leucopella) euryvitta* Gaimari & Raspi

Biology. This species has only been collected in December, as a predator of a species of *Pulvinaria* (Coccidae). The only species of *Pulvinaria* known from Eritrea is *Pulvinaria dicrostachys*, which is only known to attack *Dichrostachys nutans* (Fabaceae, subf. Mimosoideae). Species of other scale genera, such as *Ceroplastodes*, *Coccus*, *Saissetia*, and *Waxiella*, are found in Ethiopia, but no species of *Pulvinaria* are so distributed.

Distribution. This species is only known from Asmara, Eritrea, at 2400 m elevation, in the subtropical desert/semidesert ecoregion of the Eritrean highlands at the northwestern edge of the Great Rift Valley.

4. *Leucopis (Leucopella) spatula* Gaimari & Raspi

Biology. This species has been collected in January, May, and July. This species is known to attack *Pulvinaria floccifera* (Coccidae) on *Phaseolus coccineus* (Fabaceae); however, no species of *Pulvinaria* are known to attack *Phaseolus* in Eritrea, but the species *Ceroplastes rusci* is the only scale known from Eritrea, and it attacks *Phaseolus caracalla*, although *Coccus longulus* attacks other species of *Phaseolus* and occurs in Kenya and Uganda. This species is also known to attack *Saissetia oleae* (Coccidae) on *Croton macrostachyus* (Euphorbiaceae) and is known to attack *Parasaissetia nigra* (Coccidae) on an unknown host plant.

Distribution. This species is known only from Asmara and “Eztacleron” (=Adi Tekelezan), Eritrea, occurring at 2350 m elevation (Asmara), in the Eritrean highlands at the northwestern edge of the Great Rift

Valley, in a subtropical desert/semidesert ecoregion.

5. *Leucopis (Leucopella) vanharteni* Raspi & Gaimari

Biology. This species has been collected and reared in June, October, December, and February, from mealybugs on various plants, but currently, only one host species, *Paracoccus burnerae* (Pseudococcidae) has been identified, feeding on *Dodonaea viscosa* (Sapindaceae) and *Nerium oleander* (Apocynaceae). Other hosts include unidentified mealybugs on the following plants [mealybugs known from these plants on the Arabian Peninsula are listed in brackets]: *Dodonaea viscosa* [*Maconellicoccus hirsutus*, *Pseudococcus calceolariae*]; *Lantana camara* (Verbenaceae) [*Ferrisia virgata*, *Maconellicoccus hirsutus*]; *Nerium oleander* [*Maconellicoccus hirsutus*, *Nipaeococcus viridis*, *Planococcus citri*, *Planococcus ficus*]; *Solanum unguiculatum* (Solanaceae) [no known mealybugs]; *Chrysanthemum* sp. (Asteraceae) [*Maconellicoccus hirsutus*, *Nipaeococcus viridis*]; *Ligustrum* sp. (Oleaceae) [*Nipaeococcus viridis*].

Distribution. This species is known only from Yemen (Mahwir, at ~1500 m elevation, Sana'a, at 2380 m; and Ta'iz, at 1400 m), making this the only species in the subgenus present on the Arabian Peninsula, occurring in a tropical dry forest and savannah ecoregion on the edge of the undulating deserts of Ramlat al-sab'atayan.

6. *Leucopis (Leucopella) yaromi* Gaimari & Raspi

Biology. Unknown.

Distribution. This species is known only from Harena Forest in the Bale Mountains of southeastern Ethiopia, at 2800 m elevation, in a subtropical desert/semidesert ecoregion.

Diptera Systematics – Focus on Pipunculidae

Jeff Skevington

Introduction

I started work as a new employee at CDFA in August of 2002 (as an Associate Insect Biosystematist). It has been a busy year settling into a new job, starting new projects and completing projects started while I was working as a postdoctoral scientist in Ottawa, Canada and as a doctoral student in Brisbane, Australia.



Figure 1. Big-headed flies (Pipunculidae) are important parasitoids of leafhoppers and planthoppers. They are common but inconspicuous flies and are most often seen hovering amongst vegetation. On rare occasions, an individual such as this male of *Elmohardyia atlantica* can be seen sitting on a leaf.

(photo by S. A. Marshall)

My research program has several facets and focuses on one group of flies, Pipunculidae (big-headed flies). Big-headed flies are important natural control agents of leafhoppers and their relatives (Auchenorrhyncha). Females lay a single egg in host leafhoppers and the developing larva sterilizes and kills the host. Because of the importance of Pipunculidae in the natural control of leafhoppers and their relatives, their importance to agriculture is key. As new pests continue to arrive on the scene, we need to have the best understanding possible of the relationships of these potential pests and their parasitoids. Understanding the systematics of the parasitoids is the first step in this process. Once the taxonomy is no longer an impediment, populations of these flies can be maximized as part of an integrated control program (IPM), or they can be introduced as part of a biological control program.

Accomplishments in 2002

My work in 2002 advanced on three fronts:

1. Systematics of Pipunculidae. Two revisions of

Australian taxa were published in referred journals:

Skevington, J. H. (2002). Phylogenetic revision of Australian members of the *Allomethus* genus group (Diptera: Pipunculidae). *Insect Systematics and Evolution*. 33(2): 133-161.

Skevington, J. H. (2002). Revision of Australian *Eudorylas* Aczél (Diptera, Pipunculidae). *Studia dipterologica*. In press.

These papers document the diversity of three genera of Australian big-headed flies. In the first paper, the Australian species of *Allomethus* and *Claraeola* are revised and include one described species, *Claraeola erinys* (Perkins), and five new species. *Claraeola hylaea* (Perkins) is proposed to be a synonym of *C. erinys* (Perkins). A key to species is provided and male and female genitalia are illustrated. The Australian species are placed phylogenetically into a world context using available taxa within the *Allomethus* genus group. The phylogenetic relationships are discussed in light of a cladistic analysis involving 22 taxa and 60 characters.

The second paper revises Australian species of the cosmopolitan genus *Eudorylas*, one of five known Australian genera in the tribe Eudorylini. *Eudorylas* contains 407 described species that are known from all biogeographical regions. The Australian species of *Eudorylas* include two described species, *E. cinerascens* (Perkins) and *E. mutillatus* (Loew), and 14 new species. *Eudorylas hepaticolor* (Becker) and *E. cruciator* (Perkins) are proposed as new synonyms of *E. mutillatus*. A key to species is provided and male and female genitalia are illustrated. Phylogenetic relationships are discussed in light of a cladistic analysis involving 23 taxa and 70 characters.

2. Overview of true flies (Diptera). Flies (Diptera) are an important but underappreciated part of our planet's biodiversity. With over 124,000 described species, and countless more awaiting discovery, they are one of the most diverse groups of organisms on Earth. P.T. Dang and I coordinated a series of 10 review papers on Diptera in the journal *Biodiversity*. Several authors describe the diversity of dipteran lifestyles and behaviors, both as larvae and adults. They also reveal the various roles that these animals play in

the ecological interactions of the planet—countless numbers of flies feed on plants, control pest arthropods (including other flies!), break down rotting vegetation and excrement, pollinate flowers, provide food for other species, and of course, spread diseases. Indeed, because of their role as vectors of disease, flies have almost single-handedly prevented the economic development of countries in tropical Africa and South America. But flies are used in positive ways by humans, too, and several authors describe their use in forensic science, molecular research, and even as “main attractions” in the tourism industry. The intent of this series of papers is to encourage a broader interest in Diptera that, ideally, will lead to further research and conservation efforts. Citations related to my involvement in this publication follow:

- Skevington, J. H. (2002). Intimate neighbours: Parasitoids and parasites. In *Exploring the diversity of life: Diptera* (Skevington, J. H. & Dang, P. T., eds.), *Biodiversity* 3(4): pp. 8-12. Tropical Conservancy, Ottawa, Canada.
- Skevington, J. H. & Dang, P. T. E. (Editors). (2002). Exploring the diversity of flies (Diptera). *Biodiversity* 3(4): 3-27.

These articles are available as pdf files on the CDFA website for those wishing to learn more about flies and their impacts on our lives (<http://www.cdfa.ca.gov/phpps/ppd/Entomology/EntBios/JSkevington/Skevington.htm>).

3. Conference Participation. I was a participant and member of the organizing committee of the 5th International Congress of Dipterology held in Brisbane, Australia in September. This conference occurs every four years and showcases new innovations in Diptera research. Over 250 delegates from many countries attended this conference and shared insights on their research programs. After the Congress, I led an 11 day international collecting expedition to two parks in southeast Queensland. A summary of my Congress presentation and a paper providing an overview of the Congress follow:

- Skevington, J. (2002). Overview of the 5th International Congress of Dipterology – Brisbane, Australia, 29 September to 4 October 2002. *Fly Times*. 29: 4-6.
- Skevington, J. H., Cumming, J.M., Sinclair, B.J., Wiegmann, B.M., and Moulton, J.K. (2002). Molecular and morphological evidence supporting relationships within Eremoneura: Focus on Cyclorrhapha. In: “Fifth International

Congress of Dipterology. Abstracts Volume”. (Yeates, D.K., Ed.). pp. 221-222, Brisbane, Australia.

Ongoing Research—Systematics of Pipunculidae

Current revisions that I am working on or preparing for include Australian *Microcephalops* and *Collinias* and North American *Eudorylas*, *Jassidophaga*, *Nephrocerus*, and *Verrallia*. *Jassidophaga* and *Verrallia* are parasitoids of spittlebugs (Cercopidae), including pests such as the Meadow Spittlebug (*Philaenus spumarius*), Two-lined Spittlebug (*Neophilaenus lineatus*), and Saratoga Spittlebug (*Aphrophora saratogensis*). *Eudorylas* species attack a wide variety of leafhoppers in the subfamilies Deltocephalinae and Cicadellinae, many of them pests or potential pests (e.g. Glassy-winged Sharpshooter, *Homalodisca coagulata*). The hosts of *Nephrocerus* are unknown.

There are four anticipated products of this research:

1. The fauna of these genera will be described, keys will be provided, and phylogenies will be proposed.
 2. A searchable specimen database (currently containing over 13,000 geo-referenced specimens) will be updated to include all material from new revisions and will also be made available on the CDFA website.
 3. A catalogue of World Pipunculidae will be published in hardcopy through Myia (Smithsonian Museum publication, USNM) and served electronically on the CDFA and USNM websites. Databases for this catalogue are over 95% complete.
 4. The use of molecular data in classical taxonomic work will be explored and promoted. As with many insects, females and males of Pipunculidae are dimorphic and very difficult to associate. Because of this limitation, taxonomy is traditionally based on the more character-rich males. A molecular ‘barcode’ (sensu Hebert et al. 2003) will be generated for *Nephrocerus* males and we will attempt to sequence and associate females via this barcode (using mitochondrial sequences of the rapidly evolving gene cytochrome *c* oxidase I (COI)).
- Pipunculidae (big-headed flies) are exclusively endoparasitoids of Auchenorrhyncha (leafhoppers and their relatives). Over 1,300 species of these flies have been described, and it is likely that over 2,500 species exist (De Meyer 1996, De Meyer and Skevington 2000).

A recent key to the world genera of pipunculids is included in Skevington and Yeates (2001).

Many researchers have documented the effects of parasitization on Auchenorrhyncha (e.g., Whittaker (1969b), May (1979), Waloff (1980), and Yano et al. (1985)). Parasitized hoppers are sometimes recognizable by their swollen abdomens and sluggish movements. Jumping and walking are impaired by a reduction in femur length and damage to the thoracic muscles and nervous system (May 1979). Abdominal sclerites of adults may become poorly pigmented, body size may be reduced or increased, and wing venation is often aberrant. Ovipositor length and the length of claspers in males are reduced in parasitized individuals. In female hosts, the development of ovarioles is halted, and mature eggs are rarely found. Spermathecae and accessory glands are also lost. In males, the testes, spermathecal ducts and a large part of the accessory gland are often lost. Only some males are able to copulate and fertilize females (May 1979).

Most pipunculid species attack more than one species of host but show a preference for a particular set of host species. Each female big-headed fly lays only one egg inside each host and appears to be able to recognize previously parasitized hosts. Superparasitism and multiple parasitism are rare, and survival of more than one pipunculid in these cases is exceptional (Jervis 1980, Waloff and Jervis 1987, Morakote and Yano 1988).

Rates of parasitism vary from fractions of a percent to 100 percent in local populations. The importance of pipunculids as a part of the natural regulation of Auchenorrhyncha numbers is undisputedly important. However, few studies have documented the absolute importance of these flies in the control of such populations. Waloff (1975) carried out the most detailed study on host-parasitoid community assemblages involving pipunculids and Whittaker (1969a, 1973) performed what are likely the best such studies on the interaction of one species of pipunculid and its host.

Revisionary work on pipunculids is active in most biogeographical regions of the world. Research on the Nearctic Pipunculidae revolves largely around the work of Hardy (1943) with recent revisions of only a few genera (*Pipunculus* (Skevington and Marshall 1998); *Cephalops*, *Cephalosphaera*, and *Microcephalops* (De Meyer 1989), and *Dorylomorpha* (Albrecht 1990)). Hardy's work presents an adequate starting point, but his keys and illustrations are inadequate to the

task of identifying most Nearctic taxa. Expansions of collections since 1943 have also resulted in the discovery of many new species that need to be described.

Morphological and Molecular Phylogenetics of Pipunculidae (Diptera)

A phylogenetically based classification provides the solid foundation of predictability necessary for structuring and answering fundamental questions in biology. Rafael and De Meyer (1992) and Skevington and Yeates (2001) proposed quantitative hypotheses of pipunculid phylogenetic relationships. Some parts of the phylogeny are still poorly supported and additional data are required to support or refute current concepts. Molecular data presented by Skevington and Yeates (2000) produced a well-supported tree and offer a useful independent data set to test current hypotheses. As part of this preliminary hypothesis, 12 pipunculid taxa were analyzed for two mitochondrial genes (12s and 16s rDNA). I have now collected data for 30 species using these genes and two additional ones (28s and CAD nuclear DNA). All genera except *Allomethus*, *Amazunculus*, *Basileunculus*, and *Claraeola* are now represented in this dataset. To complete this project, more exemplars must be added to encompass all genera and to better represent the variation within genera. I have most of the necessary specimens stored in absolute alcohol, so all that needs to be completed now is the sequencing.

The predictive nature of this phylogeny will allow agricultural needs to be met with respect to natural or biological control priorities. For example, if a pipunculid species is known to attack a non-pest leafhopper in Europe and the same species or a related leafhopper is a pest here, we can attempt to incorporate native Nearctic pipunculids (related to the European species) into our control efforts. Knowledge of both parasitoid and host phylogenies is crucial to the success of such an effort. Because pipunculids are not strictly host specific (they tend to be lineage specific), a project such as this stands a high chance of succeeding.

