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February 14, 2014

VIA FEDERAL EXPRESS

Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835

Re: GRAS Notification for Phosphodiesterase I Produced with *Leptographium procerum*

Dear Sir or Madam:

On behalf of DSM Food Specialties ("DSM"), we are submitting under cover of this letter three paper copies and one eCopy of DSM's generally recognized as safe ("GRAS") notification for phosphodiesterase I produced with *Leptographium procerum* ("*L. procerum*"). DSM has determined, through scientific procedures, that its phosphodiesterase I enzyme preparation from *L. procerum* is GRAS for use in the production of yeast extracts and yeast autolysates, that can be applied as an ingredient in a wide range of savory foods such as soups and sauces, snacks, processed cheese, dressings and spreads, flavours and seasonings.

Phosphodiesterase I is a processing aid for the production of yeast extracts or yeast autolysates. The enzyme cleaves 5'-nucleotides (5'AMP, 5'GMP, 5'CMP and 5'UMP) from oligonucleotides originating from yeast RNA. 5'-nucleotides are known to impart flavour characteristics in food. Yeast extracts and autolyzed yeasts treated with phosphodiesterase I contain these nucleotides and consequently are a source of umami and natural taste enhancement.

Pursuant to the regulatory and scientific procedures established by proposed regulation 21 C.F.R. § 170.36, this use of phosphodiesterase I enzyme preparation from *L. procerum* is exempt from premarket approval requirements of the Federal Food, Drug and Cosmetic Act, because the notifier has determined that such use is GRAS.

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If you have any questions regarding this notification, or require any additional information to aid in the review of DSM's conclusion, please do not hesitate to contact me via email at gyingling@morganlewis.com or by telephone, (202)739-5610.

Sincerely,

(b) (6)

Gary L. Yingling

cc: DSM Food Specialties

GRAS NOTIFICATION FOR PHOSPHODIESTERASE I PRODUCED WITH A STRAIN OF *LEPTOGRAPHIUM PROCERUM*

IDENTIFIER NUMBER: DSM2034-001(#00060113)

HEALTH • NUTRITION • MATERIALS

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1. GENERAL INTRODUCTION AND CLAIM OF EXEMPTION FROM PREMARKET APPROVAL REQUIREMENTS

With this document, DSM Food Specialties (DSM) is submitting a GRAS notification for its phosphodiesterase I enzyme preparation produced by submerged fermentation of a selected, pure culture of a *Leptographium procerum* strain. Phosphodiesterase I (EC 3.1.4.15, CAS 9025-82-5) releases nucleotides from RNA fragments. DSM produces the phosphodiesterase I preparations in liquid form stabilized with sodium chloride. The enzyme is widely present in nature and can be found in plants, animals, humans and microorganisms.

Phosphodiesterase I is a processing aid for the production of yeast extracts or yeast autolysates. The enzyme cleaves 5'-nucleotides (5'AMP, 5'GMP, 5'CMP and 5'UMP) from oligonucleotides originating from yeast RNA. 5'-nucleotides are known to impart flavour characteristics in food. Yeast extracts and autolyzed yeasts treated with phosphodiesterase I contain these nucleotides and consequently are a source of umami and natural taste enhancement (Noordam, B. and Meijer, F.R., 2007). The produced yeast autolysates and extracts are intended to be used as ingredient in a broad range of savory food applications, such as soups and snacks[1].

Pursuant to the regulatory and scientific procedures established by proposed regulation 21 C.F.R. § 170.36 (see 62 Fed. Reg. 18,938, April 17, 1997), DSM has determined that its phosphodiesterase I enzyme from *Leptographium procerum* is a GRAS substance for the intended food applications and is therefore exempt from the requirement for premarket approval. Information on the enzyme and the production organism providing the basis for this GRAS determination is described in the following sections. General and specific information identifying and characterizing the enzyme, its applicable conditions for use, DSM's basis for its GRAS determination and the availability of supporting information and reference materials for FDA's review can be found here in Section 1.

