Pest Manag Sci **59**:708–717 (online: 2003)
DOI: 10.1002/ps.633

United States Department of Agriculture–Agricultural Research Service research on natural products for pest management†‡

Stephen O Duke,[1]* Scott R Baerson,[1] Franck E Dayan,[1] Agnes M Rimando,[1] Brian E Scheffler,[1] Mario R Tellez,[1] David E Wedge,[1] Kevin K Schrader,[1] David H Akey,[2] Frank H Arthur,[3] Anthony J De Lucca,[4] Donna M Gibson,[5] Howard F Harrison Jr,[6] Joseph K Peterson,[6] David R Gealy,[7] Thomas Tworkoski,[8] Charles L Wilson[8] and J Brad Morris[9]

[1]*USDA, ARS, Natural Products Utilization Research Unit, PO Box 8048, University, MS 38677, USA*
[2]*USDA, ARS, Western Cotton Research Laboratory, Phoenix, AZ 85040, USA*
[3]*USDA, ARS, Biological Research Unit, Grain Marketing and Production Research Center Manhattan, KS 66502, USA*
[4]*USDA, ARS, Food and Feed Safety Research Unit of the Southern Regional Research Center, New Orleans, LA 70124, USA*
[5]*USDA, ARS, Plant Protection Research Unit, Ithaca, NY 14853, USA*
[6]*USDA, ARS, US Vegetable Laboratory, Charleston, SC 29414, USA*
[7]*USDA, ARS, Dale Bumpers National Rice Research Center, Stuttgart, AR 72160, USA*
[8]*USDA, ARS, Appalachian Fruit Research Station, Kearneysville, WV 25430, USA*
[9]*USDA, ARS, Plant Genetic Resources Conservation Unit, 1109 Experiment Street, Griffin, GA 30223-179, USA*

Abstract: Recent research of the Agricultural Research Service of USDA on the use of natural products to manage pests is summarized. Studies of the use of both phytochemicals and diatomaceous earth to manage insect pests are discussed. Chemically characterized compounds, such as a saponin from pepper (*Capsicum frutescens* L), benzaldehyde, chitosan and 2-deoxy-D-glucose are being studied as natural fungicides. Resin glycosides for pathogen resistance in sweet potato and residues of semi-tropical leguminous plants for nematode control are also under investigation. Bioassay-guided isolation of compounds with potential use as herbicides or herbicide leads is underway at several locations. New natural phytotoxin molecular target sites (asparagine synthetase and fructose-1,6-bisphosphate aldolase) have been discovered. Weed control in sweet potato and rice by allelopathy is under investigation. Molecular approaches to enhance allelopathy in sorghum are also being undertaken. The genes for polyketide synthases involved in production of pesticidal polyketide compounds in fungi are found to provide clues for pesticide discovery. Gene expression profiles in response to fungicides and herbicides are being generated as tools to understand more fully the mode of action and to rapidly determine the molecular target site of new, natural fungicides and herbicides.
Published in 2003 for SCI by John Wiley & Sons, Ltd.

Keywords: natural product; insecticide; fungicide; herbicide; allelochemical; allelopathy; pest management; phytotoxin

1 INTRODUCTION

Novel, environmentally compatible pest-control agents are needed to replace pesticides that have been withdrawn for economic or regulatory reasons or are ineffective, due to the increasing difficulty of managing pesticide resistance. In addition, there are heightened public concerns over synthetic pesticide use, such as fears about effects on public health and negative environmental consequences. In the case of pest controls for agriculture, the withdrawal of the EPA registration of a number of pesticides has increased the need for new and effective alternatives. The need for leads for new chemical classes of pesticide is especially critical since the number of synthetic compounds

* Correspondence to: Stephen O Duke, USDA, ARS, Natural Products Utilization Research Unit, PO Box 8048, University, MS 38677, USA
E-mail: sduke@ars.usda.gov
†One of a collection of papers on various aspects of agrochemicals research contributed by staff of the Agricultural Research Service of the United States Department of Agriculture, collected and organized by Drs RD Wauchope, NN Ragsdale and SO Duke

(*Received 16 June 2002; revised version received 15 August 2002; accepted 10 September 2002*)

evaluated in order to discover a commercial product has increased dramatically. Alternative methods for pest control, including both biocontrol agents and biologically derived pesticides, are needed. Natural product-based pesticides offer advantages in that they can sometimes be specific to the target species and typically have unique modes of action with little mammalian toxicity. Furthermore, they generally do not persist in the environment.

Natural products with pesticidal activity can be produced by crops. Traditional plant breeders have striven to enhance pest resistance, often with no knowledge of the chemical ecology that they were influencing. The advent of transgenic technology offers new tools for designing crops with enhanced levels of existing natural pesticides or with new natural pesticides.

This review covers some of the more recent and significant natural product-based pest-management research carried out by the Agricultural Research Service (ARS). We do not discuss the semiochemical work of ARS, as this topic is covered by another review in this issue.

2 INSECT MANAGEMENT

The glassy-winged sharpshooter, *Homalodisca coagulata* Say, is a primary vector of *Xylella fastidiosa* Wells *et al*, the causative agent of Pierce's disease in grapevines. Pierce's disease has increased, with serious fruit and vine losses, as *H coagulata* numbers have increased, and there is an urgent need to develop short- and long-term management strategies for the latter that are economically, ecologically and socially acceptable. ARS scientists in Phoenix, Arizona evaluated natural product-based insecticides from the neem tree, *Azadirachta indica* A Juss, for control of adult *H coagulata* in grape[1] and of nymphs and adults in citrus[2] to identify potential components of management programs. One neem product was evaluated in grapes and three in citrus. Each is a natural product, registered through EPA, and available for use in conventional or organic production systems. In citrus, a series of field trials was conducted using natural *H coagulata* populations (eggs to nymphs to adults during a 6-month period). Neem products were slowly (accumulatively) effective against the development of large nymphs. Three applications of the products at weekly intervals resulted in 70–100% reduction in the number of large nymphs relative to control.[2] In grapes, the neem product had no efficacy or repellency on *H coagulata* adults (data unpublished). ARS scientists in Phoenix, AZ, conducted studies on control of silverleaf whitefly (*Bemisia argentifolia* Bellows & Perring) in cotton with the neem tree product azadirachtin.[3,4] Immature SLWF populations in azadirachtin plots were consistently lower than in the embedded control plots. Cotton treated with azadirachtin against *B argentifolia* had productive yields and was not sticky. The product is EPA registered and should have a place in integrated resistance management and integrated pest management programs. Scientists at the Beneficial Insects Research Unit at Weslaco, Texas are also evaluating natural product-based insecticides for use in conjunction with biocontrol agents.[5]

Diatomaceous earth (DE) is receiving increased interest for insect pest management in bulk-stored grains and as a surface treatment inside milling, processing and food storage facilities. Many commercial formulations are available for use throughout the world. In most areas, DE formulations are considered natural products, but regulations and certifications for specialty markets, such as organic foods, vary widely within countries and geographic regions. Researchers at the Biological Research Unit of the Grain Marketing and Production Research Center in Manhattan, Kansas, are examining the various physical and biological factors that can affect the performance of DE. In general, toxicity and efficacy of DE is negatively correlated with increases in relative humidity and positively correlated with increases in temperature.[6–8] Although DE products can kill stored-product insects, longer exposure intervals may be required to kill insects with DE compared with conventional synthetic pesticides, particularly when relative humidity increases.[6] The availability of food material also increases survival of stored-product insects exposed to DE.[9] Other inert dusts, such as kaolinite-based particle films, give results similar to those with DE.[10]

The Natural Product Utilization Research Unit in Oxford (NPURU), Mississippi, in collaboration with the Formosan Termite Research Unit in New Orleans, Louisiana, is evaluating natural sources reported to have anti-termite activity against Formosan termites, *Coptotermes formasanus* Shiraki. Biological sources investigated so far include lichens, fungi and plants, particularly woody species. For example, different fractions of tarbush (*Flourensia cernua* (L) DC) leaves were found to be highly active against termites.[11] Several quinone and essential oil components have been identified as having significant anti-termite activity.