The first attempt to construct a phylogeny for the family was made by Aczél (1948). Despite the fact that this was not based on quantitative character assessment, much of it is still in accordance with our current hypotheses. In 1990, Albrecht provided a phenetic interpretation of pipunculid evolution that has been largely discredited. The phylogeny produced by Rafael and De Meyer (1992) is one of the pivotal papers in pipunculid systematics. Their

hypotheses have contributed to a better understanding of the relationships of the genera and have stimulated considerable additional work on the family. Problems within the family (such as generic concepts in the large tribe Eudorylini) became apparent as a result of their efforts; and have stimulated further work by colleagues and myself (Skevington and Yeates 2001). Testing their hypotheses with a limited set of molecular data corroborated their findings and encouraged future efforts to expand both morphological and molecular datasets to better place problem taxa (Skevington and Yeates 2000). The goal of the current project is to complete these datasets using four genes, a large set of exemplars, and a refined morphological dataset.

Establishing Host Records of Pipunculidae

The goal of this study is to identify potential pipunculid parasitoids of pest leafhoppers and to make this information available to those working in biological control and IPM programs. Introductions of species can then be planned, or, if native species are discovered attacking pest leafhoppers, an integrated form of pest control can be planned to increase the incidence of parasitism. Nearctic leafhoppers have never been intensively surveyed for larval and adult parasitoids. Big-headed flies are key parasitoids of leafhoppers and their relatives and play important roles in the natural control of pest species in this group. We aim to survey Auchenorrhyncha for their parasitoids in several sites in North America (contingent on receiving funding from the Pierce's Disease Project). Information on host use by pipunculids will directly benefit agricultural control programs. Integrated pest management practices can be implemented to encourage target species of pipunculids in agro-ecosystems, and biological control programs can be evaluated using these flies.

Data on host ranges are available for more than 52 European species of Pipunculidae. In the Nearctic Region, only 13 species have received such documentation (Skevington and Marshall 1997). Rates of parasitism vary from fractions of a percent to nearly 100 percent in local populations. For example, Hartung and Severin (1915) found *Circulifer tenellus* (Beet Leafhopper) with up to 47% parasitism by two pipunculid species and Skevington and Marshall (1997) recorded parasitism rates of *Cuerna striata* (a relative of the pest Glassy-winged Sharpshooter) by *Eudorylas alternatus* to be as high as 89%. These high rates of parasitism suggest that Pipunculidae are crucial in controlling leafhopper numbers and that this relatively poorly known group of flies has significant economic potential. In 2001, Roman Rakitov (Illinois Natural

History Survey) supported the notion that the findings by Skevington and Marshall (1997) are not an isolated phenomenon when he noted that most specimens of *Cuerna kaloostiani* that he collected in Arizona were parasitized by an unknown species of pipunculid (pers. comm.).

The potential value of Pipunculidae for biological control has stimulated some work on the bionomics of this family. For example, research into the control of the Potato Leafhopper, *Empoasca fabae*, a major pest of alfalfa in mid-western and eastern USA and Canada, involved exploration within Europe for natural enemies to be introduced to the United States (Jervis 1992) and included rearing of *Chalarus* for release. Similarly, European species of Pipunculidae were considered for introduction into New Zealand for control of Froggatt's Apple Leafhopper, *Edwardsiana crataegi*, populations of which are insecticide resistant (Jervis 1992).

Phylogenetics of True Flies (Diptera)

Over 150,000 species of flies are known, making this one of the four megadiverse orders of insects. Resolving the phylogenetic relationships of Diptera is thus one of the foremost tasks in completing our knowledge of the tree-of-life (<http://tolweb.org/tree/phylogeny.html>). Our current research, anchored on funding to Brian Wiegmann at North Carolina State University, is focused on elucidating the phylogeny of the higher flies (Cyclorrhapha). We have assembled a 51 taxon, exemplar-based dataset and are collecting data on six genes (the four gene complex called CAD, 28s, and 12s) and morphology for these species. This dataset includes several economically important families such as Chloropidae, Empididae, Hippoboscidae, Muscidae, Phoridae, Pipunculidae, Syrphidae and Tachinidae. When complete, this data will go towards forming the most complete phylogenetic hypothesis of this group of flies to date. We hope to considerably expand this project (contingent on funding from NSF) to include all Diptera. This large collaborative effort will build on the solid foundation of more than 40 years of phylogenetic research in Diptera and an unusually well documented and researched fossil record. With a new, well-supported, phylogenetic tree as an organizing framework, the comparative research on pests, genomics, development, neurobiology, behavior, and epidemiology will flourish.

The predictive nature of this phylogeny will allow agricultural needs to be met in many ways. Whether searching for answers about the ecology of pest organisms or looking for potential predator or parasitoid taxa to apply to a pest problem, a robust phylogeny can

provide predictions for such queries. Questions about the diversity and ecology of organisms are also best asked in the framework of a well-supported phylogeny. The comparative approach has become a cornerstone of science and demands such a phylogeny.

References

- Aczél, M. L. 1948. Grundlagen einer monographie der Dorilaiden (Diptera). Dorilaiden-Studien VI. Acta Zoologica Lilloana 6: 5-168.
- Albrecht, A. 1990. Revision, phylogeny and classification of the genus *Dorylomorpha* (Diptera, Pipunculidae). Acta Zoologica Fennica 188: 1-240.
- De Meyer, M. 1989. Systematics of the Nearctic species of the genus *Cephalops* Fallén (Diptera, Pipunculidae). Bulletin de l'Institut royal des Sciences naturelles de Belgique, Entomologie 59: 99-130.
- De Meyer, M. 1996. World catalogue of Pipunculidae (Diptera). Institut Royal des Sciences Naturelles de Belgique, Documents de Travail 86: 1-127.
- De Meyer, M., and J. H. Skevington. 2000. First addition to the World Catalogue of Pipunculidae. Bulletin de l'Institut Royal des Sciences Naturelle de Belgique, Entomologie 70: 5-11.
- Hardy, D. E. 1943. A revision of Nearctic Dorilaidae (Pipunculidae). University of Kansas Science Bulletin 29: 1-231.
- Hartung, W. J., and H. H. P. Severin. 1915. Natural enemies of the sugar beet leafhoppers in California. Mon. Bull. Calif. Comm. Hort. 4: 277-279.
- Hebert, P.D.N., A. Cywinska, S.L. Ball, and J.R. deWaard. 2003. Biological identifications through DNA barcodes. Proceedings of the Royal Society of London (B) 270: 313-322.
- Jervis, M. A. 1980. Studies on oviposition behaviour and larval development in species of *Chalarus* (Diptera, Pipunculidae), parasites of typhlocybine leafhoppers (Homoptera, Cicadellidae). Journal of Natural History 14: 759-768.
- Jervis, M. A. 1992. A taxonomic revision of the pipunculid fly genus *Chalarus* Walker, with particular reference to the European fauna. Zoological Journal of the Linnean Society 105: 243-352.
- May, Y. Y. 1979. The biology of *Cephalops curtifrons* (Diptera: Pipunculidae), an endoparasite of *Stenocranus minutus* (Hemiptera: Delphacidae). Zoological Journal of the Linnean Society 66: 15-29.
- Morakote, R., and K. Yano. 1988. Biology of some Japanese Pipunculidae (Diptera) parasitizing *Nephotettix cincticeps* (Hemiptera, Deltocephalidae). Bulletin of the Faculty of Agriculture, Yamaguchi University 35: 9-22.
- Rafael, J. A., and M. De Meyer. 1992. Generic classification of the family Pipunculidae (Diptera): a cladistic analysis. Journal of Natural History 26: 637-658.
- Skevington, J., and S. A. Marshall. 1997. First record of a big-headed fly, *Eudorylas alternatus* (Cresson) (Diptera: Pipunculidae), reared from the subfamily Cicadellinae (Homoptera: Cicadellidae), with an overview of pipunculid-host associations in the Nearctic Region. The Canadian Entomologist 129: 387-398.
- Skevington, J., and S. A. Marshall. 1998. Systematics of New World *Pipunculus* (Diptera: Pipunculidae). Entomological Society of America, Lanham, Maryland. 201 pp.
- Skevington, J. H., and D. K. Yeates. 2000. Phylogeny of the Syrphoidea (Diptera) inferred from mtDNA sequences and morphology with particular reference to classification of the Pipunculidae (Diptera). Molecular Phylogenetics and Evolution 16: 212-224.
- Skevington, J. H., and D. K. Yeates. 2001. Phylogenetic classification of Eudorylini (Diptera: Pipunculidae). Systematic Entomology 26: 421-452.
- Waloff, N. 1975. The parasitoids of the nymphal and adult stages of leafhoppers (Auchenorrhyncha: Homoptera) of acidic grassland. Transactions of the Royal Entomological Society of London 126: 637-686.
- Waloff, N. 1980. Studies on grassland leafhoppers (Auchenorrhyncha: Homoptera) and their natural enemies, pp. 81-215. In A. MacFayden [ed.], Advances in Ecological Research. Academic Press Inc. Limited, London, New York.
- Waloff, N., and M. A. Jervis. 1987. Communities of parasitoids associated with leafhoppers and planthoppers in Europe, pp. 281-402. In A. Macfayden and E. D. Ford [eds.], Advances in Ecological Research. Academic Press Inc. Limited, London.
- Whittaker, J. B. 1969a. Quantitative and habitat studies of the froghoppers and leafhoppers (Homoptera, Auchenorrhyncha) of Wytham Woods, Berkshire. The Entomologist's monthly Magazine 105: 27-37.
- Whittaker, J. B. 1969b. The biology of Pipunculidae (Diptera) parasitising some British Cercopidae (Homoptera). Proceedings of the Royal Entomological Society of London. Series A. General Entomology 44: 17-24.
- Whittaker, J. B. 1973. Density regulation in a population of *Philaenus spumarius* (L.) (Homoptera: Cercopidae). Journal of Animal Ecology 42: 163-172.
- Yano, K., R. Morakote, M. Satoh, and I. Asai. 1985. An evidence for behavioural change in *Nephotettix cincticeps* Uhler (Hemiptera: Deltocephalidae) parasitized by pipunculid flies (Diptera: Pipunculidae). Applied Entomology and Zoology 20: 94-96.

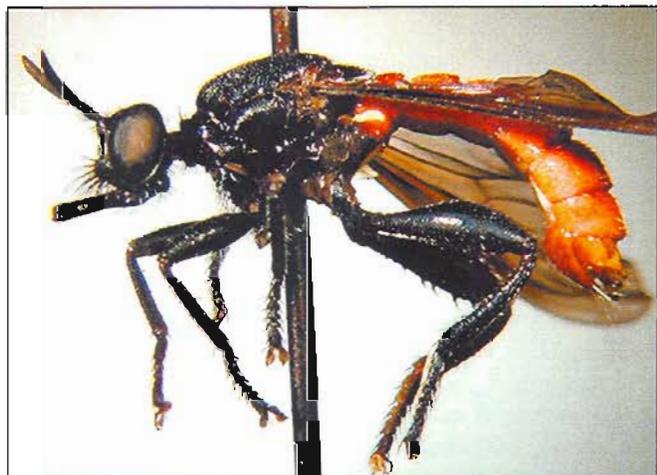
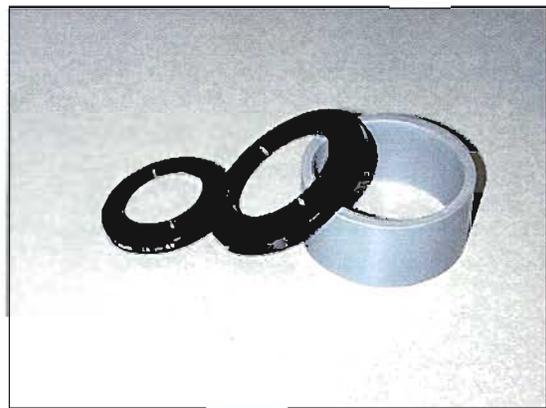
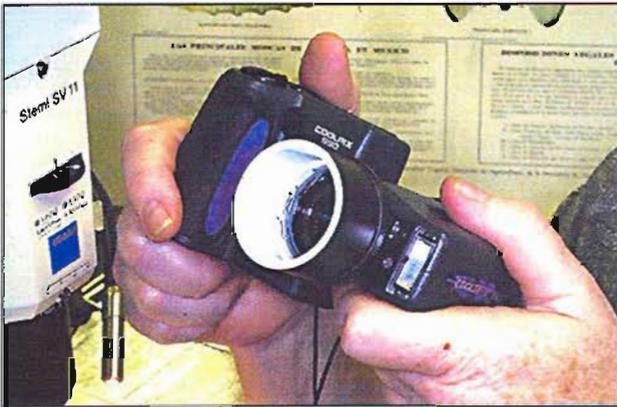
Simplified Digital Photography for Museum Work

Eric M. Fisher and Stephen D. Gaimari

We wish to share the simple and effective method for taking digital photographs through a microscope that we have developed and are using in the Entomology Lab. Our technique enables us to place a Nikon Coolpix camera against the eyepiece of a microscope (either stereo or compound), and digitally record the image in focus. We make an inexpensive plastic collar device, attach it to the fixed lens of the Coolpix, and – using the collar to hold the camera in a stable position on the microscope – take high resolution photos at maximum depth-of-field (see 4 figures).

We provide a more detailed discussion of parts, construction methods, and camera settings adapted for microscopy in a web page: “Simplified digital

photography for museum work,” CDFA site <<http://www.cdfa.ca.gov/phpps/ppd/Entomology/Diptera/digphot.htm>>. This web page was adapted from a talk presented by Steve at the 5th International Congress of Dipterology in Brisbane, Australia, on 2 Oct. 2002. Although our Coolpix-scope photo method is becoming fairly popular (especially among our Diptera colleagues!), we have been informed that most people are quite unaware of the presence of a web page on this subject.



Catalog of Leaf Beetles of America North of Mexico (in press)

Terry N. Seeno

This catalog covers the original nomenclature for the group of Coleoptera generally called leaf beetles. As used here, the term leaf beetle includes the families Megalopodidae, Orsodacnidae, and Chrysomelidae. The seed beetles, Bruchidae or Bruchinae, are now usually included in the Chrysomelidae, but are omitted from this catalogue. The bruchids have traditionally been treated as a separate family.

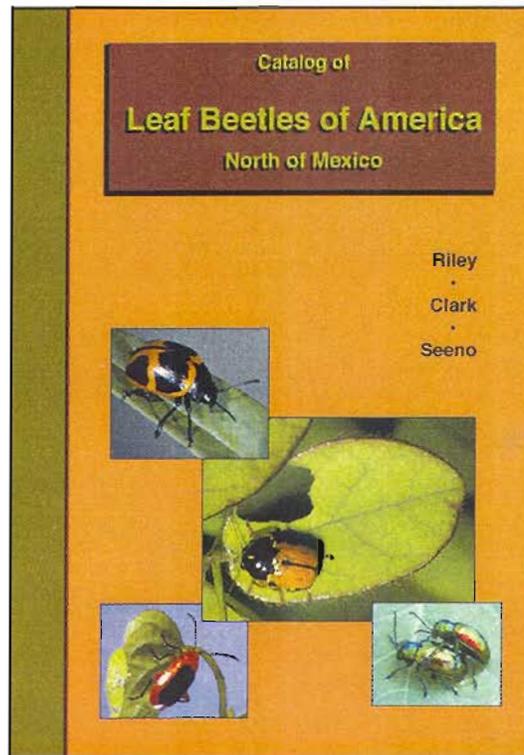
The genus-group and species-group names for non-fossil leaf beetles are cataloged for America north of Mexico. Named taxa are presented in a modern classification scheme. Valid species are shown in their current and original combinations, followed by their synonyms shown in their original combinations. Genus-group names are given with their type species and method of type-species fixation. Citations are presented for each name and for

type-species designations. State and province records from the conterminous United States, Canada, and

Alaska are given for each valid species and subspecies from the region. Species believed to have been recorded in error from the region and species names of uncertain application are presented in separate annotated lists. A complete bibliography including the cited references is given.