The production organism, *Leptographium procerum*, has a long history of safe use. The safety of the enzyme and strain are discussed in Section 7.1. Section 2 describes the origin and development of the production strain. In Section 3, the enzyme activity is described in more detail. The safety of the materials used in manufacturing, and the manufacturing process itself is described in Section 4. Section 5 reviews the hygienic measurements, composition and specifications. Section 6 provides information on the mode of action, applications, use levels of phosphodiesterase I and enzyme residues in final food products. The safety studies outlined in Section 7 indicate that phosphodiesterase I preparations from *Leptographium procerum* show no evidence of pathogenic or toxic effects. Estimates of human consumption and an evaluation of dietary exposure are also included in Section 7.

[1] Examples: crackers, popcorn, pretzels, corn chips, tortilla chips, potato chips.

1.1. Name and Address of Notifier

Notifier:

DSM Food Specialties
PO Box 1
2600 MA Delft
The Netherlands
Tel: +31 152 79 3592

Manufacturer:

DSM Food Specialties
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59472 Seclin Cédex
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Tel: 33 320964545
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Person Responsible for the Dossier:

Dr. Jack Reuvers
Regulatory Affairs
DSM Food Specialties
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2600 MA Delft
The Netherlands
Tel: +31 152 79 3739
Fax: +31 152 79 3614

1.2. Common or Usual Name of Substance

DSM's phosphodiesterase I enzyme preparation from *Leptographium procerum* is produced by submerged fermentation of a selected, pure culture of *Leptographium procerum*. The common or usual name of the substance is phosphodiesterase I. It is produced in liquid form, stabilized with sodium chloride.

1.3. Applicable Conditions of Use

The *Leptographium procerum* phosphodiesterase I preparation is intended to be used in the production of yeast extracts and yeast autolysates. The enzyme is added during the autolysis process of baker's yeast cells (*Saccharomyces cerevisiae*). The concentration, pH, temperature conditions and incubation time are optimized to achieve the desired nucleotide concentration. The enzyme dosage level can vary from 10000 to 250000 5'FDU / kg yeast expressed in dry matter.

One phosphodiesterase I unit (5'FDU) is defined as the activity needed to liberate 1 micromole phosphate per minute from 3'GMP.

After the enzymatic reaction, the yeast extract or autolyzed yeast is concentrated by evaporation and heated by ultra-high temperature processing (UHT). These heating steps inactivate the enzyme.

1.3.1. Food Products Used in

The phosphodiesterase I described in this dossier is applied specifically for the production of yeast extracts and autolyzed yeast. The aforementioned products are intended to be used in a wide range of savory products, such as soups and sauces, snacks[2], processed cheese, dressings and spreads, flavours and seasonings.

1.3.2. Levels of Use

Phosphodiesterase I can be used on yeast autolysates during the production of yeast extracts or autolyzed yeasts. The average dosage of the enzyme depends on the process conditions and on the desired properties of the yeast product.

A typical use level would be 10,000 – 250,000 5'FDU per kg yeast dry matter, or 1.6 – 41 g of phosphodiesterase I preparation described in this dossier per kg yeast dry matter.

1.3.3. Purposes

Phosphodiesterase I cleaves 5'-nucleotides (5'AMP, 5'GMP, 5'CMP and 5'UMP) from oligonucleotides originating from RNA. 5'-nucleotides are known to impart flavouring characteristics in food.
Yeast extracts and autolyzed yeasts treated with phosphodiesterase I contain these nucleotides and consequently are a source of umami sensation and natural taste enhancement (Noordam, B. and Meijer, F.R., 2007).

[2] Examples: crackers, popcorn, pretzels, corn chips, tortilla chips, potato chips.

1.3.4. Consumer Population

Phosphodiesterase I activity is widely present in nature. Its presence was demonstrated in a number of vegetable sources, such as common beet, mung beans, carrots and barley rootlets (malt sprouts). Additionally, other sources that contain phosphodiesterase I include animals, such as bovine and pigs, microorganisms, such as *Lactobacillus acidophilus* and *Penicillium* sp. and humans (Schomburg, D., 2013, Benaiges, M.D. *et al.*, 1989). The production of phosphodiesterase I by the microorganisms *Leptographium procerum* and *Penicillium citrinum* has already been described in the Pariza and Johnson paper in 2001 under "Enzymes used in food processing today" (Pariza, M.W. and Johnson, E.A., 2001).