3 PATHOGEN MANAGEMENT

Researchers at the Food and Feed Safety Research Unit in New Orleans, Louisiana, discovered that CAY-1, a fungicidal saponin (relative molecular mass 1243) isolated from the dried fruit of *Capsicum frutescens* L, is lethal for the germinating, but not to the non-germinated, conidia of several members of the *Aspergillus* genus (Fig 1).[12] These fungi can be pathogenic to some plants. CAY-1 is also lethal to *Pneumocystis carinii* and *Candida albicans* (Robin) Berkhout, which can be pathogens in immunocompromised humans. This saponin is not cytotoxic *in vitro* to mammalian cells at fungicidal concentrations,[13] nor is it active against *Fusarium* species or bacteria. However, it has not yet been determined whether it prevents diseases, *in vivo,* in *Capsicum* sp *in planta*.

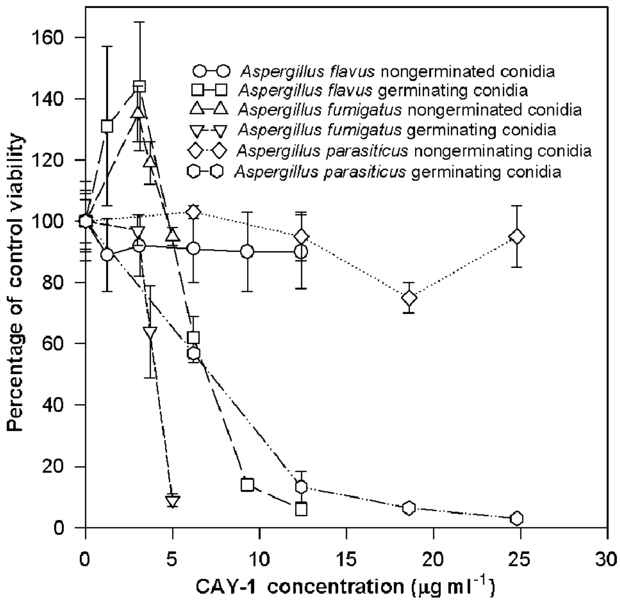

Figure 1. Effects of CAY-1 on germination of *Aspergillus* species.

Researchers at the Appalachian Fruit Research Station (AFRS) in Kearneysville, West Virginia, are conducting a multifaceted research program to discover natural alternatives to synthetic fungicides for the control of diseases of fruits and vegetables. More than 25% of the fruits and vegetables purchased by the consumer are lost due to spoilage. Thus, emphasis has been on the control of post-harvest diseases of fruits and vegetables with antagonistic yeasts, induced resistance, and natural plant- and animal-derived fungicides.[14] Research is also under way on the use of natural plant volatiles as alternatives to methyl bromide for soil fumigation.[15]

A rapid bioassay was developed to evaluate the antimicrobial activity of plant extracts for the control of post-harvest plant pathogens.[16] Through this assay, a number of essential oils and plant extracts with strong fungicidal activity against the common post-harvest pathogen *Botrytis cinerea* Pers have been identified.[17] Through a bioassay developed for volatile compounds, a number of natural products that control post-harvest pathogens such as *B cinerea, Penicillium expansum* Link, *P digitatum* (Pers) Sacc, and *P italicum* Wehmer were discovered. Utilizing an apparatus for rapid fumigant evaluation in soil, benzaldehyde was highly effective against the major soil pathogens: *Fusarium oxysporum* Schlecht, *Sclerotinia minor* Jagger and *Rhizoctonia solani* Kuehn.[15] Benzaldehyde was also found to be an effective fumigant for fruits and vegetables in reducing post-harvest decay. A time-release formulation of benzaldehyde with carbon for the fumigation of peaches and other fruit has been patented.[18]

Two natural compounds, chitosan and 2-deoxy-D-glucose, have been found to have good fungicidal activity and to enhance the efficacy of antagonistic yeasts to control post-harvest diseases of fruits and vegetables. Chitosan, an animal-derived, chitin-based polymer, forms a coating on fruits and vegetables that elicits resistance responses in the fruit.[19–21] The sugar analog 2-deoxy-D-glucose has a strong fungicidal activity against major post-harvest pathogens.[22–25] The efficacy of antagonistic yeasts for the control of post-harvest diseases of fruits and vegetables has been enhanced by the addition of chitosan or 2-deoxy-D-glucose. Patents have been issued or are pending on these technologies.

Industry has been reluctant to commercialize natural antifungal and antibacterial compounds as alternatives to synthetic pesticides because of their usually low efficacy relative to synthetic compounds and because of difficulties encountered in patenting such compounds. AFRS scientists have tried to overcome these two problems by developing combinations of natural antimicrobial compounds that might act synergistically to control plant pathogens. Some of the combinations have provided control of post-harvest pathogens comparable to that with commercially available synthetic fungicides. Also, patenting of these combinations is easier than patenting individual compounds. Utilizing this approach, a utilization patent has been filed recently covering combinations of chitosan and essential oils.

Research is being conducted at AFRS on natural antimicrobial compounds to enhance the activity of antagonistic yeasts for the control of post-harvest diseases and the use of natural antimicrobial volatiles in packaging to reduce spoilage of fruits and vegetables.[26–28] Both approaches are being pursued with industrial partners. An agreement has been developed with the Micro Flo Company (Memphis, TN, USA) to develop a post-harvest product as an alternative to synthetic fungicides for the control of post-harvest diseases. This product ('Biocure') contains the antagonistic yeast *Candida saitoana* and the enzyme lysozyme. It has been tested extensively for the past 4 years on apples and citrus in West Virginia, Florida and California, as well as internationally. The product has provided control of post-harvest diseases that is comparable to that with available synthetic fungicides. Registration of this product is being pursued with EPA and it is anticipated that 'Biocure' will be on the market by 2003. Another agreement has been developed recently between the AFRS and the Clorox Company (Oakland, CA, USA) for collaborative research and development to incorporate natural volatile compounds into packaging to extend the shelf-life of fruits and vegetables placed in these containers.

Plant pathogen management research at the NPURU in Oxford, Mississippi, focuses on the discovery and development of new natural product-based fungicides from plant and marine sources for use with minor crops. Two bioassays were developed for natural fungicide evaluations: a two-dimensional direct bioautography assay[29] and a high-throughput 96-well microbioassay.[30,31] The bioautography assay provides a format to study lipophilic compounds that are poorly soluble in the 96-well aqueous microtiter assay. Numerous pure natural compounds and extracts from terrestrial, marine and aquatic plants, as well as from sponges and algae have been evaluated.[31–34] Two promising natural fungicides effective against benzimidazole- and dicarboximide-resistant *B cinerea* and one broad-spectrum compound active against *B cinerea, Colletotrichum acutatum* Simmonds, *C fragariae* Brooks, *C gloeosporioides* (Penz) Penz & Sacc, and *F oxysporum* have been discovered with these methods. These three compounds are in the early stages of the patenting process.