This 290 page work is divided into seven sections: the catalog of taxa, annotations, names of uncertain application, excluded species, and indices to both species- and genus-group names. There are more than 40 pages of references.

Until now, the most complete catalog or listing of leaf beetles in North America has been the 1975 Wilcox List (also called the Red Version). Publication is scheduled for late Spring, 2003.



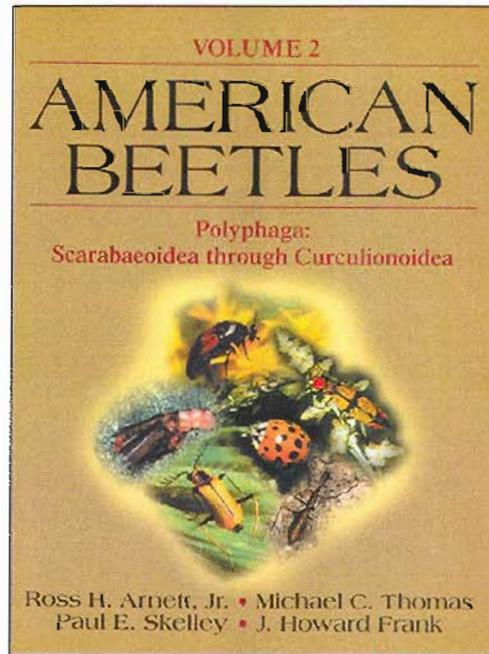
American Beetles and PPDB

C. L. Bellamy, R. E. Somerby and F. G. Andrews

The second of two volumes of *American Beetles*, the new standard guide to Coleoptera for America, North of Mexico, appeared in 2002. This new work replaces the classic *Beetles of the United States* by Ross Arnett (1960). While Arnett's original work was mostly written by himself, *American Beetles* was assembled from the contributions of more than 80 authors including several from within our ranks.

American Beetles, Volume 2: Polyphaga: Scarabaeoidea through Curculionoidea 2002.

Editors: Ross H Arnett, Jr., Michael C. Thomas, Paul E Skelley and J. Howard Frank



The following chapters were contributed from this lab:

1. Aalbu, R. L., C. A. Triplehorn, J. M. Campbell, K. W. Brown, R. E. Somerby & D. B. Thomas. Chapter 106. Tenebrionidae Latreille 1802, pp. 463-509.
2. Andrews, F. G. 2002. Chapter 95. Latridiidae Erichson 1842, pp. 395-398.
3. Bellamy, C. L. & G. H. Nelson. 2002. Chapter 41. Buprestidae Leach 1815, pp. 98-112.
4. Nelson, G. H. & C. L. Bellamy. 2002. Chapter 40. Schizopodidae LeConte 1861, pp. 95-97.

Reference cited:

Arnett, R. H. 1960. *The Beetles of the United States*. Catholic University Press, Washington, D. C., 1112 pp.

A Cooperative Project of SON and APHIS: Creation of a Non-indigenous Nematode Pest List with Associated Selection Criteria and Fact Sheets

Ke Dong

U.S. crops are susceptible to a large number of plant pathogens. Losses due to plant diseases and nematodes have been estimated to be as high as \$30 billion per year. Fortunately, not all pathogens of a given crop currently are present in the US; there are many serious pathogens that have not yet arrived or have not become established in this country. Other important plant pathogens are found in only limited areas within the country, such that most of the crop is not affected. Excluding pests from agricultural production areas is often the most effective and cost-beneficial management strategy. There is a great deal of interest in the threat of invasive species to the US; the threat is greater now than ever because of increasing international trade. Under the WTO policies, restrictions on trade must be justified on a scientific basis, rather than being an arbitrary decision by a particular country. This puts new pressure on regulatory agencies to develop sound and defensible regulations. APHIS routinely conducts thorough risk assessments for selected pathogens, but there are many potentially threatening plant pathogens of U.S. crops, and such detailed analysis could not be done timely on every non-indigenous pathogen. There are only four plant nematodes (*Bursaphelenchus cocophilus*, *Globodera pallida*, *Globodera rostochiensis*, and *Heterodera zea*) on the current APHIS list, *G. pallida* and *G. rostochiensis* are considered as significant quarantine pests. To update the pest risk-assessment protocols, APHIS is searching to form stronger partnerships with external research agencies and professional societies.

The Society of Nematologists (SON) signed a cooperative agreement with APHIS, and a project between the SON and APHIS for creating a update list of foreign (or of limited US distribution) nematode plant pests of agricultural, environmental

and regulatory significance to the US was initiated. It would be very informative to have a list of nematodes that pose a threat to U.S. crops. Such a list, with suggested criteria, pest priority ratings and nematode species fact sheets, could focus the attention of APHIS and suggest the need for detailed risk assessments, which possibly could lead to changes in trade regulations. The list also could serve as an alert to plant nematologists that certain nematodes are considered to be of importance. The list could be a useful guidance and information to suggest future research. As a scientific organization, SON contains experts on all important plant nematodes, whether the nematodes currently exist in the U.S. or not. A list of threatening plant nematodes could be prepared using this collective expertise. In addition, the list must be a 'living' list, because certain nematode species should be either added or deleted, as new information becomes available.

A working group of nematologists primarily from the SON regulatory committee, P. Lehman, J. Brito, and R. Inserra (Florida), K. Dong (California), T. Powers (Nebraska), Z. Handoo (USDA-ARS), and L. Millar (USDA-APHIS), has been conducting the project. Paul S. Lehman, the regulatory nematologist from Florida, is the leading person. As the SON regulatory committee chair this year, I am also a member in this working group. The working group held a 2-day meeting February 12-13, 2002 in Gainesville, FL.

A preliminary nematode pest list with a total of 68 nematode species was discussed at that meeting and several conference calls. This preliminary list was also posted on the SON web-site to solicit input from SON members. The selection of nematodes on the list was considered based on the primary factors: absence from or limited distribution

of the nematode in the US; known economic damage caused by the nematode; host range; and the distribution and economic importance of the nematode's hosts in the US. Introduction potential and impact on exports were also considered as secondary factors. Introduction and establishment potential would need to be investigated further in a complete pest risk assessment completed by APHIS. In addition, the rankings for nematodes on the list (high, moderate, low, and very low) were suggested. The working group rated the nematodes based on: (1) high, very important pest and must be kept out. (2) moderate, moderate to large threat and have strong economic impact. (3) low, small threat but should not be interpreted as being equivalent to a low risk for US agriculture or that minimal effort should be made to exclude these nematodes. (4) very low, either there are few pathways for their introduction into the US or because currently there is limited evidence that these nematodes damage economically important crops. The threatening nematode pest list and rankings provide suggested guidelines or priority ratings for APHIS to develop a timeframe for conducting complete pest risk assessments. Complete pest risk assessments are needed for all of the nematodes on the list, but this task requires resources and time beyond the scope of the current project.

A decision was made at the meeting in Florida to prepare two lists of threatening nematodes:

(1) Nematodes not believed to be in the U.S. at

this time; and (2) Nematodes of very limited distribution within the U.S., such that the nematode does not currently affect most of the crop and containment/eradication may still be possible. There are seven nematode species rated to be high, 27 as moderate and 18 as low in the lists. In addition, 16 candidate nematodes suggested from the preliminary list are given a very low priority ranking based on the Working Group consensus. The species fact sheet with information of taxonomy, identification, distribution and host range is prepared for each nematode.

This report contains a brief summary of my activities with the SON-APHIS regulatory working group. For detail information about the threatening plant nematodes and the development of nematode pest lists, please review the web-site at <http://nematode.unl.edu/son/projectpest.htm>.

References Cited:

Lehman, P. S., Brito, J. A., Dong, K., Handoo, Z. A. Inerra, R., Powers, T., and Millar, L. 2002. Creation of a List of Exotic Nematode Plant Pests of Agricultural and Environmental Significance to the United States with Associated Selection Criteria and Fact Sheets. SON web. <http://nematode.unl.edu/son/projectpest.htm>

Madden, L. V. 2001. What are the Non-indigenous Plant Pathogens that Threaten U.S. Crops and Forests? APSnet. <http://www.apsnet.org/online/feature/exotic/>

Chitambar Report (see pages 23-25)

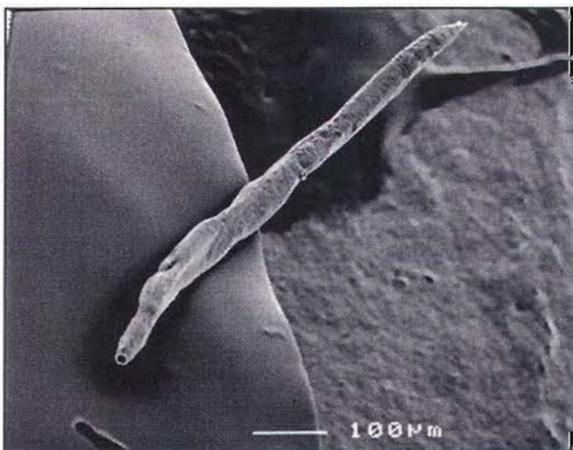


Figure 1. *Diploenteron* sp. Female: SEM view of total body. Arrows indicate oral aperture (mouth) at lower left and vulva at mid body

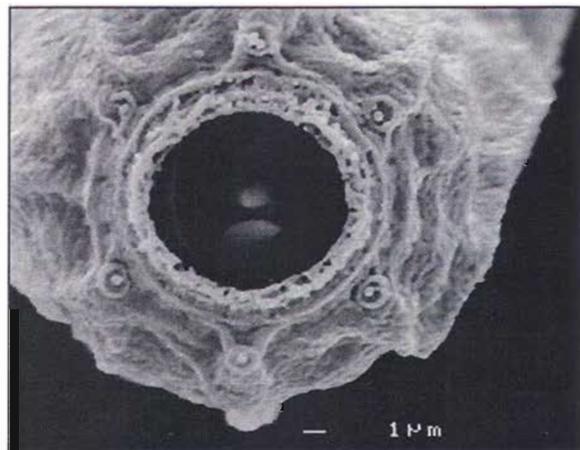


Figure 2. *Diploenteron* sp. Female: SEM face view showing oral aperture (mouth) with two internal teeth within cavity. Aperture is surrounded by six sensory papillae.

Preliminary Studies on the Culturing and Feeding Activity of a Predatory Nematode, *Diplenteron* sp.

John J. Chitambar

Terrestrial predaceous nematodes are commonly found in cultivated and non-cultivated soils and form an important component of the micro fauna ecosystem. The extent to which these predators feed on nematodes and other microorganisms has a direct effect on the quantitative and qualitative balance of micro faunal life in the ecosystem. This balance is often disrupted in cultivated agricultural soils as agronomic and pest management practices not only affect harmful nematode communities but also beneficial ones. An understanding of the behavior of predaceous nematodes is essential for a clearer definition of their role in the environment, and their potential as biological control agents.

Studies on predaceous nematodes have been largely taxonomic in nature (Zullini & Loof, 1980; Chitambar & Noffsinger, 1989; Chitambar, 1990). Biological information is known for relatively few species, based mostly on laboratory tests using synthetic media (Yeates, 1969; Small & Grootaert, 1983; Chitambar & Noffsinger, 1989). Information thus gained, has been presumed representative of all members within a genus or family. Most terrestrial predaceous nematodes belong to the orders Mononchida and Diplogasterida. The taxonomy and biology of predaceous nematodes has been studied more extensively within the Mononchida than the Diplogasterida.

The current study was conducted to obtain a preliminary understanding of the predaceous activity of a *Diplenteron* sp. against plant parasitic nematodes in synthetic culture medium. Furthermore, this preliminary information formed an important basis for an on-going study on the biological control potential of predaceous nematodes against plant parasitic nematodes in plants grown in controlled environments.

Materials and Methods

In July 2002, four individual adult *Diplenteron* sp. were extracted from a soil sample collected around roots of *Vitis vinifera* grown in San Joaquin County, California.

Culturing: Initially collected *Diplenteron* sp.

adults were transferred to a 1.5% water agar plate. Approximately, 50 free-living bacterivorous nematodes, *Rhabditis* sp. were also added as prey to the same plate. Additional individuals of the same *Rhabditis* sp. were added to 1.5% water agar, 25% corn meal, 25% oatmeal agar, and 25% nutrient agar media. Culture plates were incubated at 25 ± 0.5 C and observed after five to six days for increases in nematode numbers.

Feeding activity: The predaceous effect of *Diplenteron* sp. was studied on two plant parasitic nematode species, namely, *Aphelenchoides fragariae* (the Strawberry Bud and Foliar Nematode) and *Ditylenchus dipsaci* (the Stem and Bulb Nematode) as test prey. The latter species were obtained from carrot callus cultures maintained in the laboratory. Treatments comprised of i) 100 *A. fragariae* in 0.32 ml sterile water and 5 hand-picked, gravid female *Diplenteron* sp., ii) 70 *D. dipsaci* in 0.20 ml sterile water and 5 hand-picked, gravid *Diplenteron* sp., iii) 5 *Diplenteron* sp. alone (control), and iv) 100 *A. fragariae* alone (control). The nematodes were transferred to 2-in-diam petri plates containing 1.5% water agar. Three sets of treatments were incubated at 25 ± 0.5 C for 5, 15 and 20 days. Treatments were replicated five times per test prey nematode species per incubation period. In addition, two sets of a) 5 female *Diplenteron* sp. and 100 *A. fragariae*. and b) 10 female *Diplenteron* sp. and 100 *A. fragariae* were added to a single, callused carrot disc in 1.5% water agar in a 4-in-diam petri plate and incubated at 25 ± 0.5 C for 20 days. Each set was replicated three times. Controls comprised inoculations of 5 predator and 100 prey species alone.

The nematodes in media were daily observed microscopically. Nematode counts were made after the respective incubation periods and analyzed statistically by analysis of variance.

Results

Culturing: *Diplenteron* sp. readily fed on *Rhabditis* sp. and increased in numbers (egg laid) five days after inoculation. Increases were not quantified, but observed. Similar increases of both prey and predator were also observed in oatmeal and cornmeal agar. However, at 25% concentration, nutrient agar did

not support increases in predator and prey populations, but instead, supported non-identified bacterial growth that was detrimental to the nematode population.

Feeding Activity:

1. *Diplenteron* sp. (predator) + *Aphelenchoides fragariae* (prey) in water agar.

Numbers of prey individuals decreased rapidly below the inoculated levels for all treatments (including control), and no significant differences in prey numbers were observed between treatments after 5, 15 and 20 days (data not shown). Significant increases in numbers of predator individuals in presence of prey, were observed between 5 and 15 days ($P = 0.05$), however these values were not significantly different from that obtained 20 days after inoculation, or from control values after 15 and 20 days.

2. *Diplenteron* sp. (predator) + *Aphelenchoides fragariae* (prey) in carrot callus.