Many of the naturally occurring foods containing phosphodiesterase I, as described in the reference given above, are part of the human diet. Since the phosphodiesterase I produced in *Leptographium procerum* is a normal protein (composed of natural amino acids), it will be digested in the human gastrointestinal tract just as any other food protein/enzyme would.

The substrates for phosphodiesterase I are 3'-hydroxy termini of 3'-hydroxy-terminated oligonucleotides originating from RNA. Oligonucleotides are small RNA molecules consisting of a chain of linked nucleotides. Phosphodiesterase I successively releases 5'-nucleotides (5'AMP, 5'GMP, 5'CMP and 5'UMP) from the oligonucleotides (Schomburg, D., 2013). These free nucleotides can be found in naturally occurring RNA in both vegetable and animal products, such as barley, soya, maize, and fish meal (Sauer, N. and Bauer, E., 2011), and occur in relatively high amounts in milk (Kobata, A. *et al.*, 1967, Sauer, N. and Bauer, E., 2011, Schlimme, E. *et al.*, 2000). Also, yeast, and consequently processed yeast, naturally contain nucleotides (Sijben, J. *et al.*, 1998, Tibbets, G.W., 2007). The use of phosphodiesterase I merely increases the nucleotide content. There is increasing evidence that dietary nucleotides play a beneficial role in human health (Sauer, N. and Bauer, E., 2011, Schlimme, E. *et al.*, 2000). Consequently, adverse effects on nutrients are not expected.

It can thus be concluded that the substrate occurs in many food products, from animal as well as plant origin, and the enzymatic conversion by phosphodiesterase I creates reaction products which are common constituents of the human diet. Hence, there is no basis to believe that the use of phosphodiesterase I will have a significant effect on processed foods or on the human body.

As is shown in Section 6.4 of this dossier, the amount of enzyme TOS[3] in the final food is expected to be approximately 4.9-122 mg/kg in a wide range of savory products, such as soups and sauces, snacks[4], processed cheese, dressings and spreads, flavours and seasonings.

Since phosphodiesterase I is present in the final food products at very low levels, it is inactivated after the autolysis of yeast. Because it is a naturally occurring substance in cells and tissues commonly ingested by humans without any harm, it is unlikely that the consumer population will be affected by the presence of phosphodiesterase I in foodstuffs when used as processing aid.

1.4. Basis for GRAS Determination

Pursuant to 21 C.F.R. § 170.30, DSM has determined, through scientific procedures, that its phosphodiesterase I enzyme preparation from *Leptographium procerum* is GRAS for use in the production of yeast extracts and yeast autolysates, that can be applied as an ingredient in a wide range of savory foods such as soups and sauces, snacks[4], processed cheese, dressings and spreads, flavours and seasonings.

---

[3] TOS: Total Organic Solids

[4] Examples: crackers, popcorn, pretzels, corn chips, tortilla chips, potato chips.

1.5. Availability of Information for FDA Review

The data and information that are the basis for DSM's GRAS determination are available for the FDA's review, and copies will be sent to FDA upon request. Requests for copies and arrangements for review of materials cited herein may be directed to:

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2. PRODUCTION MICROORGANISM

2.1. Name and Designation

The strain used for the production of the phosphodiesterase I belongs to the species *Leptographium procerum*.

2.2. Source of the Organism

The wild-type strain was isolated from wood pulp, and after classical strain improvement, the phosphodiesterase I production strain (abbreviated as FDA) was deposited in the DSM culture collection under accession number DS 14595. This strain was taxonomically identified in July 1989 by the Dutch Culture Collection, the "Centraalbureau voor Schimmelcultures" (CBS), using known methodologies. The CBS is an independent, internationally recognised laboratory, established in Baarn, The Netherlands. As can be seen in Annex 2.2.A, the strain has been identified as *Verticicladiella procera Kendrick*, a synonym for *Leptographium procerum* (Wingfield, M.J., 1985).