Sweet potato (*Ipomoea batatas* Poir) is a genetically diverse species, and genotypes with high levels of resistance to soil insects, fungi, bacteria and nematodes have been identified. These resistances have been incorporated into several varieties developed at the US Vegetable Laboratory in Charleston, SC. Ongoing research is directed towards elucidating the biochemical bases of pest resistances. The resin glycosides are moderately inhibitory to insects and pathogenic fungi, but other root components are more active in bioassays.[35,36] Components isolated from sweet potato cortex are highly inhibitory in insect feeding bioassays, suggesting that they may be involved in insect resistance. Preliminary evidence indicates that they are complex esters that are not similar to periderm resin glycosides. Phenolic compounds also contribute to sweet potato root defense. Sweet potato genotypes vary in periderm caffeic acid content. Levels ranged from undetectable to over 1% dry weight, with indications that levels may be affected by environmental and genetic influences. Caffeic acid is inhibitory to root rot fungi in Petri-dish bioassays at concentrations similar to those found in some varieties, indicating that it may function in disease resistance and allelopathy. Other phenolic compounds found in sweet potato roots include *p*-coumaric acid, scopoletin, scopolin, chlorogenic acid, isochlorogenic acid and other caffeoyl quinates. Levels of these compounds also vary between sweet potato genotypes and environments. Current efforts are directed toward determining the role of these compounds in the complex chemical defense systems in sweet potato and characterizing the components of cortex tissue that may confer insect resistance.

Research at the Plant Genetic Resources Conservation Research Unit in Griffin, GA, determined that dried tissues of semi-tropical leguminous plants reduced the number of root knot nematode galls on tomato when added to nematode-infested soil.[37] Tissues of *Canavalia ensiformis* (L) DC, *Crotalaria retusa, Indigofera hirsuta, I nummularifolia, I spicata, I suffructicosa* Mill, *I tinctoria* L and *Tephrosia adunca* were effective in reducing galling on tomato by 80–90%.

4 VEGETATION MANAGEMENT

The ARS research on using natural products for weed management can be put into two categories: natural products as herbicides and allelopathy.

4.1 Natural products as herbicides

Researchers at the NPURU are evaluating known compounds and extracts of organisms for phytotoxicity.[32,34,38] Some of this work involves biosassay-directed isolation and discovery approaches. For example, preliminary work on extracts from roots of allelopathic rice variety Taichung Native 1 (TN1) resulted in the identification by GC-MS of two peaks found to be present in two fractions that were phytotoxic to barnyardgrass (*Echinochloa crus-galli* [L] Beauv).[39] Work is continuing to characterize further the allelochemical(s) from TN1. Minor phytotoxic constituents were isolated from the root exudates of *Sorghum bicolor* Moench and were determined to be analogues of the major allelochemical sorgoleone.[40] The biosynthesis of sorgoleone is being elucidated, and incorporation of the labeled substrates has been established using ^{13}C-NMR. Biosynthetic studies are being integrated with studies to genetically engineer sorgoleone production.

The second largest cause of economic losses to channel catfish (*Ictalurus punctatus* Raf) producers in the USA is off-flavor problems. The most frequently encountered off-flavors are musty and earthy, caused by the absorption of 2-methylisoborneol (MIB) and geosmin into the flesh of the catfish, thereby rendering them unpalatable and unmarketable. These compounds are produced by cyanobacteria (blue–green algae) that can form blooms in the catfish ponds. The application of synthetic compounds (copper-based products and diuron) to catfish ponds is currently the most frequently used management approach by catfish producers to prevent the growth of cyanobacteria. These synthetic compounds have several drawbacks, including low selective toxicity towards cyanobacteria, the public's negative perception to the use of synthetic compounds in aquaculture ponds, and environmental safety issues. The discovery of environmentally safe natural compounds that are selective in killing the undesirable cyanobacteria would greatly benefit the catfish industry.

A rapid bioassay[41] has been used over the past 5 years to screen hundreds of natural compounds and thousands of extracts from plants to discover a safe, selective algicide for use in catfish aquaculture. Of the compounds screened so far, several quinones have been found to be the most selective towards the MIB-producing cyanobacterium *Oscillatoria perornata*.[42,43] Anthraquinone, one of the most promising quinones, has been found to inhibit photosynthesis in *O perornata*.[44] Promising natural compounds are also undergoing efficacy testing within circular enclosures (limnocorrals) that are placed in catfish production ponds.[45] A quinone derivative found to be effective in selectively killing *O perornata* at 125 μg litre^{-1} in catfish ponds is currently being patented.

At the AFRS, the use of crude essential oil mixtures for weed management is being studied. Phytotoxic essential oils are extracted from plants and thus may be useful as a 'natural product herbicide' for organic farming systems. Laboratory and greenhouse experiments determined the herbicidal effects of plant-derived essential oils and identified the active ingredient in one oil with herbicide activity.[46] Twenty-five oils were applied to detached leaves of dandelion (*Taraxacum officinale* Weber) in the laboratory. Essential oils (1% v/v) from red thyme (*Thymus vulgaris* L), summer savory (*Satureja hortensis* L), cinnamon (*Cinnamomum zeylanicum* Nees) and clove (*Syzgium aromaticum* (L) Merr & Perry) were the most phytotoxic, leading to cell death. These essential oils plus two adjuvants (nonionic surfactant and paraffinic oil blend at 2 ml litre^{-1}) were sprayed on shoots of common lambsquarters (*Chenopodium album* L), common ragweed (*Ambrosia artemisiifolia* L) and johnsongrass (*Sorghum halepense* (L) Pers) growing in the greenhouse. They caused shoot death within 1 h to 1 day when applied at aqueous concentrations from 50 to 100 ml litre^{-1}. Essential oil of cinnamon, rich in eugenol (2-methoxy-4-prop-2-enylphenol) (84%), had high herbicidal activity. Dandelion leaf disk and whole-plant assays verified that eugenol was the active ingredient in essential oil of cinnamon.

There are many potential target sites for herbicides, but commercial products are directed toward only a few molecular targets. For several reasons, there is a pressing need to develop new weed management tools that affect new molecular sites. Natural products often have unique mechanisms of action.[47] The NPURU has discovered two new molecular targets using physiological and biochemical approaches.[48] Asparagine synthetase was found to be the target site for 1,4-cineole and related structures (Fig 2A).[49] A structural analogue of 1,4-cineole, cinmethylin, is a commercial herbicide. Cinmethylin was found to be bioactivated to become a potent inhibitor of asparagine synthetase. The phytotoxic *Fusarium solani* (Martius) Sacc product, 2,5-anhydro-D-glucitol, has been found to be an inhibitor of fructose-1,6-bisphosphate aldolase, once it is bioactivated by phosphorylation (Fig 2B).[50] The phytotoxic lichen component, usnic acid, was found to be a potent inhibitor of *p*-hydroxyphenylpyruvate dioxygenase (HPPD).[51,52] Many natural phytotoxins inhibit HPPD,[52] with usnic acid and sorgoleone being the most potent.