In predator with prey treatments, numbers of predator individuals increased significantly over control, whereas, significant decreases in numbers of prey individuals were observed between the same treatments ($P = 0.01$) (Table 1). No significant differences were observed in predator and prey numbers between treatments with 5 and 10 predator individuals inoculated.

3. *Diplenteron* sp. (predator) + *Ditylenchus dipsaci* (prey) in water agar.

Numbers of prey individuals decreased below the inoculated levels for all treatments (including control). *Diplenteron* sp. significantly decreased numbers of prey below the control values at 15 and 20 days after inoculation ($P = 0.05$) (Table 2). Numbers of predator individuals in presence of prey were significantly greater than control and inoculated values after 5 days, however, there was no significant difference between predator numbers after 5, 15, and 20 days.

Discussion

The successful rearing of *Diplenteron* sp. on *Rhabditis* sp. in laboratory cultures renders the former species as a prime tool for biological studies. Bacteria-feeding *Rhabditis* sp carry sufficient bacteria in their bodies to maintain increases in prey populations for several generations in water agar. Increases in predator population were directly related to the number of prey available for feeding. Although increases in prey and populations were not quantified, it was observed that *Diplenteron* sp. was a voracious feeder capable of eliminating its prey population (> 500) within 24 hours. Cornmeal and oatmeal agar sufficiently supported prey and predator cultures, however, over time the rigidity

of the media broke down, thereby, inhibiting the free motility of the predators and their subsequent feeding and reproductive capabilities. Because of this and also their natural opaqueness, cornmeal and oatmeal agar media are not suitable for microscopic observations of nematode activity.

The effect of *Diplenteron* sp. on *A. fragariae* in water agar was not conclusive largely due to the inability of the prey to remain alive and motile in water agar over time. *Aphelenchoides fragariae*, the Strawberry Bud and Foliar Nematode, is a facultative parasite capable of feeding, reproducing and surviving on fungi and host plants. In the absence of its hosts the nematode species soon died or became inactive, thereby, resulting in significant decreases below the inoculum level in all treatments including control. Apparently, the rate of decrease was similar for all treatments and durations of days, except at five days where significant difference was observed between the control and combination values. However, even these values were well below the inoculum level to be of any practical significance. On carrot callus, *A. fragariae* was able to feed and increase significantly above the inoculum level. Increases in prey density and motility provided increased number of prey per unit area of predator, thereby, increasing the number of contacts between predator and prey. As a result, prey populations were soon decreased by actively feeding predators. The direct effect of prey density on predation has been reported for other predaceous nematodes (Chitambar & Noffsinger, 1989; Yeates, 1969).

Ditylenchus dipsaci, the Stem and Bulb Nematode, is also a facultative parasite capable of feeding on fungi and plants hosts. Similar to the Foliar Nematode, numbers of *D. dipsaci* soon decreased below the inoculated level in all treatments including control. However, the rate of decrease was slower than *A. fragariae* and sufficient to support significant increases in predator population after five days.

While the effects of *Diplenteron* sp. on the two prey species was by no means conclusive in this preliminary study, it does shed light on the need for careful interpretation of data on effects of predaceous nematodes on facultative and obligate plant parasitic nematodes in synthetic media in the absence of their natural hosts. To some extent, in the above water agar experiments, increases in predator numbers were due to inocula comprised of already gravid female *Diplenteron* sp. Both prey species are readily fed on by *Diplenteron* sp. under growth conditions conducive for both predator

and prey. Both prey species are endoparasitic, thereby, indicating a specific predator and target prey selectivity. This is true of some predaceous nematodes (Chitambar & Noffsinger, 1989; Esser & Sobers, 1964).

This study is possibly the first report on the biological activity of *Diplenteron* sp. Although the information obtained is preliminary, it has provided a launching pad for future research.

References

Chitambar, John J. 1990. Description of a new predaceous nematode, *Monobutlerius macrogubernaculum* n. sp. (Nemata: Diplogasteridae). *Revue de Nématologie* 13: 369-373.

Chitambar J. J., and E. Mae Noffsinger. 1989. Taxonomy and postembryonic stages of the nematode predator *Odontopharynx longicaudata* de Man, 1912 (Diplogasterida). *Journal of Nematology* 21: 189-201.

Chitambar J. J. and E. Mae Noffsinger. 1989. Predaceous behavior and life history of *Odontopharynx longicaudata* (Diplogasterida). *Journal of Nematology* 21: 284-291.

Esser, R. P., and E. K. Sobers. 1964. Natural enemies of nematodes. *Proceedings of Soil and Crop science Society of Florida* 24: 326-353.

Small, R. W., and P. Grootaert. 1983. Observations on the predation abilities of some soil dwelling predatory nematodes. *Nematologica* 29: 109-118.

Yeates, G. W. 1969. Predation by *Mononchoides potohikus* (Nematoda: Diplogasteridae) in laboratory culture. *Nematologica* 15: 1-9.

Zullini, A., and P. A. A. Loof. 1980. Systematic notes on some species of *Diplogasteridae* (*Rhabditida*). *Nematologica* 26: 17-26.

Table 1. Effects of *Diplenteron* sp. (predator)¹ on *Aphelenchoides fragariae* (prey)² in carrot callous 20 days after inoculation.

Treatments	Final population densities of Prey ³
Prey alone (Control)	6,630.3 a
Prey with 5 Predator	201.3 b
Prey with 10 predator	29.6 b
Final population densities of Predator ³	
Predator alone (Control 1)	0.0 a
Predator alone (Control 2)	0.0 a
Predator (5) with prey	135.0 b
Predator (10) with prey	110.3 b

¹Initial inocula of predator = 5 and 10 females; ²initial inoculum of prey = 100 individuals. ³Numbers are means of three replicates. Means in columns followed by the same letter are not significantly different ($P = 0.01$) according to Duncan's Multiple-range test.

Table 2. Effects of *Diplenteron* sp. (predator)¹ on *Ditylenchus dipsaci* (prey)² in water agar.

Treatments	Final population densities of Prey ³		
	5 days	15 days	20 days
Prey alone (Control)	22.0 a	54.8 a	33.6 a
Prey with Predator	11.4 a	0.6 b	0.8 b
Final population densities of Predator ³			
Predator alone (Control)	1.0 a	51.0 a	22.6 a
Predator with Prey	33.2 b	54.0 a	39.0 a

¹Initial inocula of predator = 5 females; ²initial inoculum of prey = 70 individuals. ³Numbers are means of five replicates. Means in columns followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's Multiple-range test.

The 34th and 35th Annual California Nematology Workshops

Robert W. Hackney

The CDFA Nematology Program (CDFANP) has co-sponsored 35 consecutive annual California Nematology Workshops along with the Departments of Nematology at the University of California, Davis and Riverside. During 2002 the 34th California Nematology Workshop was held at the University of California, Davis. I convened the Steering Committee and laid the foundation (program planning, venue, invited speakers, etc.) for the 35th California Nematology Workshop in San Diego. Since the California Nematology Workshop's recent funding through a grant from the University of California Division of Natural and Agricultural Resources (DANR) Nematology Work Group, a tradition has been established that at least one or more of the invited speakers will be selected from outside California and/or the United States. Participants frequently come from the international scientific community (i.e., outside the United States) as well as from California and other states. Continuing education credits are always available for the participants (i.e., pest control advisers and operators, growers and farmers, retail and wholesale nursery employees, arborists, landscapers, municipal and state employees, parks and recreation personnel, educators and consultants) who register to claim those units.

The 34th California Nematology Workshop at U.C., Davis on March 27, 2002, emphasized the important interactions between nematodes and weeds. There were sessions on species identification of lesion nematodes and the use of a virtual microscope to capture and view nematode morphology and anatomy. The morning's lecture style session covered the surprising interactions of Nutsedge and Root-knot nematodes; an update on methyl bromide alternatives for weeds, nematodes and plant pathogen control in strawberries; clarification of the buffer zones currently required for fumigant nematicides; and updates on the latest research being conducted by graduate students and post-graduate researchers from U.C., Davis and U.C., Riverside. During a combination extended morning

break and poster session, posters presented at recent professional meetings were on display; their authors were on hand to discuss the results. In the afternoon seven hands-on breakout sessions covered the following topics: 1) Nematode symptoms on and damage to weeds; 2) A comparison of nematode and herbicide damage symptoms on several crops; 3) A tutorial, utilizing a five-headed microscope, on identification of important California species of lesion nematodes; 4) Entomopathogenic (insect-parasitic) nematodes for biological control programs; 5) Video capture and editing of nematodes (Vcenema); Nematology teaching and research through the virtual microscope; 6) The world through the eyes of a nematode (videos on nematode biology); and 7) Sampling for nematodes. Complete details of the 34th California Nematology Workshop's program including speakers, their professional affiliations and their presentation titles/topics are archived on the CDFANP's web site.

Detailed information for the 35th California Nematology Workshop in San Diego on March 4, 2003, is available on the CDFANP's web site. The 35th California Nematology Workshop offers pest management professionals and growers the latest information on problems caused by plant-parasitic nematodes and potential solutions. The theme topic is invasive nematode pests. Afternoon breakout sessions will give the audience an opportunity to sharpen their skills in nematode identification, disease diagnostics, sampling procedures, and surfing the Internet for information on plant parasitic nematodes. Posters will inform the participants about the latest Nematology research activities at the University of California, CDFA, USDA and industry. The invited speakers' presentations will cover concept and principles of invasion biology, detection surveys, the Western Regional Diagnostics Network, molecular diagnostics of invasive nematode pests, management strategies for invasive plant-parasitic nematodes and current research on invasive nematodes at U.C., Riverside.

(Footnotes)

¹ <http://www.cdfa.ca.gov/phpps/ppd/Nematology/NemaIndexPage.htm>

Regulatory Diagnostics of *Meloidogyne mayaguensis* Using Molecular Technology

Robert W. Hackney

¹Florida recently reported finding the root-knot nematode, *Meloidogyne mayaguensis* Rammah and Hirschmann, 1988 (Nematoda: Tylenchida). Its occurrence throughout several counties in Florida (March 1, 2002, a commercial tomato field, LaBelle, Hendry County, FL; March 7, 2002, ornamental plants in a nursery, Boynton Beach, Palm Beach County, FL; March 7, 2002, tropical fruit trees in a nursery, Redlands, Dade County, FL; and ornamental plants in a nursery, Miami, Broward County, FL) constitutes new records of this exotic pest in the continental United States.

Because the nematode has such a wide host range of ornamentals and field crops, overcomes resistance in several field crops and can reproduce on commercial tomatoes containing the *Mi* gene for resistance, it is important to keep this Exotic Pest out of California. California has no record of it ever being detected anywhere in the State.

Incoming shipments of plants and/or soil containing this Exotic Pest would not be allowed to enter California. This could mean rejecting numerous Nursery shipments from Florida, Texas, Arizona, Hawaii, Puerto Rico, South Africa, Europe and elsewhere. If the nematode were to become established in California, then many California Nursery shipments could be rejected from destinations, anywhere in the world, that are free from *M. mayaguensis*. Locally as well as internationally the potential dollar losses resulting from rejecting shipments of plants or soil contaminated with *M. mayaguensis* are enormous. For example in fiscal year 2001/2002, the value of floral products produced in California was \$376 million. During the same period the value of California's floral and nursery products was \$3.2 billion.

In 1988, the original published concept of the species, *M. mayaguensis*, was based upon host range, morphology, cytogenetics and biochemistry. Today (i.e., 2002 and later) a definitive differential diagnosis of this species from all of the other valid *Meloidogyne* spp. requires the following three components:

- 1) Morphological (i.e., emphasis on the perineal pattern);
- 2) Biochemistry (i.e., emphasis on the isozyme phenotype); and
- 3) Molecular (i.e., emphasis on a DNA analysis).

Host range and nematode cytogenetics can provide valuable supplemental information. However, DNA analysis is the most appropriate tool in screening large numbers of samples for *M. mayaguensis*, and it is decisive for reporting infestations of *M. mayaguensis*. When diagnostics are reported for regulatory action, DNA analysis is mandatory to determine whether plants or soil contain the nematode, because visual inspection and/or laboratory sampling using microscopes (i.e., light and electron) are not sufficient.

²A set of laboratory protocols for the differential diagnosis of *M. mayaguensis* using molecular diagnostics has been subjected to peer review from the international scientific community and ³published.



One single infection site in tomato, (*Lycopersicon esculentum* Linn., 'Rutgers'), roots containing the Southern root-knot nematode, *Meloidogyne incognita* (Kofoid and White, 1919) Chitwood, 1949 is shown. After staining intact roots with acid fuchsin-lactophenol, the following microscopic plant anatomy and nematode morphology are visible: the saccate egg laying female enclosed within a single gall on a tomato root; the female's head and neck inserted into giant cells, which were formed within the root's stele; and a single egg mass, which is composed of eggs within a gelatinous matrix protruding through an opening in the root gall (i.e., appearing slightly within the gall, but mostly external to the galled root).

Microphotograph by R. W. Hackney

Those protocols were originally developed for the basic research, which was published. The international scientific community is currently adapting those laboratory protocols/techniques for use in applied research.

Regulatory diagnostics usually involves a transfer of information from basic research into a specific diagnostic laboratory using pertinent information from applied research plus additional applied research in that specific laboratory. The CDFA Nematology Program (CDFANP) is currently using applied research (Powers,

2002) and pertinent information from in-house and/or collaborative applied research.

Positive controls for the molecular diagnostics of *M. mayaguensis* exist as known/confirmed populations of *M. mayaguensis* from within the Continental United States. Facilities are nearing completion that will allow the CDFANP to accumulate and maintain numerous published positive controls from basic research that exhibit minor, insignificant differences among populations from known/confirmed populations of *M. mayaguensis* outside the United States.

(Footnotes)

- ¹ Brito, Janete, Renato Inserra, Paul Lehman and Wayne Dixon. 2002. Florida Department of Agriculture and Consumer Services, Division of Plant Industry. <http://doacs.state.fl.us/~pi/enpp/nema/m-ayaguensis.html>
- ² Powers, T. O. 2002. Personal communication.
- ³ Blok, Vivian C., Wishart, Jane, Fargette, Mireille, Berthier, Karine and Phillips, Mark. 2002. Mitochondrial DNA differences distinguishing *Meloidogyne mayaguensis* from the major species of tropical root-knot nematodes, *Nematology*, Vol. 4(7), 773-381.
- ⁴ Blok, Vivian C. 2002. Personal communication.

Determination of a Potential Recombinant Potato Virus Y(PVY) Using Serological and Nucleic Acid Based Analysis

Tongyan Tian, Joyce Tuttle, and Terra Irving

Potato virus Y (PVY) is one of economically important viruses for potato production worldwide. Based on disease symptoms, PVY has been divided into three different strains. PVY-o usually causes leaf mottle and necrosis on potato and is mild to symptomless on tobacco. In contrast, PVY-n induces only mild symptoms on potato but severe vein necrosis on tobacco. In addition, PVY-ntn induces tuber necrotic ringspot on potato and also vein necrosis on tobacco. PVYn and PVY-ntn are closely related and considered as the PVY-n group.