2.2.1. *Information on Reproductive Cycles (sexual/asexual) of the Classical Production Organism*

The FDA strain is not a genetically engineered strain, and no specific phenotypic or genotypic characteristics are known. The only relevant parameter in this respect is its ability to produce sufficient phosphodiesterase I enzyme.

The fungus *Leptographium procerum* belongs to the Deuteromycotina or Fungi Imperfecti. These fungi do not have a sexual state; consequently mating compatibility among strains cannot be used to discriminate between species. However, the extent of morphological variation within a species is ill-defined for most asexual genera. .

Leptographium procerum is an aerobic fungus which produces dark conidiophores with conidia (conidiospores) produced in a slimy matrix. The fungus is a saprophyte which is mostly found in nature on the surface of wood or in wood-infesting insects (Kendrick, 1962; Wingfield, 1985). Representatives of the fungus *Leptographium procerum*, present in various strain collections, have been isolated as blue stain fungus from wood pulps or infected pine-trees in many regions of the world, such as Finland, Croatia, South-Africa, Scotland, Canada and the USA. Thus, the fungus appears to be widely distributed in nature. In line with its preference for pine-wood, it is found predominantly in mild to cold environments.

The fungus grows in mycelium form, and when grown submerged without nutrient-deprivation, it will not significantly sporulate. Very little published information is available on the physiology of *Leptographium procerum*, but based on the origin of isolation of the various *Leptographium procerum* strains found in public culture collections, it can be concluded that the fungus is a mesophilic organism and prefers to grow at moderate temperatures and at an acidic to neutral pH.

2.3. Strain Improvement

The wild-type strain was isolated from wood pulp, and was selected in a research project, where culture filtrates of microorganisms were screened for their ability to degrade RNA into 5'-GMP. A strain improvement program, using classical mutation and selection techniques, yielded strain DS 14595, which was identified by the CBS in Baarn, The Netherlands as *Verticicladiella procera* Kendrick (see annex 2.2.A), a synonym for *Leptographium procerum*.

2.4. The Classical Taxonomy

The formal classification of *Leptographium procerum* is:

Kingdom	: **Fungi**
Division	: **Eumycota**
Subdivision	: **Deuteromycotina**
Class	: **Hyphomycetes**
Order	: **Moniliales**
Family	: **Dematiaceae**
Subfamily	: **Leptographium complex**
Genus	: **Leptographium**
Species	: **Leptographium procerum**

Kendrick (Kendrick, W.B., 1962) describes a number of species from the Leptographium complex in detail, including *Leptographium procerum*. He also describes the struggle among taxonomists on how to name these types of fungi (*Hantzchia, Leptographium, Scopularia, Phialocephala, Verticicladiella).*

2.5. Molecular Biological Taxonomy

An in-depth literature search yielded no references regarding the use of molecular biological tools in the taxonomy of *Leptographium procerum* (See Annex 7.1.A).

2.6. Stability of Classical Production Organism in Terms of Relevant Genetic Traits

As stated in 2.4, the original strain was subjected to a classical strain improvement which yielded DS 14595. From this strain, working cell banks have been prepared by single colony isolation and mass propagation. These working cell banks are in use up to the present day without any indication of deterioration in growth rate and/or phosphodiesterase (FDase) activity.

2.7. Nature of Pathogenicity and Virulence, Infectivity, Toxicity and Vectors of Disease Transmission

There is evidence from the literature that the wild *Leptographium procerum* strains found in nature can infect trees that are stressed or in damaged conditions (Klepzig, K.D. *et al.*, 1996). Therefore, the fungus may be termed an "opportunistic" phyto-pathogenic fungus. However, the eight strains of *Leptographium procerum* deposited in the renowned ATCC Culture Collection are all classified as Biosafety Level 1 (ATCC, 2013). Moreover, literature reveals no evidence of the fungus being a pathogen for animals or humans (see Annex 7.1.A for literature search results).