Members of the Plant Protection Research Unit at Ithaca, New York, in collaboration with Rosemary Loria of Cornell University, have taken both a screening and a genetic approach to characterize the family of thaxtomins in order to evaluate their potential usefulness as commercial herbicides. Thaxtomins are a family of modified non-ribosomally synthesized, dipeptide phytotoxins produced by plant pathogenic *Streptomyces* sp that cause 'scab' diseases on potato tubers, sweet potato storage roots and expanded tap roots of radish, beet and similar crops.[53] Toxin production occurs in diseased tissue and can also be elicited under appropriate culture conditions *in vitro*.[54] All of the pathogenic species produce

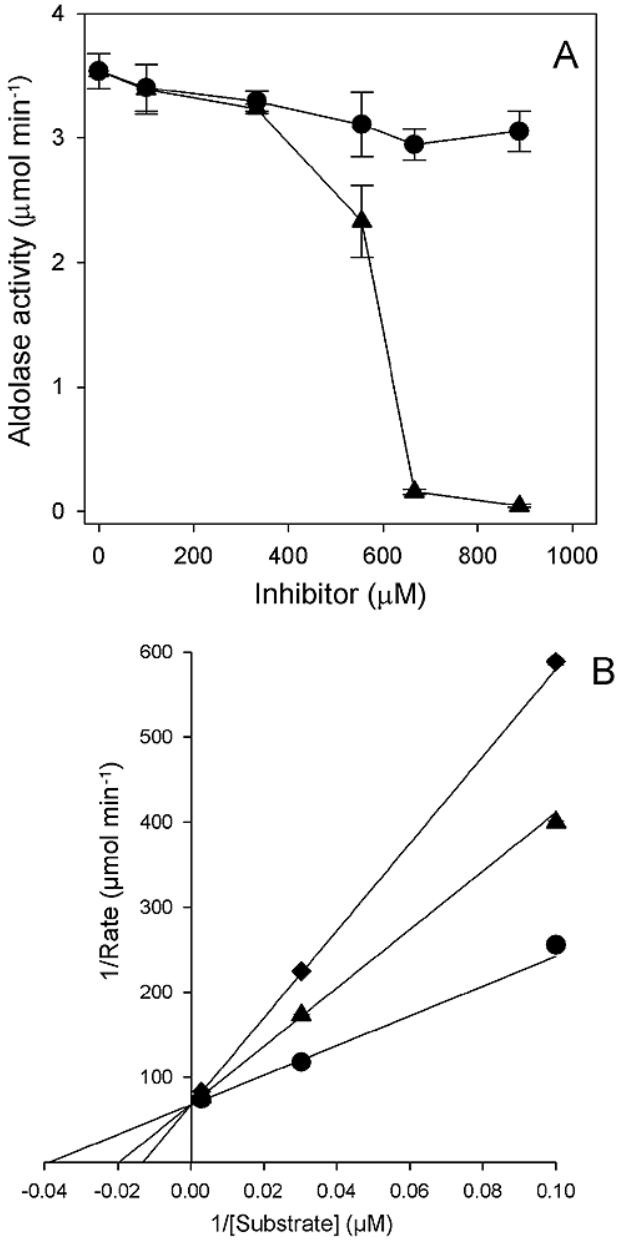

Figure 2. (A) Inhibitory activity of (●) 2,5-anhydro-D-glucitol and (▲) 2,5-anhydro-D-glucitol bisphosphate on spinach Fru-1,6-bisP aldolase. (B) Kinetics of spinach aldolase activity in the presence of (♦) 0, (♦) 100 and (●) 200 μM 2,5-anhydro-D-glucitol. From Reference 45.

one or more members of the thaxtomin family, while non-pathogenic species lack toxin production.[55] Concentrations of less than 1 μM of thaxtomin A cause cell swelling, necrosis and growth inhibition in monocotyledonous and dicotyledonous seedlings.[56] Effects of thaxtomin A are similar to injuries caused by the commercial herbicides dichlobenil and isoxaben, known to be cellulose synthesis inhibitors.

Nucleotide sequence analysis of the thaxtomin synthetase genes from *S acidiscabies* 84.104 identified a putative P-450 monooxygenase gene, *txtC*, adjacent to the 3′ end of *txtB*.[56] Thaxtomin A has two hydroxyl groups on the phenylalanyl residue, one on the α-carbon (C14) and one on the phenyl ring carbon (C20) *meta* to the juncture with the β-carbon.

This suggests that a functional monooxygenase is a component of the synthetase gene cluster, and contributes one or both hydroxyl groups to thaxtomin A. A series of P-450 mutants were generated via gene disruption experiments and were screened for production of thaxtomins. Thaxtomin D, a 14,20-didehydroxy derivative, was isolated in high yield as the principal thaxtomin produced by these mutants.[57]

Both thaxtomin A and thaxtomin D, at equivalent doses, have marked activity as pre- or post-emergent herbicides. Biological activity was assessed by adding concentrations of thaxtomins prepared in 1 ml of acetone to 5.5-cm filter discs, air-drying the filters, and then placing 20 lettuce seeds onto the filter pre-wetted with 1 ml of water. Although there was some seed germination at doses at $1\,\mu g\ ml^{-1}$ and lower, roots remained short in length, with browning of the root tips (less than 5 mm), at applied doses as low as $0.5\,\mu g\,ml^{-1}$ (R Loria and DM Gibson, unpublished observations). Although thaxtomins do not act systemically, they may provide useful leads for development of novel herbicides.

4.2 Allelopathy

At the US Vegetable Laboratory, corn spurry (*Spergula arvensis* L), a winter annual weed in the southern USA, was observed to greatly suppress the growth of cole crops despite its 'non-aggressive' growth habit. Field, greenhouse and laboratory studies indicated that the suppression was largely due to allelopathy.[58] Corn spurry leaves are covered with trichomes that contain high levels of a number of phytotoxic sugar–fatty acid esters commonly called sugar esters.[59,60] Sugar esters have been reported in wild tobacco relatives, and have been developed as insecticides against soft-bodied insects.

The ability of some sweet potato varieties to suppress weeds led to investigation of the allelopathic effect of sweet potato on yellow nutsedge (*Cyperus esculentum* L) and other species at the US Vegetable Laboratory. Field studies demonstrated that sweet potato was highly suppressive to nutsedge, and greenhouse studies showed that the suppression was not due to light, water or mineral nutrient competition.[61–63] The inhibition of yellow nutsedge by extractable components of the sweet potato periderm was mostly attributed to a group of partially characterized components called resin glycosides.[64] These are complex fatty acid–sugar esters with high molecular weights, similar to compounds from sweet potato and *Ipomoea tricolor* characterized by researchers in Japan and Mexico. Sweet potato genotypes vary greatly in periderm resin–glycoside contents, and glycoside contents were highly correlated with the relative inhibition of yellow nutsedge by fourteen sweet potato clones in the greenhouse.[65]

At the Dale Bumpers National Rice Research Center in Stuttgart, Arkansas, hundreds of rice germplasm lines have been identified as giving various degrees of suppression against rice weeds.[66] Allelopathy appears to contribute significantly to the suppression in some of these lines. PI 312 777 was originally produced at the International Rice Research Institute in Manila from the cross, T65*2/Taichung Native 1, (Taiwan parentage) (GRIN 2002).[67] Taichung Native 1 is also in the parentage of the weed-suppressive cultivar, PI 338 046, and is, itself, suppressive to barnyardgrass (*Echinochloa crus-galli* [L] Beauv) (Plate 1).[68] In field studies, a number of foreign weed-suppressive rice entries reduced density and growth of barnyardgrass and produced about 20% more grain yield compared to non-suppressive rice cultivars.[69] Weed suppressive activity in Arkansas field trials was found in 412 rice accessions against ducksalad (*Heteranthera limosa* [Sw] Willd), 145 accessions against redstem (*Ammannia coccinea* Rottb) and 94 accessions against barnyardgrass.[70]

Some weed suppressive cultivars can even moderately suppress red rice (*Oryza sativa* L). In a replacement series study, tiller production, leaf area and relative yield of Kaybonnet (commercial rice cultivar) were greatly reduced by a short-statured red rice (suspected commercial rice–red rice cross) and by a tall red rice ecotype, while those for the weed-suppressive cultivar, PI 312 777, were similar to both red rice types. These results indicate that Kaybonnet was much less competitive than PI 312 777 against red rice and that high tillering capacity may be a useful trait when breeding for weed suppressive rice cultivars.[70] Teqing, a Chinese indica type cultivar with barnyardgrass suppression similar to that of PI 312 777[71,72] controlled both propanil-susceptible barnyardgrass[73] and propanil-resistant barnyardgrass (M Lovelace 2001, personal communication) alone or with reduced rates of thiobencarb better than did US cultivars.