In 2001, we reported detection of a PVY-n strain from potatoes collected in Santa Barbara County. Here, we report our finding of a potential recombinant PVY in potato tubers submitted by UC Extension. According to information provided to us, the potatoes were probably produced in Washington State.



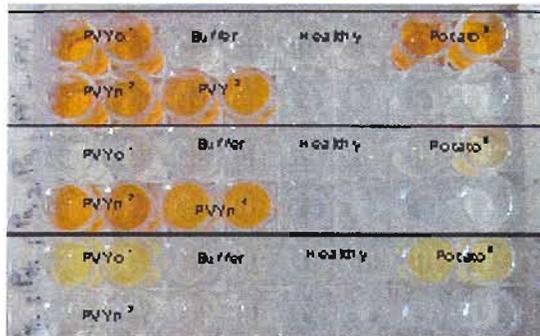
Figure 1. Potato tuber sample obtained in November 2001. Note typical necrotic ringspots on the surface.

Symptoms: The potato tubers clearly showed necrotic ringspots (Figure 1).

We inoculated tobacco plants using the potatoes as the source of inoculation. The tobacco plants later showed vein necrosis symptoms.

Figure 2. ELISA tests using antibodies against PVY, PVY-n, and PVY-o.

- 1 PVYo obtained in 2001 from Santa Barbara Co.
- 2 PVYn obtained in 2001 from Santa Barbara Co.
- 3,4 Positive controls provided by Agdia.
- 5 Potato sample from UC Extension.



ELISA Tests:

We tested the potato sample using ELISA and antibodies against PVY (all strains), PVY-n, and PVY-o. The potato sample reacted positively to antibodies against PVY and PVY-o, but did not react to antibodies against PVY-n (Figure 2).

RT-PCR and Nucleotide Sequence Analysis:

We conducted RT-PCR to amplify PVY sequences corresponding to the P1 and coat protein (CP) coding regions (Figure 3). No product was detected when strain specific primers against PVY-o P1 region were used for RT-PCR. However, DNA fragment of expected size was detected when PVY-n specific primers were used for the same region (data not shown).

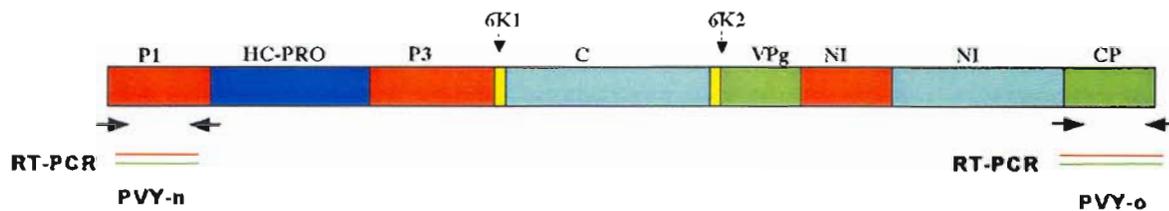


Figure 3. Schematic representation of PVY genome. Arrow pairs indicate oligo primers.

We obtained nucleotide sequences of the RT-PCR products for both P1 and CP region and conducted sequence analysis using Blast search. Our analysis indicated the sequence of P1 coding region belonged to PVY-n group. However, the CP coding region belonged to PVY-o group.

Summary:

The results of our ELISA tests and RT-PCR indicated that there was only one strain of PVY in the potato sample. RT-PCR against the P1 coding region was positive only for PVY-n, however, ELISA and RT-PCR against the CP coding region were positive only for PVY-o. Therefore, a mixed infection by both PVY-n and PVY-o was unlikely. Based on these analyses, we believe that this particular isolate of PVY is a recombinant PVY possessing PVY-n sequence for the P1

region, but PVY-o sequence for the CP region.

Recently, several PVY-n isolates have been detected in the northwestern United States (Crosslin et al., 2002). One of the isolate was described to possess both PVY-n and PVY-o sequences and only reacted to antibodies against PVY-o, not PVY-n. However, this particular isolate was reported to be associated with tubers showing internal brown rings in potato cv. Alturas. We only observed external necrosis on our potato sample. We are not sure whether this particular PVY isolate is the same as reported by Crosslin et al., 2002. We were unable to obtain the cultivar's information for these potatoes or conduct bioassays on potatoes.

Although we have obtained the nucleotide sequences from both ends of the PVY genomic RNA, a large portion of the sequence between P1 and CP remains unknown for this particular isolate. Additional biological and molecular characterization will be extremely helpful for understanding the nature of recombination and its potential threat to potato production.

References Cited:

Crosslin, J. M.; Eastwell, K. C.; Thornton, R. E.; Brown, C. R.; Corsini, D.; Shiel, P. J.; and Berger, P.H. 2002. First report of the Necrotic strain of Potato virus Y (PVYn) on potatoes in the Northwestern United States. *Plant Disease* Vol. 86. No. 10: 1177.

Laboratory Diagnosis of *Phytophthora ramorum* from Field Samples

Cheryl Blomquist¹ and Tom Kubisiak²

Introduction. Diagnosis of a plant disease should never be done on the basis of a single test. Using as much information as possible leads to the most informed diagnosis. The species of host plant, its symptoms, the location of the plant, the status of the county or state (known infested versus not infested with the pathogen), the culture results, and the results of DNA tests should all be used to make the determination. In the case of Sudden Oak Death (SOD), caused by *Phytophthora ramorum* (*Pr*), different kinds of proof are required depending upon whether a sample comes from an infested county versus a county or state not yet known to be infested. In an infested county, recognized host plants with characteristic symptoms and an unequivocal DNA test result are adequate to confirm the presence of *Pr*. To confirm *Pr* in a previously uninfested county or state, or infecting a new host species, the pathogen must be grown in culture and identified using morphological characteristics and DNA sequence analysis. To be unequivocally confirmed, the sequence of the intergenic transcribed spacer (ITS) region of the ribosomal DNA of the suspect organism must match exactly to that of *Pr*.

Symptoms associated with *Pr* infection look different in nurseries than in the wildlands of California. Nursery infections are characterized by large necrotic spots on



Figure 1. Rhododendron leaves infected with *Phytophthora ramorum*. Notice the large necrotic lesions with a diffuse margin. Other *Phytophthora* spp. cause identical symptoms on rhododendron.

rhododendron (*Rhododendron* spp.), typical of infection by many *Phytophthora* spp. (Figure 1). Dieback symptoms on *Viburnum* spp. also occur; however, to date, *Pr* has only been found on *Viburnum* in Europe. In the wildlands of California, symptoms include leaf tip necrosis with angular spotting in California bay laurel (*Umbellularia californica*) (Figure 2) and bleeding in oaks (*Quercus* spp.) (Figure 3). Large necrotic spots are symptoms in California coffeeberry (*Rhamnus californica*) and

toyon (*Heteromeles arbutifolia*), and edge necrosis in big leaf maple (*Acer macrophyllum*) and California buckeye (*Aesculus californica*). However, other plant pathogens including other *Phytophthora* species cause identical symptoms on these host plants. Therefore, laboratory tests are necessary to determine if *Pr* is present.

Culturing *Pr*. Plant pathologists are fortunate to have a number of different laboratory tools to help them diagnose plant diseases. Classically, pathologists have plated infected plant tissue on general or selective media and used morphological characteristics to identify the pathogenic oomycete or fungus that grew into the media. For *Phytophthora* spp. (Phylum Oomycota), a selective medium called PARP is used. PARP includes two antibiotics and an antifungal agent to prevent growth of competitive species of saprophytic bacteria and fungi. Figure 4 shows colonies growing out of lesion margins excised from 4 symptomatic California bay laurel leaves that were plated onto PARP media three to five days earlier. Three to five days after colonies first appear, they are examined by light microscopy for *Pr*'s characteristic hyphae and chlamydospores. Culturing *Pr* from infected host plants seems to be dependent on the environmental conditions



Figure 2. California bay leaves infected with *P. ramorum*. Notice the leaf tip necrosis bordered by an uneven margin and the scattered squarish spots. A small plant sample is assayed from several symptomatic leaves including the margin between the necrotic and healthy tissue. *P. ramorum* can also be detected in some of the scattered spots.



Figure 3. Coast live oak bleeding due to *P. ramorum* infection. If the outer bark is peeled away, a canker with a defined edge is exposed and assayed for *P. ramorum*.

where the samples were collected, on host response, and on the presence of competing organisms in the specific plant species (4). In some infected host plants such as Douglas fir (*Pseudotsuga menziesii*), *Pr* can be cultured only from samples collected during a few weeks in the spring. Except on rare occasions, culturing samples from host plants like Douglas fir during other times of the year yields negative results. With these “hard-to-culture from” plant species, DNA-based technologies to determine if *Pr* is present in a symptomatic plant are more convenient, and plants can be tested during much of the year.

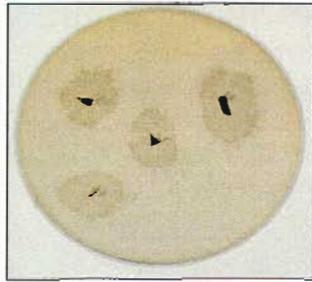


Figure 4. Symptomatic California bay leaf pieces plated on PARP selective media after 5 days.

with any of the *Phytophthora* spp. tested below 10 pg of DNA/ μ l of reaction. We found the presence of *Pr* to still be faintly detectable with as little as 2.5 fg DNA/ μ l of reaction (4000 times less DNA). The cross-reactivity of the primers with certain isolates of *Phytophthora* has since been reduced by further optimization of the PCR technique (M. Garbelotto, pers. comm.). In general, the technique appears to be highly specific for *Pr* especially in the range of DNA concentrations one might expect from extractions performed on symptomatic host tissues collected in the field.

DNA extraction from host tissues.

Reliable use of a DNA test to assay for *Pr* requires that pathogen DNA be extracted from symptomatic plant tissues. Foliar samples from infected host species that support abundant sporulation, such as rhododendron and California bay laurel (2) or bark tissues around the margin of bleeding oaks, appear to be the most reliable source of tissue for diagnosis. However, *Pr* is known to infect many other host species (4). In general, plants produce polyphenolic compounds along with tannins and other natural-occurring compounds (especially polysaccharides) that make the extraction of quality DNA difficult. Plants and oomycetes have strong cell walls that must be broken before the DNA can be released. Cells are usually broken manually with a mortar and pestle, mechanically with a dental amalgamator, or by more high-throughput technologies. Most leaves are easy to grind, while most woody tissue is difficult.

DNA-based diagnosis of *P. ramorum*. Recently, a DNA-based method for the detection of *Pr* was developed (3). This method takes advantage of the sequence divergence between *Pr* and all other *Phytophthora* spp. (for which sequence data was available) in the ITS region of the nuclear ribosomal DNA gene repeat. We recently tested the first-round primers (Phyto1 and Phyto4) on pure DNAs obtained from a number of *Phytophthora* spp. including *P. boehmeriae*, *P. cambivora*, *P. cinnamomi*, *P. cryptogea*, *P. erythroseptica*, *P. gonapodyides*, *P. lateralis*, *P. megasperma*, *P. palmivora*, *P. parasitica*, *Pr*, and *P. syringae*. Using the polymerase chain reaction (PCR) this primer pair was known to amplify a band of 687 base pairs in size from *Pr*. At high DNA concentrations, we observed some cross-reactivity in the expected size range with *P. cambivora*, *P. cinnamomi*, *P. lateralis* and *P. syringae*. Cross-reactivity is a real concern and can lead to a false-positive identification for *Pr*. In general, the cross-reactive bands were fainter than the band observed for *Pr*, and additional diagnostic bands were often amplified (Figure 5). As the DNA concentration was reduced, the cross-reactivity of the primers was no longer an issue. Given the standard 35-cycle PCR protocol, there was no detectable cross-reactivity observed

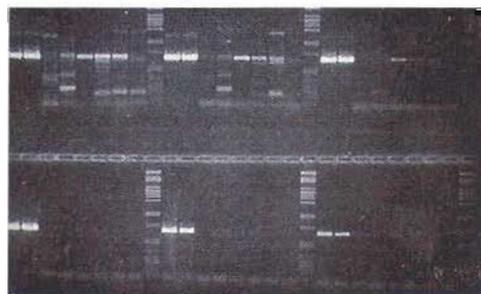


Figure 5. DNAs from eight different pure *Phytophthora* isolates (order on gel: *P. ramorum* 016; *P. ramorum* 013; *P. syringae* 442; *P. cinnamomi* 447; *P. cambivora* 444; *P. cambivora* 443; *P. lateralis* 452; and *P. lateralis* 440) were amplified using the standard 35 cycle PCR protocol and the first-round primers Phyto1 and Phyto4 (Garbelotto et al. 2002) at 6 different DNA concentrations (from upper left to lower right: 1000 pg/ μ l of reaction; 100 pg/ μ l; 10 pg/ μ l; 1 pg/ μ l; and 0.1 pg/ μ l). Genomic lambda digested with the restriction enzyme *Pst*I was used as the size standard.

Many protocols for DNA extraction have been published. We have tested several of the more common protocols on *Pr* infected leaf disks from several host species including bay laurel, rhododendron, and mountain laurel (*Kalmia latifolia*) and have found some to be superior to others. One method found to work well across the host species tested was a nonionic detergent, cetyltrimethylammonium bromide (CTAB)-based, extraction protocol (Figure 6). Polysaccharides, polyphenolic compounds, and other enzyme-inhibiting contaminants often found in plant cells are generally removed, as most do

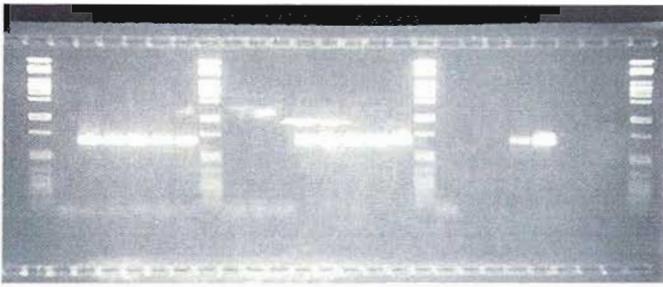


Figure 6. DNAs extracted from three different host species (bay laurel, rhododendron, and mountain laurel) infected by *P. ramorum* were extracted using three different DNA extraction techniques [CTAB; DNeasy® kit (Qiagen Inc. Valencia, California, USA); and PhytoPure™ kit (Amersham International plc, Buckinghamshire, England)] and amplified using the standard 35 cycle PCR protocol and the first-round primers Phyto1 and Phyto4 (Garbelotto et al. 2002). Genomic lambda DNA digested with the restriction enzyme *Pst*I (λ -*Pst*I) was used as the size standard. Lanes are: λ -*Pst*I; H₂O control; infected bay laurel/CTAB; infected bay laurel/CTAB; infected rhododendron/CTAB; infected rhododendron/CTAB; infected mountain laurel/CTAB; λ -*Pst*I; H₂O control; uninfected mountain laurel/DNeasy®; uninfected mountain laurel/DNeasy®; infected bay laurel/DNeasy®; infected bay laurel/DNeasy®; infected rhododendron/DNeasy®; infected rhododendron/DNeasy®; infected mountain laurel/DNeasy®; λ -*Pst*I; H₂O control; uninfected mountain laurel/PhytoPure™; uninfected mountain laurel/PhytoPure™; infected bay laurel/PhytoPure™; infected bay laurel/PhytoPure™; infected rhododendron/PhytoPure™; infected rhododendron/PhytoPure™; infected mountain laurel/PhytoPure™; λ -*Pst*I.

not precipitate with CTAB during the extraction process (1). Some of the more practical benefits of the protocol are that large quantities of reagents and solutions can be prepared at a fraction of the cost of commercially available kits, it is fairly simple, and is easily scaled from milligrams to grams of tissue. Unfortunately, it is not very amenable to high throughput techniques (~50 samples per technician day). Another method found to work well across the host species tested was a commercial column or membrane-based DNA extraction kit (Figure 6). This protocol allows for the adsorption of DNA to a special membrane, thus, allowing for the optimal removal of polysaccharides, polyphenols, and other unwanted plant metabolites and constituents. The main benefit associated with this protocol is that it is highly amenable to high-throughput technologies (~400 samples per technician day). Despite this, the cost is significantly more expensive per sample than the CTAB-based protocol. A final method tested was a commercial resin-based DNA extraction kit. This protocol employs a resin that specifically binds and precipitates unwanted polysaccharides. Using this technique, DNAs obtained from two of the three host species tested were not amenable to enzyme manipulation (Figure 6). This protocol appears to be less reliable for the detection of *Pr* in infected host tissues than either the CTAB or

membrane-based methods.