With this and a history of more than three decades of domestication of the strain (Noordam, B. and Meijer, F.R., 2007), we conclude that our *Leptographium procerum* FDA strain lineage is not plant pathogenic. Consequently, no specific containment measures to prevent dissemination of the fungus into the environment have been necessary in the production process at DSM, formerly Gist-brocades, in Seclin, France.

For further details on the safety of the production strain is referred to Section 7.1

2.8. Natural Habitat, Geographic Distributions and Climatic Characteristics of the Original Habitats

The blue stain fungus *Leptographium procerum* has been isolated from pine-trees (soft-wood) from many regions of the world, such as Finland, Croatia, South-Africa, Scotland, Canada and the USA. The fungus appears to be widely distributed in nature. Due to its preference for pine-wood, it is predominantly found in mild to cold environments.

2.9. Absence of the Production Organism in the Product

Good Manufacturing Practice is not the only reason to assure absence of the production organism in the final products. For many reasons, it is very important for each enzyme producer that the final commercial product does not contain viable production organisms. Therefore all traces of the production organism are removed during the manufacturing process (see Section 4.4), ensuring that the enzyme preparation is free from the production organism *Leptographium procerum*.

2.10. Absence of Toxins

For several decades, *Leptographium procerum* has been used in the commercial production of phosphodiesterase I (Noordam, B. and Meijer, F.R., 2007). During this long history of use, there has been no evidence that this enzyme would contain toxins derived from the species.

The DSM *Leptographium procerum* strain has been tested to determine if it produces any known toxins under growth conditions that are known to induce toxin production in general. These tests showed that the strain does not produce any known toxins under these conditions (see Annex 2.11.A). Furthermore, Annex 2.11.B demonstrates that the strain does not produce any known toxins under commercial fermentation conditions.

Toxicological tests confirm that the DSM *Leptographium procerum* strain also does not produce any unknown toxins (See Section 7.4 of this dossier).

3. ENZYME IDENTITY

3.1. Enzyme Identity

- Systematic name : oligonucleotide 5'-nucleotidohydrolase
- Common name : phosphodiesterase I
- Other names : 5'-exonuclease; phophodiesterase I; 5'-nucleotide phosphodiesterase, Alkaline phosphodiesterase I, Exonuclease I, nuclease
- Enzyme Commission No. : 3.1.4.1
- CAS number : 9025-82-5

Within the enzyme nomenclature, phosphodiesterase I belongs to the family of phosphoric diester hydrolyases, part of the hydrolases acting on ester bonds.

3.2. Amino Acid Sequence

The amino acid sequence of the phosphodiesterase from *Leptographium procerum* has not been determined. Based on SDS-gel determination, it can be concluded that the Molecular Mass of the enzyme protein is about 39 kDa.

3.3. Enzymatic Activity

3.3.1. Main Enzymatic Activity

Phosphodiesterase I cleaves 5'-nucleotides (5'AMP, 5'GMP, 5'CMP and 5'UMP) from oligonucleotides originating from RNA. The enzyme is an exonuclease which needs a free end of an oligonucleotide chain for its activity. It hydrolyses both ribonucleotides and deoxyribonucleotides and has low activity towards polynucleotides. A 3'-phosphate terminus on the substrate inhibits hydrolysis.

DSM developed a simple, accurate and reproducible method to measure the enzyme activity in phosphodiesterase I enzyme preparations using 3'GMP as substrate (see Annex 3.3.A). This method is also used for standardizing the enzyme preparation. The enzyme activity described in this method is expressed in so-called 5'-Fdase Units (5'FDU). Under the described conditions, one 5'FDU is defined as the activity needed to liberate 1 micromole phosphate per minute from 3'GMP.

The biochemical properties of phosphodiesterase I from *Leptographium procerum* have been investigated. Phosphodiesterase I exhibits activity from pH 3 - 8. The optimum pH depends on the substrate, e.g. the optimum is pH 7.2 with 3'-GMP as a substrate, whereas with RNA as substrate the pH optimum ranges from 5.0 to 5.6. The temperature optimum, measured with 3'-GMP as a substrate and at pH 5.6, is 65°C.