Recent studies in Arkansas and Japan have indicated that allelochemicals may contribute to weed-suppressive activity in certain rice cultivars, including PI 312 777.[74,75] Probable allelochemicals with varying activity have been obtained from leaves of rice.[74,75] One QTL on chromosome six and five QTL overall have explained 16% and 37%, respectively, of the total variation in 'allelopathy' observed when a mapping population (PI 312 777 × Rexmont) was assayed against lettuce root growth.[76] The degree to which allelopathy may or may not contribute to weed suppression in the field is not known. Anatomical characteristics, such as root biomass and tillering, probably account for much of the weed suppression observed in these cultivars. Weed suppressive cultivars have produced several times the root biomass and tiller density of non-suppressive cultivars[66,77] and can deplete soil nitrate nitrogen levels more extensively than can commercial cultivars during the extended period between seedling emergence and application of the permanent flood (DR Gealy, unpublished data), suggesting that aggressive competition for soil nutrients may play a role in the success of some weed suppressive cultivars.

The most effective use of weed suppressive rice cultivars may ultimately be in combination with reduced rates of existing herbicides. In a 3-year study in Arkansas, PI 312 777 and other Asian cultivars suppressed barnyardgrass more effectively and at lower propanil rates than did commercial US cultivars, indicating the potential economic benefit of using suppressive rice cultivars in combination with reduced herbicide rates.[70,71] A rain-free period of more than 1 week after planting has increased weed-suppressive activity of PI 312 777 and other rice cultivars in the field,[78] suggesting that post-planting soil moisture management could be key to the optimization of natural weed suppression.

The ultimate application of molecular biology techniques to weed science might not be the development of new herbicides or herbicide-resistant crops. Instead, it could be used to engineer crops with properties that would make them more competitive or more allelopathic against weeds.[79–81] Current research at the NPURU emphasizes the allelopathy component of interference. Although allelopathy has been extensively studied for almost a century, little effort has been focused on the manipulation or introduction of known allelochemicals in crop plants—despite the identification of several potentially useful allelochemical targets. This lack of effort is perhaps not surprising, given the current emphasis placed by industry on the development of herbicide-resistant crops. This situation is also a reflection of the fact that the use of synthetic herbicides has been extremely cost-effective in controlling weeds. Nevertheless, allelopathy might be more environmentally friendly than the application of herbicides and would be very beneficial for countries or societies where the use of herbicides is cost-prohibitive.

Much research must be done to develop useful allelopathy in crops. In many cases, a plant is known to be allelopathic, but the specific compound has not been identified. In the few cases where an allelochemical has been identified, there is limited or no knowledge of the biosynthetic pathway. Additional problems that need to be addressed include the lack of basic information regarding the sequestration and secretion of allelochemicals, and the identification of gene promoters which direct expression of the corresponding biosynthetic enzymes to the appropriate cell type. While these pose significant challenges to the development of allelopathic crops, genomics-based approaches such as mining of expressed sequence tag (EST) data sets should accelerate the discovery of genes involved in allelopathic pathways. Additional technical aspects concerning the development of genetically modified crops with these traits have been addressed in recent reviews.[79–81]

5 BASIC PESTICIDE RESEARCH

The Ithaca, New York-based ARS Bioprospecting and Exploration Research Team, in collaboration with Olen Yoder, Gillian Turgeon and Jon Clardy of Cornell University, is probing entomopathogenic fungi for polyketides with potential as pesticides. The principal source materials are from the USDA ARS Collection of Entomopathogenic Fungal Cultures (ARSEF), the world's largest germplasm repository for fungal pathogens of invertebrates. This unique resource is also the principal source for screening efforts to identify and characterize novel polyketide chemistries for biobased, environmentally friendly pesticides. Growing many of these genetically and ecologically diverse fungi in sufficient amounts to isolate compounds is a daunting task. Little is known of the chemistry profiles of many of the isolates of this collection, which includes fungi in parasitic or saprophytic associations with insects, nematodes and other fungi. An alternative approach used by this group has been to identify genes for polyketide synthases (PKS) from these organisms to determine those that might have unique PKS, and therefore are more likely to produce unique and interesting polyketides.

Lee *et al*[82] described the use of a degenerate PCR primer approach to detect and characterize PKS fragments in a genetically diverse group of insect- and nematode-associated fungi for which the capacity to produce polyketides is largely undescribed. Out of a starting group of 157 fungal isolates representing 73 genera, they were able to detect a putative KS domain in each of 92 isolates. Thus, the PCR-based screening may be a rapid, efficient technique to identify PKS fragments from among a wide distribution of fungi. Sequence analysis of the fragments derived from the highly conserved KS domain of PKS genes indicated groupings of fungal genes distinct from those already reported in Genbank. This work showed that PKS genes are widespread and diverse among insect- and nematode-associated fungi. The clustering of the entomophagous fungi within clades hints that they may have a distinct grouping of PKS genes. Relating the PKS-encoding genes to polyketides produced by these organisms is now in progress.

A novel antimicrobial, akanthomycin, was isolated from the entomopathogenic fungus *Akanthomyces gracilis* Samson & HC Evans; direct application of akanthomycin (250 ng applied to a filter disc) onto an agar plate containing *Staphylococcus aureus* Rosenbach was sufficient to inhibit growth. This activity is slightly less potent than that of a related analog, 8-methylpyridoxatin.[83] This study is the first step in determining whether these novel PKS genes and their polyketide products are functionally dedicated to the specialized activities of these organisms.

Discovery of molecular target sites by conventional means is a time-consuming and costly part of biocide development. It can take anything from several months to years of intensive research to determine a given compound's target site. The NPURU is developing cDNA microarray systems that will aid in determining a natural product's target site. The medical profession has been able to use cDNA microarrays to fingerprint