Although CTAB has been the standard for DNA extraction from leaf tissues, to date we have been unable to obtain reliable first-round PCR amplification from infected oak bark using this method. We have, however, obtained good quality DNA from a membrane-based technique. Freeze-drying woody tissue before grinding makes the tissue brittle and thus facilitates cell disruption.

Conclusion. Detection of a plant pathogen with quarantine status can have severe economic consequences. Therefore, diagnoses should be made using all available information. Until *Phytophthora* spp. more closely related to *Pr* are found, morphological identification and current DNA tests seem to provide reliable means of detection without a high number of false positive results. To minimize the number of false-negatives, a robust DNA extraction procedure is recommended, i.e., one that is applicable across the widest range of host species and tissues. The current ITS-based PCR technique (described above) is highly specific for *Pr* at low DNA concentrations and has proven useful for its detection in infected host tissues. There are also other DNA-based methods being developed for the detection of *Pr*; these include assays based on the sequences for other nuclear genes such as b-tubulin, or sequences for organellar or mitochondrial genes such as *cox2*. A promising new technique called single strand conformation polymorphism (SSCP) analysis may eventually allow for the detection and unambiguous identification of most, if not all, *Phytophthora* spp.. In the future, we look forward to additional molecular assays for detecting and identifying *Pr*.

References:

- Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and Stuhl, K. 1987. *Current protocols in molecular biology*. Wiley, New York.
- Davidson, J.M., Rizzo, D.M., Garbelotto, M., Tjosvold, S., and Slaughter, G.W. 2002. *Phytophthora ramorum* and sudden oak death in California: II. Transmission and survival. USDA Forest Service Gen. Tech. Rep. PSW-GTR-184 p741-749.
- Garbelotto, M., Rizzo, D.M., Hayden, K., Meija-Chang, M., Davidson, J.M., and Tjosvold, S. 2002. *Phytophthora ramorum* and sudden oak death in California: III. Preliminary studies in pathogen genetics. USDA Forest Service Gen. Tech. Rep. PSW-GTR-184 p765-774.
- Rizzo, D.M., Garbelotto, M., Davidson, J.M., Slaughter, G.W., and Koike, S.T. 2002. *Phytophthora ramorum* and sudden oak death in California: I. Host relationships. USDA Forest Service Gen. Tech. Rep. PSW-GTR-184 p733-739.

Nursery Testing of Prune Dwarf Virus, Prunus Necrotic Ring Spot Virus, Grapevine Fanleaf Virus, and Grapevine Leafroll Associated Viruses

YunPing Zhang and Umesh Kodira

Prune dwarf virus (PDV) and prunus necrotic ring spot virus (PNRSV) are both ilarviruses and infect a wide range of *Prunus* species. Although they are not serologically related, they do share many properties such as: seed and pollen borne, readily graft-transmitted. Due to some of these characteristics, they are readily transmitted to a large number of stone fruit trees and can cause heavy crop loss.

Grapevine fanleaf virus (GFLV) is a nepovirus, nematode transmitted, graft-transmittable, causing various symptoms on grapevine and yield loss. Grapevine leafroll associated viruses (GLRaV) are closteroviruses. There are seven types of leafroll-associated closteroviruses reported. Type 2 and 3 are most common in California. These leafroll-associated viruses have been reported to be transmitted by mealybugs.

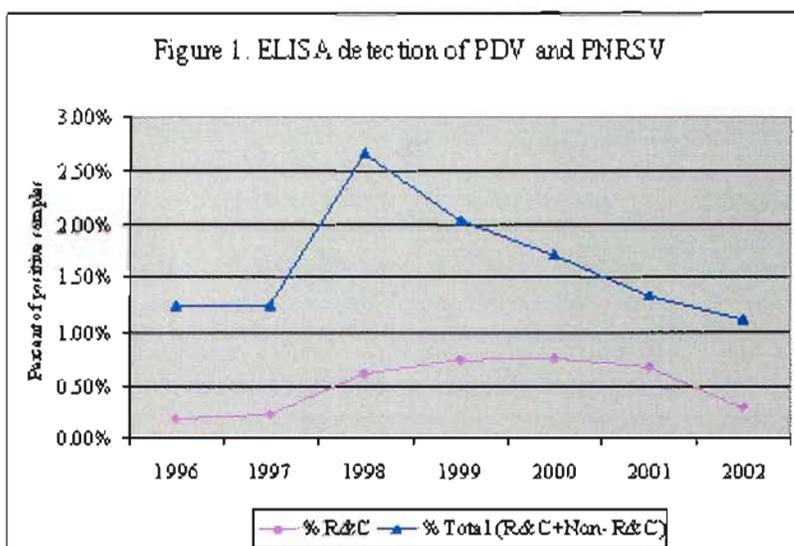
California Department of Food and Agriculture nursery program tests these viruses annually to ensure the healthy production of California fruit tree and grapevines. A registration and certification program was established to test various fruit trees, nut trees, and grapevines for the participating nurseries. In the laboratory these viruses are tested by Enzyme-Linked Immunosorbent Assay (ELISA).

The ELISA test is being performed using polyclonal and monoclonal antibodies in a combined format for both PDV and PNRSV. The ELISA plates are first coated with a mixture of polyclonal antibodies of both PNRSV and PDV, followed by incubation with sample extracts. Monoclonal antibodies of both PNRSV and PDV, along with alkaline phosphatase conjugated goat anti-mouse antibody, were then added to react with viral antigen. Positive samples are detected by addition of substrate for alkaline phosphatase and color change. A positive sample is scored by the detection of either one of the two viruses or both.

For the detection of GFLV, GLRaV 2 and 3, a F(ab)₂ ELISA system is used. F(ab)₂ antibody is used

to coat the plates, and then virus specific antibodies are used for detection of the virus. Protein-A alkaline phosphatase conjugate is then used to react with the virus specific antibody. The major steps are the same as for the detection of stone fruit viruses.

A total of 45,992 stone fruit samples from 16 nurseries were tested by ELISA for the two ilarviruses for the year 2002. Of the stone fruit tree samples (40,467 R&C samples and 5,525 service samples)



tested, 516 samples (1.12%) were tested positive for the 2 ilarviruses. There were only 120 (0.30%) of the R&C samples tested positive for the same viruses while 395 (7.17%) of the service samples were tested positive, which is a much higher infection by the viruses (Figure 1). The stone fruit trees being tested included: peach, nectarine, almond, apricot, cherry, plum, and prune.

We also tested 1,318 grapevine samples (composite of 6,590 grapevines) for grapevine fanleaf virus and 1,540 grapevine samples for grapevine leafroll associated viruses 2 & 3. All these samples were tested negative.

Acknowledgements: This project is supported by California Fruit Tree, Nut Tree, and Grapevine Improvement Advisory Board, Pest exclusion biologists, and participating nurseries.

The Epidemiology of Pierce's Disease

Barry L. Hill and Jennifer Hashim

INTRODUCTION

The epidemiology of Pierce's Disease (PD) changed dramatically in California with the arrival of the glassy-winged sharpshooter (GWSS) about 15 years ago. Before that time infections that persisted and resulted in vine death were the result of primary spread, i.e., from inoculum sources outside the vineyard. The disease caused losses, but the spread was linear, not logarithmic, and the damage was a gradual linear accumulation resulting in the loss of a small percentage of vines. With the exception of some traditional "hotspot" areas, losses from PD were important but not severe enough to preclude grape production. With the arrival of the GWSS, however, the transmission of the causal bacterium appears to be both primary and secondary (from vine to vine) and subsequent disease spread has become logarithmic, such that entire vineyards can be destroyed in as little as 3 to 5 years (Perring, Farrar et al., 2001; Blua, Phillips et al., 1999; Purcell and Saunders 1999). To cope with this development there have been extensive field studies to determine methods to control the glassy-winged sharpshooter. However, our understanding of how to control the disease and the characterization of the changes in the epidemiology of PD when the causal bacterium is transmitted by GWSS has been based largely on anecdotal information and general observations with limited actual field data.

This project will use field data from large numbers of vineyards over several years time to assess the impact of the glassy-winged sharpshooter on the epidemiology of Pierce's Disease. The field season of 2002 was the first year of a proposed 5 year project. The resulting improved understanding of PD epidemiology may also enable CDFA and UCCE to propose some preliminary recommendations for disease-based control strategies that growers can implement.

Two critical issues are: 1. How much economic loss can be expected where GWSS occurs or when the insect moves into new viticulture areas? and 2. What disease-based control methods can be employed in areas already infested with GWSS? The current

economic loss models for GWSS are not based on empirical data but on arbitrary projections. Empirical mapping and disease tracking data that enables the comparison of various epidemiological factors (such as cultivar and susceptibility, vineyard age, proximity to GWSS hosts, cultural and control practices in grapes and other crops, etc.) are needed to make better informed projections. Current epidemiological models based on other native sharpshooter vectors (Purcell 1981) are not adequate to account for vine-to-vine spread when GWSS is the vector. Historically, mapping the incidence and vine locations of PD and tracking the spread over a few consecutive years has led to key conclusions regarding the sources of PD spread (Hewitt and Houston 1941, Purcell 1974) and the effectiveness of various control methods (Purcell 1979, Hewitt, Frazier et al., 1949). For example, these previous efforts paid off in identifying the highest risk areas to be avoided with new grape plantings.

OBJECTIVES

1. Develop a model for PD epidemiology when Xf is vectored by GWSS that evaluates the importance of epidemiological factors such as GWSS population size, vine age, cultivar susceptibility, control practices, and GWSS control treatments in vineyards and nearby GWSS hosts or habitat.
2. Develop PD identification and management strategies for use by growers to reduce risk and damage. Update and provide educational materials to assist vineyard managers, pest control advisors, and county, state, and federal staff involved in advising growers and area-wide management plans.
3. Create a central data processing facility to compile the data from these projects in a GIS format. Share the resulting data, maps, and information with collaborating plant pathologists, statistical analysts, agricultural economists, and other legitimate researchers to maximize the opportunity to understand the changed epidemiology of Pierce's disease, to manage the disease, and to generate projections for potential economic consequences and risk assessment.

RESULTS AND CONCLUSIONS

Two projects with shared methods and objectives were pursued cooperatively to avoid duplication and make the most efficient use of management and field personnel,

equipment and other resources. 1. Epidemiological Assessments of Pierce's Disease (BLH) 2. Monitoring and Control Measures for Pierce's Disease in Kern County (JH). Field surveys were conducted between early August and the end of November, 2002, after which the data compilation began. A field crew composed of CDFA and UC people was trained, and the surveys were done using all terrain vehicles.

Another project using identical methods and funded by private sources was conducted by Gisela Wittenborn, and her data was made available to the overall project. Every vine displaying possible PD symptoms was identified, tagged, mapped, and a sample was taken and sent to the CDFA diagnostic laboratory in Sacramento and tested by ELISA for *Xf*, more than 3100 samples total. In all more than 250 blocks (> 6000 acres total) in Kern county and more than 60 blocks (>3000 acres total) in Tulare county were surveyed and mapped. More than 30 growers participated in the project. As the data is compiled these participants will be provided with mapped survey results for their vineyards to assist in disease control.

The following cultivars were included in the study: Red varieties include Christmas Rose, Crimson Seedless, Flame Seedless, Redglobe, Ruby Seedless. White varieties include Calmeria, French Columbard (wine), Jade Seedless. Muscat, Perlette, Thompson Seedless, Superior Seedless. Purple varieties include Autumn Royal, Black Emerald, Fantasy Seedless. A data center at the Center for the Assessment and Monitoring of Forest and Environmental Resources (CAMFER) at University of California, Berkeley is beginning to compile the data and create a GIS based data set that will be used in these projects and made

available to other legitimate researchers. The sites that were surveyed were selected to enable a wide range of comparisons within the data set to enable the evaluation of epidemiological variables, projection of disease progression over time, and the effectiveness of disease control practices.

References:

- Blua, M. J., Phillips, P. A. and Redak, R. A. 1999. A new sharpshooter threatens both crops and ornamentals. *California Agriculture* 53(2): 22-25.
- Hewitt, W. B., Frazier, N. W. and Freitag, J. H. 1949. Pierce's disease investigations. *Hilgardia* 19: 207-264.
- Hewitt, W. B. and Houston, B. R. 1941. Association of Pierce's disease and alfalfa dwarf in California. *Plant Dis. Rep.* 25: 475-476
- Perring, T. M., Farrar, C. A. and Blua, M. J. 2001. Proximity to citrus influences Pierce's disease in Temecula Valley vineyards. *California Agriculture* 55: 13-18.
- Purcell, A. H. 1974. Spatial patterns of Pierce's disease in Napa Valley. *Amer. J. Enol. Vitic.* 25: 162-167.
- Purcell, A. H. 1979. Control of the blue-green sharpshooter and effects on the spread of Pierce's disease of grapevines. *J. Econ. Entomol.* 72: 887-892.
- Purcell, A. H. 1981. Vector preference and inoculation efficiency as components of resistance to Pierce's disease in European grape cultivars. *Phytopathology* 71: 429-435.
- Purcell, A. H. and Saunders, S. R. 1999. Glassy-winged sharpshooters expected to increase plant disease. *California Agriculture* 53(2): 26-27.

2002 Calendar Year Annual Report Items

T. E. Tidwell

A devastating new rust disease of *Vinca major* and *Vinca minor* from San Mateo County was reported and published in Plant Disease:

First report of *Puccinia vincae* on *Vinca* spp. In California. J. R. Hernandez, USDA ARS Systematic Botany and Mycology Laboratory, Beltsville, MD 20705, M.E. Palm Hernandez, APHIS, Systematic Botany and Mycology Laboratory, Beltsville, MD 20705, and T.E. Tidwell California Department of Food and Agriculture, Sacramento, 95832. Plant Disease 86:75, 2002.