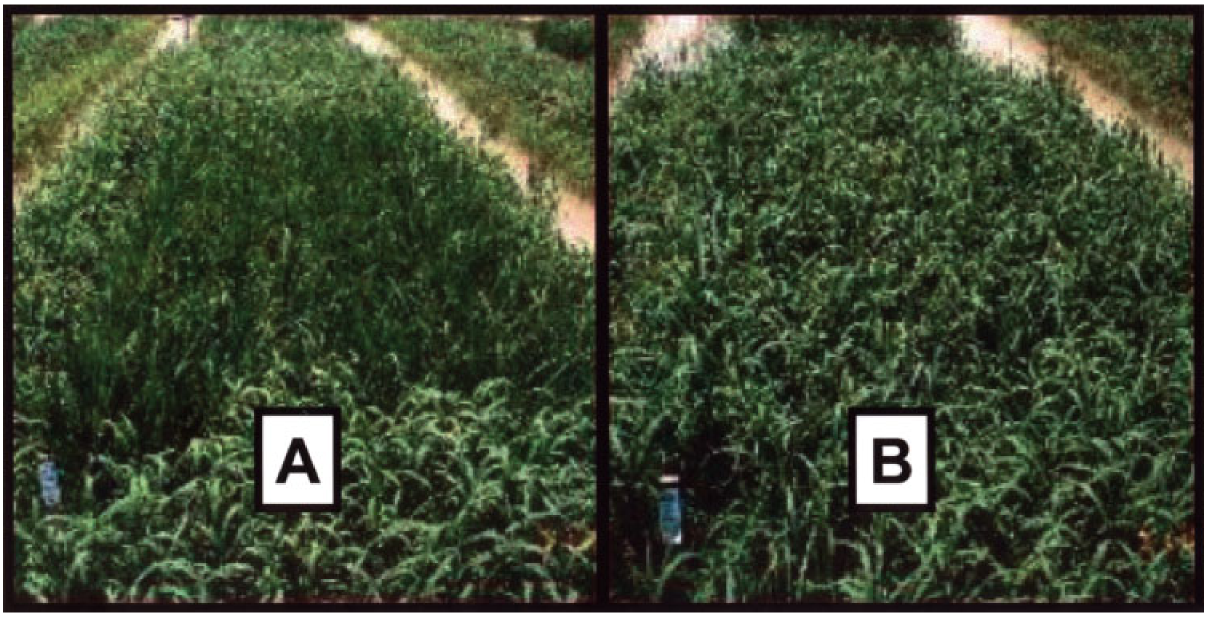

Plate 1. Suppression of barnyardgrass in (A) PI 312 777 rice versus lack of control in (B) conventional Kaybonnet cultivar.

certain illnesses, resulting in more effective treatments. A similar technique has been used to characterize the response of yeast (*Saccharomyces cerevisiae* Meyer ex Hansen) to anti-fungal compounds and one herbicide. The NPURU group is using a similar approach to fingerprint natural anti-fungal agents and phytotoxins. If an organism is treated with a specific sub-lethal concentration of a biocide it will respond by altering the expression of its genes, thus creating an expression fingerprint for the compound. By creating a fingerprint database for all known modes of action, it will be possible to test a new biocide with an unknown target site to see if its expression fingerprint is similar to that of a herbicide with a known target site. While this procedure does not determine mode of action with complete assurance, it provides information that can direct physiological and biochemical studies to identify a target site more efficiently, leading to significant reductions in time and research costs.

REFERENCES

1 Akey DH, Henneberry TJ and Toscano NC, Insecticides sought to control adult glassy-winged sharpshooter. *Calif Agric* **55**:22–27 (2001).
2 Akey DH, Blua M and Henneberry TJ, Potential of conventional and biorational insecticides for glassy-winged sharpshooter control, in *Pierce's Disease Research Symposium, Dec 5–7, 2001*, ed by Tariq A, Oswalt S and Esser T, Calif Dept Food Agric, Sacramento, CA, USA, pp 42–43 (2001).
3 Akey DH and Henneberry TJ, Control of silverleaf whitefly with the neem product azadirachtin as Bollwhip in upland and cotton in Arizona, in *Proc Beltwide Cotton Prod Res Conf*, ed by Dugger P and Richter D, National Cotton Council of America, Memphis, TN, pp 914–918 (1999).
4 Akey DH and Henneberry TJ, Azadirachtin (as Bollwhip™), a biorational agent against the silverleaf whitefly, *Bemisia argentifolii*, in field trials in upland cotton in Arizona, Section C: Chemical Control, Biorationals And Pesticide, in *Silverleaf Whitefly: National Research, Action, and Technology Transfer Plan, 1997–2001 (Formerly Sweetpotato Whitefly, Strain B): Annual Review of the Second 5-Year Plan*, ed by Henneberry TJ and Faust RM, USDA, ARS, 1999–01, Natl Tech Info Service, Springfield, VA (2000).
5 Elzen GW and James RR, Responses of *Plutella xylostella* and *Coleomegilla maculata* to selected insecticides in a residual insecticide bioassay. *Southwest Entomol* **26**:365–372 (2001).
6 Arthur FH, Impact of food source on survival of red flour beetles and confused flour beetles (Coleoptera: Tenebrionidae) exposed to diatomaceous earth. *J Econ Entomol* **93**:1347–1356 (2000).
7 Arthur FH, Immediate and delayed mortality of *Oryzaephilus surinamensis* (L) exposed on wheat treated with diatomaceous earth: effects of temperature, relative humidity, and exposure interval. *J Stored Prod Res* **37**:13–21 (2001).
8 Arthur FH, Survival of *Sitophilus oryzae* (L) on wheat treated with diatomaceous earth: Impact of biological and environmental parameters on product efficacy. *J Stored Prod Res* **38**:305–313 (2002).
9 Arthur FH, Toxicity of diatomaceous earth to red flour beetles and confused flour beetles: effects of temperature and relative humidity. *J Econ Entomol* **93**:526–532 (2000).
10 Arthur FH and Puterka GJ, Evaluation of kaolinite-based particle films to control *Tribolium* species (Coleoptera: Tenebrionidae). *J Stored Prod Res* **38**:341–348 (2002).
11 Tellez M, Estell R, Fredrickson E, Powell J, Wedge D, Schrader K and Kobaisy M, Extracts of *Flourensia cernua* (L): volatile constituents and antifungal, antialgal, and antitermite bioactivities. *J Chem Ecol* **27**:2263–2273 (2001).
12 De Lucca AJ, Bland JM, Vigo CB and Selitrennikoff CP, Fungicidal saponin, CAY-1, and isolation thereof from *Capsicum* species fruit, *US Patent 6 310 091* (2001).
13 De Lucca AJ, Bland JM, Vigo CB, Cushion M, Selitrennikoff CP and Walsh TJ, CAY-1, a fungicidal saponin from *Capsicum* sp fruit. *Med Mycol* **40**:131–137 (2002).
14 Wilson CL, El Ghaouth A and Wisniewski ME, 1999. Prospecting in nature's storehouse for biopesticides. *Rev Mex Fitopathol* **17**:49–53 (1999).
15 Wilson CL, Fravel DR, Solar JM and El Ghaouth A, Benzaldehyde as a soil fumigant, and an apparatus for rapid fumigant evaluation. *HortScience* **34**:681–685 (1999).
16 Wilson CL, Solar JM, El Ghaouth A and Wisniewski ME, Rapid evaluation of plant extracts and essential oils for antifungal activity against *Botrytis cinerea*. *Plant Dis* **81**:204–210 (1997).
17 Wilson CL, Franklin JD and Otto BE, Fruit volatiles inhibitory to *Monilinia fructicola* and *Botrytis cinerea*. *Plant Dis* **71**:316–319 (1987).
18 Solar J, Wilson CL and El Ghaouth A, Controlled release fumigation of harvested agricultural commodities, *US Patent 5 958 490* (1999).
19 El Ghaouth A, Smilanick JL and Wilson CL, Enhancement of the performance of *Candida saitoana* by the addition of glycolochitosan for the control of postharvest decay of apple an citrus fruit. *Postharvest Biol Technol* **19**:103–110 (2000).
20 El Ghaouth A and Wilson CL, Bioactive coating for harvested commodities, *US Patent 5 633 025* (1997).
21 El Ghaouth A, Wilson CL and Benhaou N, Biochemical and cytochemical interactions of chitosan and *Botrytis cinerea* in Bell pepper fruit. *Postharvest Biol Technol* **12**:183–194 (1997).
22 El Ghaouth A, Smilanick JL, Brown EG, Ippolito A and Wilson CL, Control of decay of apple and citrus fruits in semicommercial tests with *Candida saitoana* and 2-deoxy-D-glucose. *Biol Control* **20**:96–101 (2001).
23 El Ghaouth A, Smilanick JL, Wisniewski M and Wilson CL, Improved control of apple and citrus fruit decay with combination of *Candida saitoana* and 2-deoxy-D-glucose. *Plant Dis* **84**:249–253 (2000).
24 El Ghaouth A and Wilson CL, Antifungal activity of 2-deoxy-D-glucose on *Botrytis cinerea, Penicillium expansum*, and *Rhizopus stolonifer*: ultrastructural and cytological aspects. *Phytopathology* **87**:772–779 (1997).
25 El Ghaouth A, Wilson CL and Wisniewski M, Sugar analogs as potential fungicides for postharvest pathogens of apple and peach. *Plant Dis* **79**:254 (1995).
26 El-Neshawy SM and Wilson CL, Nisin enhancement of biocontrol of postharvest diseases of apple with *Candida oleophila*. *Postharvest Biol Technol* **10**:9–14 (1997).
27 Wilson CL, El Ghaouth A and Wisniewski M, Synergistic combinations of natural compounds that control decay of fruits and vegetables and reduce contamination by foodborne human pathogens, *US Patent Application DN 0145.00* (2001).
28 Wilson CL and El Ghaouth A, Composition containing 2-deoxy-D-glucose and *Candida saitoana* and a method of use for the biological control of postharvest diseases, *US Patent 5 591 427* (1997).
29 Wedge DE and Nagle DG, A new 2D-TLC bioautography method for the discovery of novel antifungal agents to control plant pathogens. *J Nat Prod* **63**:1050–1054 (2000).
30 Wedge DE and Kuhajek JM, A microbioassay for fungicide discovery. *SAAS Bull Biochem Biotech* **11**:1–7 (1998).
31 Wedge DE, Galindo JCG and Macias FA, Fungicidal activity of natural and synthetic sesquiterpene lactone analogs. *Phytochemisty* **53**:747–757 (2000).
32 Tellez MR, Dayan FE, Schrader KK, Wedge DE and Duke SO, Composition and some biological activities essential oil of *Callicarpa americana* (L). *J Agric Food Chem* **48**:3008–3012 (2000).
33 Tellez MR, Estell RE, Fredrickson E, Powell JE, Wedge DE, Schrader KK and Kobaisy MM, Extracts of *Flourensia cernua*

(L): volatile constituents and antifungal, antialgal, and antitermite activities. *J Chem. Ecol* **27**:2263–2273 (2001).
34 Kobaisy MM, Tellez MR, Webber D, Dayan FE, Schrader KK and Wedge DE, Phytotoxic and fungitoxic activities of essential oil of kenaf (*Hibiscus cannabinus* L) leaves and its composition. *J Agric Food Chem* **49**:3768–3771 (2001).
35 Peterson JK, Harrison Jr HF and Muckenfuss AE, Interference of sweetpotato glycosides with growth and survival of diamondback moth larvae (Lepidoptera: Plutellidae). *Internat J Allelopathy* **5**:43–52 (1998).
36 Harrison HF, Peterson JK, Clark CA and Snook ME, 2001. Sweetpotato periderm components inhibit in vitro growth of root rotting fungi. *HortScience* **36**:927–930 (2001).
37 Morris JB and Walker JT, Semi-tropical legumes as potential soil amendments for nematode control. *J Nematol* (in press).
38 Dayan FE, Hernandez A, Allen SN, Moraes RM, Vroman JA, Avery MA and Duke SO, Comparative phytotoxicity of artemisinin and several sesquiterpene analogues. *Phytochemistry* **50**:607–614 (1999).
39 Rimando AM, Olofsdotter M, Dayan FE and Duke SO, Searching for rice allelochemicals: An example of bioassay-guided isolation. *Agron J* **93**:16–20 (2001).
40 Rimando AM, Dayan FE, Czarnota MA, Weston LA and Duke SO, A new photosystem II electron transfer inhibitor from *Sorghum bicolor*. *J Nat Prod* **61**:927–930 (1998).
41 Schrader KK, de Regt MQ, Tucker CS and Duke SO, A rapid bioassay for selective algicides. *Weed Technol* **11**:767–774 (1997).
42 Schrader KK, de Regt MQ, Tidwell PR, Tucker CS and Duke SO, Selective growth inhibition of the musty-odor producing cyanobacterium *Oscillatoria* cf *chalybea* by natural compounds. *Bull Environ Contam Toxicol* **60**:651–658 (1998).
43 Schrader KK and Harries MD, Compounds with selective toxicity towards the noxious, musty-odor cyanobacterium *Oscillatoria peronata*. *Bull Environ Contam Toxicol* **66**:801–807 (2001).
44 Schrader KK, Dayan FE, Allen SN, de Regt MQ, Tucker CS and Paul RN Jr, 9,10-Anthraquinone reduces the photosynthetic efficiency of *Oscillatoria peronata* and modifies cellular inclusions. *Interant J Plant Sci* **161**:265–270 (2000).
45 Schrader KK, Tucker CS, de Regt MQ and Kingsbury SK, Evaluation of limnocorrals for studying the effects of phytotoxic compounds on plankton and water chemistry in aquaculture ponds. *J World Aquacult Soc* **31**:403–415 (2000).
46 Tworkoski T, Herbicide effects of essential oils. *Weed Sci* **50**:425–431 (2002).
47 Duke SO, Dayan FE and Romagni JG, Natural products as sources for new mechanisms of herbicidal action. *Crop Protect* **19**:583–589 (2000).
48 Dayan FE, Romagni JG and Duke SO, Investigating the mode of action of natural phytotoxins. *J Chem Ecol* **26**:2079–2094 (2000).
49 Romagni JG, Duke SO and Dayan FE, Inhibition of plant asparagine synthetase by monoterpene cineoles. *Plant Physiol* **123**:725–732 (2000).
50 Dayan FE, Rimando AM, Tellez MR, Scheffler BE, Roy T, Abbas HK and Duke SO, Bioactivation of the fungal phytotoxin 2,5-anhydro-D-glucitol by glycolytic enzymes is an essential component of its mechanism of action. *Z Naturforsch* **57c**:645–653.
51 Romagni JG, Meazza G, Nanyakkara NPD and Dayan FE, The phytotoxic lichen metabolite, usnic acid, is a potent inhibitor of plant *p*-hydroxyphenylpyruvate dioxygenase. *FEBS Lett* **480**:301–305 (2000).
52 Meazza G, Scheffer BE, Tellez MR, Rimando AM, Romagni JG, Duke SO, Nanayakkara D, Khan IA, Abourashed EA and Dayan FE, The inhibitory activity of natural products on plant *p*-hydroxyphenylpyruvate dioxygenase. *Phytochemistry* **59**:282–288 (2002).
53 King RR, Lawrence CH, Calhoun LA and Ristaino JB, Isolation and characterization of thaxtomin-type phytotoxins associated with *Streptomyces ipomoeae*. *J Agric Food Chem* **42**:1791–1794 (1994).
54 Loria R, Bukhalid RA, Creath FA, Leiner FH, Olivier M and Steffens JC, Production of thaxtomins by pathogenic *Streptomyces* species in vitro. *Phytopathology* **85**:537–541 (1995).
55 King RR, Lawrence CH and Gray JA, Herbicidal properties of the thaxtomin group of phytotoxins. *J Agric Food Chem* **49**:2298–2301 (2001).
56 Healy FB, Wach M, Krasnoff SB, Gibson DM and Loria R, The *txtAB* genes of the plant pathogen *Streptomyces acidiscabies* encode a peptide synthetase required for phytotoxin thaxtomin A production and pathogenicity *Molecul Microbiol* **38**:794–804 (2000).
57 Healy FG, Krasnoff SB, Wach M, Gibson DM and Loria R, Involvement of a cytochrome P450 monooxygenase in thaxtomin A biosynthesis by *Streptomyces acidiscabies*. *J Bacteriol* **184**:2019–2029 (2002).
58 Harrison HF Jr and Peterson JK, Inhibitory effects of corn spurry (*Spergula arvensis* L) on cole crops and English pea. *Allelopathy J* **4**:283–290 (1997).
59 Peterson JK, Snook ME and Harrison HF Jr, Isolation, structural identification and allelopathic potential of sucrose esters from corn spurrey (*Spergula arvensis*). *J Chem Ecol* **24**:1803–1816 (1998).
60 Peterson JK, Harrison HF Jr and Chortyk OT, Effects of various synthetic sucrose esters on weed seed germination and crop growth structure-activity and dose-response relationships. *J Food Agric Chem* **45**:4833–4837 (1998).
61 Harrison HF Jr and Peterson JK, Allelopathic effects of sweet potatoes (*Ipomoea batatas*) on yellow nutsedge (*Cyperus esculentus*) and alfalfa (*Medicago sativa*). *Weed Sci* **34**:623–627 (1986).
62 Peterson JK and Harrison HF Jr, Differential inhibition of seed germination by sweetpotato (*Ipomoea batatas*) root periderm extracts. *Weed Sci* **39**:119–123 (1991).
63 Harrison HF and Peterson JK, Sweetpotato periderm components inhibit yellow nutsedge (*Cyperus esculentus* L) growth. *Weed Technol* **8**:168–171 (1994).
64 Peterson JK and Harrison HF Jr, Isolation of substance from sweet potato (*Ipomoea batatas*) that inhibits seed germination. *J Chem Ecol* **17**:943–951 (1991).
65 Peterson JK, Harrison HF and Snook ME, Evaluation of approaches to the quantitative estimation of multiple resistances in sweetpotato germplasm *Internat J Allelopathy* **6**:201–208 (1999).
66 Dilday RH, Mattice JD, Moldenhauer KA and Yan W, Allelopathic potential in rice germplasm against ducksalad, redstem and barnyardgrass. *J Crop Prod* **4**:287–301 (2001).
67 Germplasm Information Resources Network (GRIN), (2002). [online database], USDA, ARS, National Genetic Resources Program, National Germplasm Resources Laboratory, Beltsville, MD, available at http://www.ars-grin.gov
68 Gealy DR and Dilday RH, Progress in suppressing barnyardgrass (*Echinochloa crus-galli*) with foreign and domestic rice cultivars, in *BR Wells Rice Research Studies—2000 Series 485*, ed by Norman RJ and Meullenet J-F, Arkansas Agric Exp St, University of Arkansas, Fayetteville, AR, USA, pp 62–68 (2001).
69 Dilday RH, Moldenhauer KA, Yan WG and Gealy DR, Allelopathic activities to barnyardgrass in rice and yield reduction due to barnyardgrass infestation, in *BR Wells Rice Research Studies—1997. Series 460.* ed by Norman RJ and Johnson TH, Arkansas Agric Expt Stn, University of Arkansas, Fayetteville, AR, USA, pp 27–31 (1998).
70 Estorninos LE Jr, Gealy DR and Talbert RE, Growth response of rice (*Oryza sativa*) and red rice (*O. sativa*) in a replacement series study. *Weed Technol* **16**:401–406 (2002).
71 Chavez RSC, Gealy DR and Black HL, Reduced propanil rates and naturally suppressive cultivars for barnyardgrass control in drill-seeded rice, in *BR Wells Rice Research Studies—1998 Series 468*, ed by Norman RJ and Johnson TH, Arkansas Agric Expt Stn, University of Arkansas, Fayetteville, AR, USA, pp 43–50 (1999).
72 Gealy DR, Wailes EJ and Chavez R, Efficacy and economics of reduced propanil rates to control *Echinochloa crus-galli*