A new Lily disease to North America, *Botryotinia spaherosperma*, was detected in Santa Barbara County by Santa Barbara County Plant Pathologist, Dr. Heather Scheck, and identified by CDFA PPDC mycologist, Diana Fogle. This is a very destructive disease of lily plants and produces a long-lived sclerotial survival stage that can contaminate soil of growing grounds and carry the pathogen over from crop to crop.

Daylily rust, *Puccinia hemerocallidis*, was ultimately confirmed from all the Southern California Counties. Commissioner Bill Gillette initiated a move to downgrade the rating from Q to C.

In 2002 Groundsel rust, *Puccinia lagenophorae*, was confirmed from all coastal counties from San Diego to Oregon. PPDC plant pathology staff initiated a move to downgrade the rating from Q to C.

FOV testing of cotton seed from Australia. In a combined effort with scientists from UC Davis and UC Berkeley, CDFA scientists tested samples of linted cotton seed, imported for feed purposes by the California cattle industry, for the soil and seed-borne pathogen, *Fusarium oxysporum* f. sp. *vasinfectum* (FOV). The FOV strains present in Australia are highly virulent and very different in their biology from the strains currently known in California. Much effort was expended for the second consecutive year via seed health testing and mycological studies to insure that the seed was not still harboring the FOV pathogen, despite having been fumigated on a very large scale basis.

A prospective publication for UC Extension on the topic of Chrysanthemum white rust was reviewed by PPDC staff scientists. The publication was subsequently deemed to be inaccurate and of inadequate quality to be published by UC Extension.

Seed Health testing:

Several PPDC scientists participated in a workshop to train seed scientists in procedures to detect genetically modified organisms (GMO) in seed, and consequently received certification for GMO testing.

Contributions were made by PPDC to the ongoing collection of National Seed Health System (NSHS) seed health testing protocols; PPDC scientists participated in the review and decisions on protocols to be published in USDA NSHS Reference manual B.

Report for the Year 2002

Dan Opgenorth

In consideration of the need for rapid and accurate response to diagnose regulated plant diseases, I have continued to pursue the development of Real Time PCR. The expanding global economy and free trade agreements together with an increase in international travel make the interception of accidentally and deliberately introduced plant pathogens of great importance. To determine if PCR-based assays are useful for routine detection of plant pathogens in dry plant materials at port facilities, we evaluated a real-time PCR assay for on-site detection of the citrus canker bacterium (*Xanthomonas citri*). Using previously intercepted samples, dried materials were prepared and tested on-site in about one hour with approximately 90% positive at LAX and 50% positive at SFO. The same samples were later used for culture analysis at Fredrick, MD and positive *X. citri* was isolated from each group. Using the Smart Cycler (Cepheid) provides a rapid, sensitive and simple method to make on-site determinations of suspect materials. I would like to thank Norman Schaad at Fredrick, MD, the USDA and CDFA people at LAX and SFO for making this project possible. A portion of the funding for travel came from CDFA for work on exotic pests. I would hope that these resources would be continued and enhanced so that similar methods for detection of additional target pests could be developed. More information can be found in a recently published article using this technique to detect Pierce's Disease (Phytopathology 92(7):721, 2002).

Devastation of the corn forage crops in the central valley continue to be a problem with losses in tonnage in individual fields approaching 50%. Since custom harvesters are paid by the ton, many of these fields may go unharvested and can thus result in a complete loss. Corn Stunt caused by *Spiroplasma citri* seems to be the predominant cause of this disease problem. Our laboratory is doing ELISA testing in cooperation with UC Parlier to verify the presence of disease and

continue to investigate the problem. A PCR technique has also been implemented in our lab which has increased the sensitivity of detection and can be used on the leafhopper vector. Presently, we can detect the spiroplasma in 5-10 leafhopper species. Since these vectors are now found through the San Joaquin Valley and have now been found in Sacramento and Butte counties, we can potentially expect the spread of the disease. Using PCR on insects collected on yellow traps last season, we hope to evaluate the extent of the problem and to predict what growers may expect next year. A special thanks to Charlie Summers from the UC Parlier station and Farm Advisors and Ag Commissioner staff in Kings, Tulare, Fresno and Sacramento counties for their help in working on this project.

Another developing project involves the detection of Phytoplasma in Strawberry Nurseries. Red plants with curled leaves have been on the increase in the last several years. We usually can detect Phytoplasma from the leaf petioles of these plants using PCR and generic primers. Presently, additional information is being developed that would allow us to further characterize these Phytoplasma. If these are classified as Astor Yellows the disease condition may be a local and isolated occurrence. If exotic Phytoplasma are found, it could potentially be a large problem for the entire industry.

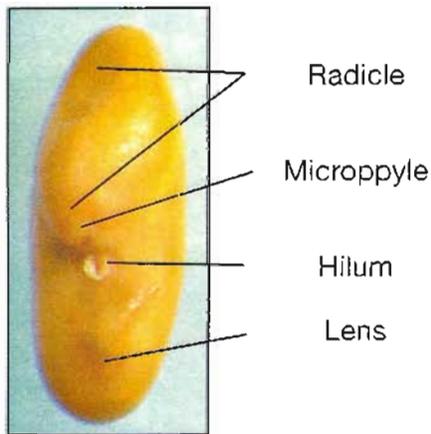
On January 16, 2002 The California Plant Disease Conference was held at the Diagnostic Center. This was the ninth conference with the goal of informing our constituents in the counties about additions to our staff, newly developed assays and special projects. The afternoon session focused on newly emerging biotechnology and regulation of transgenic crops. As usual we had a sellout crowd with several County Commissioners participating.

Seed Technologist Training

Jim Effenberger, Elaine Harris, Deborah Meyer, Paul Peterson, Evelyn Ramos, Marian Stephenson and Connie Weiner

Seed technologists' responsibility is to quantify the quality of seed lots. Their laboratory analyses serve as the basis for seed trade and thus, the exchange of millions of dollars in seed sales. Standardization of laboratory test procedures is key to the success of the seed industry. Providing training via seed workshops (including supervision of individual study) is one PPDC Seed Lab's mission, with the goal of promoting standardization among laboratories.

The Seed Laboratory hosted two one-day workshops in 2002 and provided training to several visiting seed technologists. Teaching materials were prepared on identification of wheatgrass florets, Lamiaceae nutlets, seeds and some fruits of Solanaceae and Convolvulaceae, corn, brassica, onion and cucurbit seedling evaluation, and tetrazolium testing of wheat and alfalfa.



Psathyrostachys juncea
Russian wildrye

Identifying Characters

Lemma lance shaped, scabrous, usually copiously pubescent

Palea has sunken appearance, densely pubescent and scurfy

Rachilla stout



The traditional fruit type for Lamiaceae is a schizocarp splitting into four nutlets.



Nutlets of *Lamium amplexicaule*, henbit



Developing nutlet of *Lavandula*

Important seed identification characters for species of Convolvulaceae include size, shape color, texture, hilum type, and embryo shape.



Longitudinal section of *Convolvulus arvensis*, field bindweed.



Size, shape and color of *Cuscuta gronovii*, dodder.

Customized Tests Provide Expanded Description of Planting Seed Quality

Jim Effenberger and Marian Stephenson

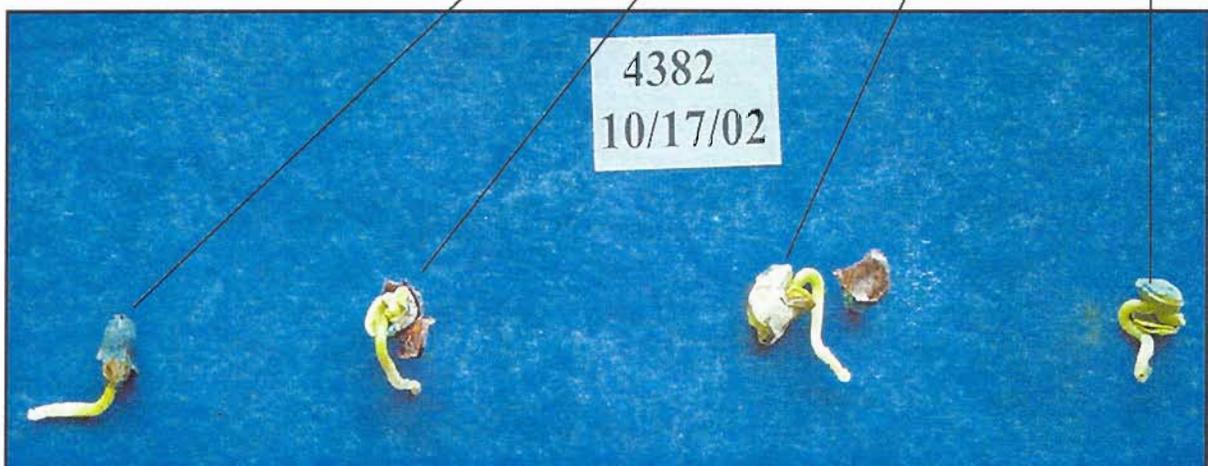
Standardized procedures for describing the purity and germinative potential of seed under optimum conditions form the basis for seed trade. The results of such tests are expressed in percentages and lists of contaminating species. Clients may request customized examinations and reports to obtain more information about a seed lot. The following data from customized examinations provide an enhanced description that suggests the cause of poor field performance of a cottonseed lot.

An examination of a sample of the seed lot revealed mechanical damage to the seed. Severely damaged seeds were removed and placed on germination media for evaluation of germinative potential. While the incidence of mechanically damaged seed is not reflected in the reported percentage of pure seed (99.93% for the sample considered below), mechanical damage is known to result in reduced emergence and seedling vigor.

Pure seed with Damaged Seed Coats

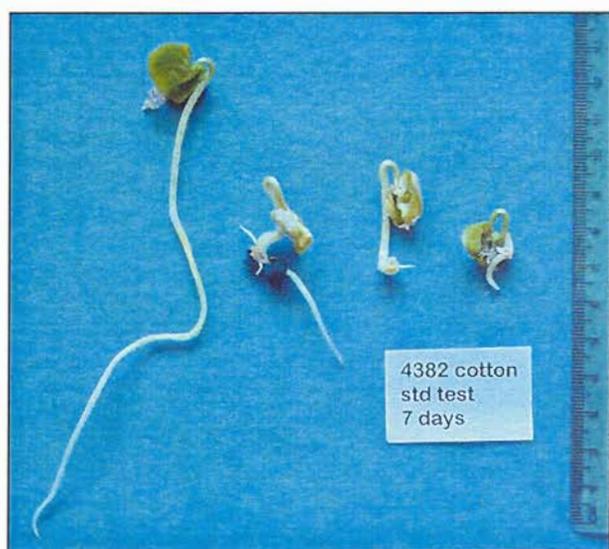


Pure Seed Severely Damaged



Severely damaged seed germinated on top of blotters at 20-30 °C with light for 7 days produced seedlings with insufficient root systems as well as shortened hypocotyls.

The standard germination test for cotton is conducted by placing 400 seeds on damp medium (rolled germination paper) at temperatures alternating between 20°C (68°F) for 16 hours and 30°C (86°F) for 8 hours. Care is taken in standard germination tests to provide adequate but not excessive moisture. Seedlings develop within 12 days; those that possess the structures essential to produce a normal plant under favorable conditions are considered “normal.” A normal cotton seedling has half or more of cotyledon tissue attached and free of necrosis or damage, an elongated hypocotyl without lesions affecting conducting tissue, and sufficient roots--either primary or secondary--to anchor the seedling. Non-uniformity of seedling lengths is not a factor in the standard germination result. The final



germination for the example above was 90%, indicating that the lot the sample represents is capable of producing an adequate stand under favorable field conditions

In a “cool” test, cottonseed is tested in distilled water-saturated rolled towels in the dark at 18°C (64.4° F) for 7 days. Only seedlings with root-hypocotyls

measuring at least 4 cms are counted. Only 18% of the seeds tested from the cotton sample illustrated above met that standard. The cool germination result (18%) indicates that the lot the sample represents is not vigorous; it would not be suitable for planting in cool soil.



References Cited:

- Association of Official Seed Analysts. 2002. Rules for testing seeds. 166 pp.
- Association of Official Seed Analysts. 2002. Seedling evaluation handbook. Contribution no. 35 to the handbook on seed testing. 128 pp.
- Association of Official Seed Analysts. 1983. Seed vigor testing handbook. pp 63-64
- Douglas, A.G., O.L. Brooks & E.E. Winstead. 1965. Effect of mechanical harvester damage on germination and vigor of cottonseed. Proceedings of the 55th annual meeting, Association of Official Seed Analysts. pp 97 - 103..

Effect of Temperature on Germination of Blue Wildrye, *Elymus glaucus* Buckley

Marian Stephenson, Evelyn Ramos and Jaime Sallee

Germination requirements of the native grass *Elymus glaucus*, blue wildrye have not been documented nor has a procedure for laboratory testing been adopted by the Association of Official Seed Analysts or other seed testing organizations, i.e., the International Seed Testing Association (ISTA). Five samples of seed submitted to the PPDC Seed Laboratory as regulatory samples were tested at three temperature regimes under light. Four 100-seed replicates were tested at each temperature. Final germination percentages at 15-25° C, 20° C, and 20-30° C were significantly different ($\alpha=.05$) for only one sample, where germination was negatively affected at 20-30° C. Seven-day prechill followed by incubation at the above temperatures was performed on three of the samples; there were no significant differences in final germination results.



Germination of <i>Elymus glaucus</i> , blue wildrye at three temperatures, with and without prechill						
Sample	20-30 C	15-25 C	20 C	20-30 C prechill	15-25 C prechill	20 C prechill
1916	89	86	95	89	92	89
	88	93	91	91	90	90
	84	92	92	90	94	93
	88	88	90	91	91	93
1986	89	89	88	85	93	89
	91	91	88	92	91	95
	91	90	90	90	93	93
	88	88	90	89	93	84
2105	86	87	80	78	76	83
	82	84	81	81	89	84
	84	79	87	85	79	79
	80	87	87	78	93	81
2553	89	91	89			
	83	91	93			
	86	89	91			
	86	94	88			
2758	90	94	93			
	94	95	92			
	93	93	96			
	93	95	95			

Laboratory Germination of a Potentially Invasive Plant Pest, *Sesbania punicea*

Jaime Sallee and Marian Stephenson

Sesbania punicea (Fabaceae), an ornamental in California gardens, is an invasive woody species in South Africa, Florida, Georgia and Texas. The woody shrub has recently been found “out of place” along the American River Parkway in Sacramento, CA as well as in Suisun Marsh and on the shore of the Oroville Dam north forebay. Seeds of the plant have an impermeable seed coat, prohibiting imbibition of water and, thus, germination. Four treatments (manual scarification, hot water soak, and tumbling in water or in water with wet sand) followed by incubation between damp germination blotters at 20-30° C, were applied to seeds collected from trees in Sacramento.

Scarification Method	Germination (%)
Shaken for 6 hours/water	4
Shaken for 6 hours/sand & water	0
Shaken for 30 hours/water	4
Shaken for 30 hours/sand & water	8
Heated for 2 Minutes	20
Manual scarification w/ a razor blade	100*

*25 seeds; other treatments were applied to 4 replicates of 25 seeds each.