in naturally suppressive *Oryza sativa* cultivars. *Weed Sci* (in press).

73 Lovelace ML, Talbert RE, Dilday RH, Scherder EF and Buerhing NW, Use of allelopathic rice with reduced herbicide rates for control of barnyardgrass (*Echinochloa crus-galli*), in *BR Wells Rice Research Studies—2000. Series 485*, ed by Norman RJ and Muellenet J-F, Arkansas Agric Expt Stn, University of Arkansas, Fayetteville, AR, USA, pp 75–79 (2001).

74 Ebana K, Yan W, Dilday RH, Namai H and Okuno K, Variation in the allelopathic effect of rice with water soluble extracts. *Agron J* **93**:12–16 (2001).

75 Mattice JD, Dilday RH, Gbur EE and Skulman BW, Barnyardgrass growth inhibition with rice using high-performance liquid chromatography to identify rice accession activity. *Agron J* **93**:8–11 (2001).

76 Ebana K, Yan W, Dilday RH, Namai H and Okuno K, Analysis of QTL associated with the allelopathic effect of rice using water-soluble extracts. *Breeding Sci* **51**:47–51 (2001).

77 Gealy DR, Determination of root distribution of weed-suppressive rice (*Oryza sativa*) and barnyardgrass (*Echinochloa crus-galli*) in field soil using ^{13}C isotope analysis, in *BR Wells Rice Research Studies—2000 Series 485*, ed by Norman RJ and Muellenet J-F, Arkansas Agric Expt Stn, University of Arkansas, Fayetteville, AR, USA, pp 69–74 (2001).

78 Gealy DR, Dilday RH and Rutger JN, Interaction of flush irrigation timing and suppression of barnyardgrass with potentially allelopathic rice lines, in *BR Wells Rice Research Studies—2000 Series 460*, ed by Norman RJ and Muellenet J-F, Arkansas Agric Expt Stn, University of Arkansas, Fayetteville, AR, USA, pp 49–55 (1998).

79 Duke SO, Scheffler BE, Dayan FE and Ota E, Strategies for using transgenes to produce allelopathic crops. *Weed Technol* **15**:826–834 (2001).

80 Scheffler BE, Michel A, Scheffler JA and Duke SO, Molecular methods in weed science. *Z Plfanzenkrankh Pflanzenshutz* **28**:45–56 (2002).

81 Scheffler BE, Duke SO, Dayan FE and Ota E, Crop allelopathy: enhancement through biotechnology. *Rec Adv Phytochem* **35**:257–274 (2002).

82 Lee T, Yun S-H, Hodge KT, Humber RA, Krasnoff SB, Turgeon GB, Yoder OC and Gibson DM, Polyketide synthase genes in invertebrate-associated fungi. *Appl Microbiol Biotechnol* **56**:181–187 (2001).

83 Wagenaar MM, Gibson DM and Clardy J, Akanthomycin, a new antibiotic pyridine from the entomopathogenic fungus *Akanthomyces gracilis*. *Org Lett* **4**:671–673.