Comparison of Purity Testing Methods of Weeping Alkaligrass (*Puccinellia distans* (Jacq.) Parl.).

D. J. Lionakis Meyer and J. Effenberger

The use of seed blowers in the laboratory to estimate seed quality (i.e., percentages of pure seed and inert material) for certain grass species has been studied by numerous seed researchers since the late 1950's. Seed blower methods employ a calibrated air stream within a General Seed Blower (Figure 1) to separate pure seed from inert material (Everson, et al., 1983), effectively eliminating the factor of personal bias that could affect results of the hand separation method (HSM). In most of these studies purity test results among laboratories were less variable with the seed blower methods as opposed to the HSM. Additionally, test efficiency was improved with the seed blower methods. The seed blower method referred to as the Uniform Blowing Procedure (UBP) is the internationally accepted method for purity testing of Kentucky bluegrass (*Poa pratensis*), rough bluegrass (*P. trivialis*) and orchardgrass (*Dactylis glomerata*) (AOSA 2001, CFIA 1997, ISTA 1999). In the United States the UBP is also the standard purity testing procedure for Canada bluegrass (*P. compressa*), Pensacola bahiagrass (*Paspalum notatum* 'Pensacola'), blue grama (*Bouteloua gracilis*) and side-oats grama (*B. curtipendula*) (AOSA 2001). The UBP requires the seed blower to be calibrated using a calibration sample available from either the Association of Official Seed Analysts (AOSA) or the International Seed Testing Association (ISTA). Rather than use calibration samples specific for each species, for Canada bluegrass, rough bluegrass, blue grama and side-oats grama the UBP employs a mathematical factor associated with the Kentucky bluegrass calibration sample.

Weeping alkaligrass (*Puccinellia distans*) is a small-seeded grass species similar in size to Kentucky bluegrass and rough bluegrass. The purpose of this study was to establish an efficient and non-subjective UBP that closely replicates purity results from the labor intensive HSM for weeping alkaligrass currently used by laboratories. For the HSM these pure seed units were required to contain at least one caryopsis (grass fruit containing a single seed), as determined by microscopic examination. Under the UBP seed units retained in the heavy fraction following blowing were considered pure seed, and those in the light fraction were considered inert matter, irrespective of whether they contained a caryopsis.

The study was divided into two experiments. In Experiment I, pure seed percentages from the HSM were compared with results from the UBP of five blower settings at or near those established in the AOSA Rules

for Kentucky bluegrass and rough bluegrass (AOSA Rules 2001). Additionally, to determine if the UBP improved consistency in the germination test (percentage of normal seedling produced), the percentage of pure live seed (PLS) was determined for the pure seed obtained from each blower setting of the UBP and by the HSM. PLS is the product of the pure seed and germination percentages divided by 100. Experiment I was conducted at the California Department of Food & Agriculture Seed Laboratory. The UBP that compared best with the HSM and demonstrated the least amount of variability in Experiment I was selected for comparison with the HSM in Experiment II as a collaborative validation study among nine laboratories.

METHODS AND MATERIALS

Experiment I Sub-samples of weeping alkaligrass were taken from each of 12 commercial seed lots and were randomly assigned to one of six testing methods: one sub-sample from each lot was assigned to the HSM, and the remaining 5 sub-samples were each assigned to one of the five UBP blower settings.

Each sub-sample was separated into two categories: pure seed units consisting of single or multiple florets or spikelets with attached pedicels (Figure 2), and all contaminants (i.e., other crop, weed seed, and inert matter). Sub-samples tested by the HSM were examined under a microscope, and slight pressure was applied to each seed unit to determine if a caryopsis was present. Each sub-sample tested by the UBP was blown for 3 minutes at the appropriate blower setting. Seed units (i.e., single or multiple floret or spikelet) remaining in the heavy portion were considered pure seed, and those in the light portion were considered inert matter. Inert matter not attached to the seed units and seeds of other species were removed from the heavy portion and added to the inert matter from the light portion. The final separation for both methods resulted in two categories; pure seed and all contaminants. The time required to complete each method was recorded.

Germination tests were conducted to examine the effectiveness of the UBP at removing empty or immature seed units. Four hundred seed from each purity test were planted (Chirco and Turner 1986), and the number of normal seedlings produced was determined at 21 days. In order to compare overall influence of the purity method on germination across all methods, the percentage of PLS was determined for each lot and treatment.

In this experiment the HSM was considered the standard method, because it is the current purity testing method used among laboratories that routinely test weeping alkaligrass. The statistical analysis compared percent pure seed and PLS for each UBP against the standard established by the HSM.

Experiment II Sub-samples from five commercial seed lots of weeping alkaligrass were prepared for the two test treatments. One sub-sample designated for each treatment from each lot was randomly distributed to each of the nine laboratories. No association was made between the sub-samples for each lot to avoid bias. The laboratories were instructed to test the sub-samples designated for the HSM by the procedure described in Part I. For the UBP laboratories were instructed to first calibrate their General type blower with an AOSA Kentucky bluegrass calibration sample (Everson, et al., 1983). All sub-samples designated for the UBP were to be blown at a factor of 0.76 of the Kentucky bluegrass blowing point (as determined by calibration) for three minutes. The heavy and light portions were treated by the procedure described in Part I. Participants recorded the time required to make the purity separations for both methods. Germination tests on the pure seed from each lot and method were conducted as described in Part I.

RESULTS AND DISCUSSION

Experiment I Statistical analysis showed that mean pure seed percentages for all but one of the UBP were not significantly different ($p=0.05$) from the HSM. Mean PLS percentages across all lots from two of the five blower settings were not significantly different ($p=0.05$) from those of the HSM. Although certain pure seed and PLS results were found to be statistically significant, in practical laboratory or regulatory applications these differences would be considered acceptable within applied tolerance levels (AOSA 2001, USDA 2000). In general, the UBP produced lower percent pure seed and higher percent PLS than the HSM. The blower setting with a factor 0.76 of the Kentucky bluegrass calibrated blowing point was the least variable and most closely replicated the HSM for both pure seed and PLS results. Figure 3 shows the comparison of mean times to perform purity tests using the HSM and UBP. While the UBP and HSM produced similar results, the UBP required less than one half the time to complete as the HSM.

Experiment II Least squares means (LSM) for each method indicate the two methods produced virtually identical results for pure seed (Table 1). Although the difference in LSM for PLS percentages were found to be statistically significant ($p=0.05$) (Table 1), in practical application these differences would be considered acceptable (USDA 2000). Mean pure seed percentages from the UBP were slightly more variable than those from the HSM, and mean PLS percentages from the UBP were less variable than those from the HSM, however, overlap

in the confidence intervals of the two methods indicates there is no significant difference in the variability between the two methods among laboratories. A comparison of the mean times among all laboratories to complete purity tests using the HSM and the UBP are shown in Figure 4. While the two purity methods produced similar results the mean UBP test duration was 42% more time efficient.

CONCLUSIONS

The requirement for examination of the seed units for the presence of a caryopsis in the HSM renders this procedure prone to subjectivity and possible seed unit damage due to the manipulative nature of the HSM. Damaged seed units may still be considered pure seed, however, they may have poor germination potential. The UBP eliminates this subjective and potentially harmful seed unit examination. Comparison of the UBP to the standard HSM in Experiments I and II demonstrated that acceptable pure seed and PLS results could be obtained with greater efficiency using the UBP.

NOTE

A detailed report of this study has been submitted to the journal *Seed Technology*. In addition, the UBP for weeping alkaligrass has been submitted to the Association of Official Seed Analysts for potential adoption by its membership into the AOSA Rules for Testing Seeds.

ACKNOWLEDGEMENTS

We would like to thank Evelyn Ramos, Connie Weiner, Monica Negrete, James Modar and Jaime Salle from the California State Seed Laboratory for preparing the samples. Thank you to Kirk Remund (Monsanto) and Larry Prentice (Nebraska Crop Improvement Association) for performing the statistical analysis. Finally, we would like to thank the following laboratories for participating in the referee portion of this project: Agri Seed Testing, California Dept. of Food & Agriculture Seed Lab, Georgia Dept. of Agriculture Seed Lab (Atlanta), Michigan Dept. of Agriculture Seed Lab, Mid-West Seed Services, Oregon State University Seed Lab, Tangent Seed Lab Intl., Utah Dept. of Agriculture & Food Seed Lab, and Washington Dept. of Agriculture Seed Lab.

REFERENCES

- AOSA. 2001. Rules for Testing Seeds. Association of Official Seed Analysts.
- CFIA. 1997. Canadian Methods and Procedures for Testing Seed. Canadian Food Inspection Agency. 115 pp.
- Chirco, E.M. and T.L. Turner. 1986. Species without AOSA testing procedures. Association of Official Seed Analysts News Letter 60:2-70.
- Everson, L.E., A.L. Larson and S.B. Glassman. 1983. The Uniform Blowing Procedure. Contribution No. 24 to the Handbook on Seed Testing. Association of Official Seed Analysts. 20 pp.
- ISTA. 1999. International Rules for Seed Testing. *Seed Science and Technology* 27, Supplement. 333 pp.
- USDA. 2000. Federal Seed Act Regulations Part 201. USDA, Agricultural Marketing Service, Livestock and Seed Division, Wash., D.C. 73 pp.

Lot	Mean % Pure Seed		Mean % Pure Live Seed	
	HSM	UBP	HSM	UBP
1	99.49	99.56	94.18	94.80
2	98.95	99.33	93.56	93.81
3	97.76	97.61	90.27	91.21
4	99.28	99.03	93.65	94.85
5	99.55	99.51	94.79	95.53
LS Means	99.0047	99.0062	93.2916	94.0420
Standard Error	0.3504	0.3521	1.0865	1.0693
F-value	0		5.36†	

† significant at 0.05 level.

Table 1. Percentage of pure seed and pure live seed from the Hand Separation Method (HSM) and the Uniform Blowing Procedure (UBP) within and across lots, least squares means, standard error and F-value for comparison between test methods.

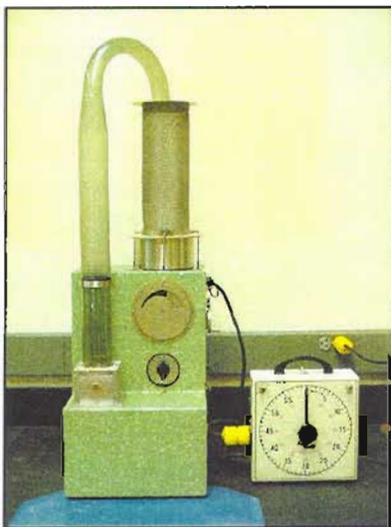


Figure 1. General Seed Blower:
A - heavy sample fraction collection site;
B - light sample fraction collection site;
C - air stream separation chamber; and
D - airflow regulator.

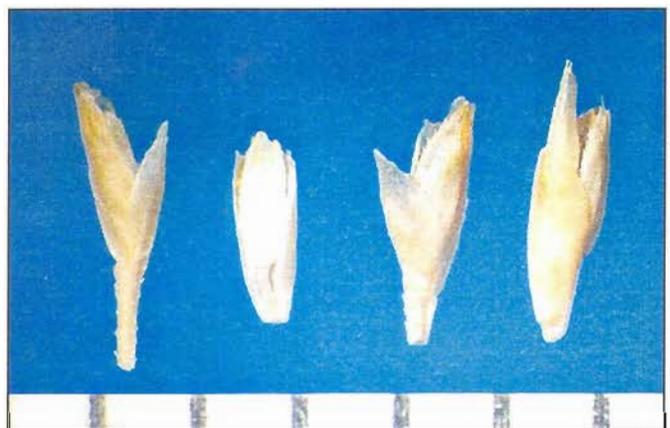


Figure 2. Examples of pure seed units of weeping alkaligrass:
A- and C- spikelet (floret and glumes) with attached pedicel,
B- single floret, and D- multiple floret.

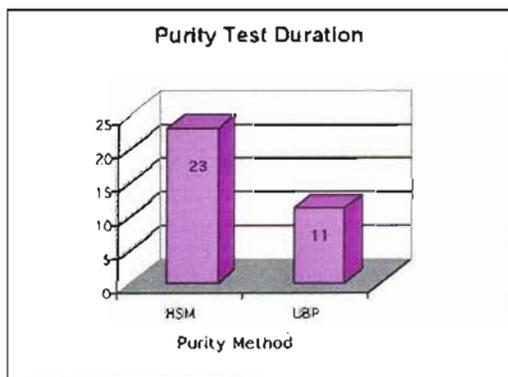


Figure 2. Experiment I - Comparison of mean times to complete purity tests of weeping alkaligrass using the hand separation method (HSM) and the Uniform Blowing Procedure (UBP).

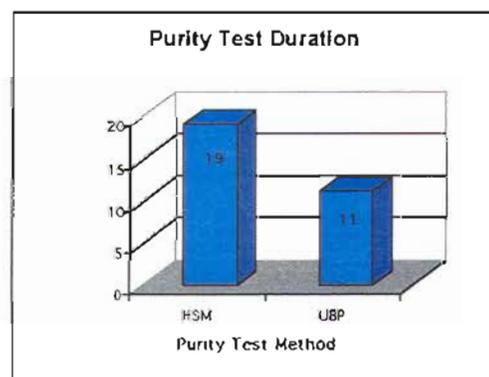


Figure 3. Experiment II - Comparison of mean times among laboratories to complete purity tests of weeping alkaligrass using the hand separation method (HSM) and the Uniform Blowing Procedure (UBP).

USDA-GDFA Collaboration

Julia Scher

Federal noxious weed (FNW) propagules (“seeds”) are prohibited from entering the U.S. or being transported in interstate commerce. USDA, Animal and Plant Health Inspection Service (APHIS), Plant Protection and Quarantine (PPQ) inspectors detect and intercept seeds from imported commodities at U.S. ports of entry nationwide, and PPQ identifiers ascertain whether these are FNW and thus actionable. PPDC seed botanists examine samples of seed lots offered for sale in California to determine if any FNW are present.

Resources with which to identify the species listed as federally noxious (7 C.F.R. 360) are insufficient. A unique federal-state collaboration has recently been established between USDA/APHIS/PPQ, Center for Plant Health Science and Technology (CPHST) and CDFA/PPDC to rectify this problem. An employee was hired by CPHST in September 2002 to work at the Meadowview site with seed lab personnel to create a taxonomic key to the FNW seeds.

The key will be created using Lucid software. Lucid keys are easy to use, computer-based, multi-access keys. Users can select characters to examine and are, thus, not hampered by the structure of a traditional,

paper-based, dichotomous key. Identification is facilitated by multimedia (images, video, sound) attached to taxa and characters.

Once identified, users may view detailed information about a particular species attached to each taxon, including links to websites for further information.



Asphodelus fistulosus, a noxious weed seed most often found in shipments of cumin seed from India, and less frequently from other countries such as Pakistan, Singapore, Mexico and Germany. A *fistulosus* has been intercepted at numerous U.S. ports, including Brooklyn, NY, Dallas, TX, and San Francisco, CA.

distributed, and provide a valuable identification tool for both partners in this collaboration.

CDFA’s extensive seed collection, library, catalog of images, and the expertise of seed lab botanists, are reasons why CPHST chose to locate the project here. It is hoped that the Lucid key to FNW seeds will be widely