The enzyme activity is reduced to 1 % in 30 minutes at 65°C. When treated by UHT (135 °C), no enzyme activity can be found after 14 seconds.

3.3.2. Subsidiary Enzymatic Activities

Like any other living organism, the phosphodiesterase I production organism produces many other enzymes needed for the breakdown of nutrients and buildup of cell material. The phosphodiesterase I enzyme preparation will therefore contain minor, non-standardized amounts of these other enzymes. These amounts do not have an effect in the application.

4. MANUFACTURING PROCESS

4.1. Overview

Phosphodiesterase I from DSM is produced by a controlled submerged fermentation of a selected, pure culture of *Leptographium procerum* (see Section 2). The production process includes the fermentation process, recovery (downstream processing) and formulation of the product. A flow sheet of the different steps involved is given in Annex 4.1.A.

4.2. Raw Materials

All raw materials meet predefined quality standards that are controlled by the Quality Assurance Department of DSM. The raw materials used for the fermentation and recovery of the product are suited for the intended use; this leads to the required safety status of the product. The safety is confirmed by the toxicological studies performed (see Section 7.5 of this dossier). The raw materials used for the formulation are of food grade quality and meet FCC specifications. The antifoam and flocculent are listed in the FDA September 11, 2003 letter to ETA as acceptable for use in enzyme manufacturing (Annex 4.2.A).

4.3. Fermentation Process

Biosynthesis of phosphodiesterase I occurs during the main fermentation. To produce the enzyme of interest, a submerged, aerobic fed batch fermentation process is employed, using a stirred tank fermentor. The fermentor is equipped with devices to measure for pH, temperature, oxygen and antifoam control, a top-mounted mechanical agitator and a bottom air sparger.

All fermentation equipment is carefully designed, constructed, operated, cleaned, and maintained so as to prevent contamination by foreign microorganisms. During all steps of fermentation, physical and

chemical control measures are taken and microbiological analyses are done to ensure absence of foreign microorganisms and to confirm strain identity.

The fermentation process consists of three steps: inoculum preparation, seed fermentation and main fermentation. The whole process is performed in accordance with Good Food Manufacturing Practice (see Section 5.2).

Growth of the production organism and increase of enzyme production are checked at the end of the main fermentation by analysis of aseptically collected samples. After the fermentation has been stopped downstream processing will start.

4.4. Recovery Process

The cell material is separated from the enzymes by means of a simple membrane filtration process. A flocculent and filter aid is used to facilitate the process. Subsequently, the remaining particles are removed with a polish filtration and a germ filtration. Next, the enzyme preparation is concentrated by ultrafiltration (UF), and then purified by first applying diafiltration, and then chromatography.

4.5. Formulation and Standardization Process

The purified concentrate is stabilised with approximately 20% sodium chloride and standardized at an enzyme activity range of 4000-6000 5'FDU, subjected again to polish and germ filtration, and subsequently stored at 4 – 6°C.

4.6. Quality Control of Finished Product

In accordance with the general specifications for enzyme preparations used in food processing as established by the Joint Expert Committee of Food Additives (JECFA) of the FAO/WHO in 2006 and the FCC (8th edition, (FCC 8, 2012)), the final phosphodiesterase I preparations from *Leptographium procerum* meet the following specifications:

In addition, the following specifications were set:

Parameter	Specification limit
Lead	Not more than 5 mg/kg
Total Coliforms	Not more than 30/g
Salmonella	Absent in 25g
Escherichia coli	Absent in 25g
Antimicrobial activity	Absent by test

Parameter	Specification
Appearance	Liquid
phosphodiesterase I activity	4000 – 6000 5'FDU/g

5. COMPOSITION AND SPECIFICATIONS

5.1. Composition

The common starting material before formulation is the UF (ultra-filtration) concentrate. Apart from the enzyme complex, the phosphodiesterase I preparations will also contain some substances derived from the microorganism and the fermentation medium. These harmless impurities consist of polypeptides, proteins, carbohydrates and salts. Since the enzyme is purified by chromatography, the amount of impurities derived from the micro-organism and the fermentation medium is very low when compared to other enzyme preparations used in food.

The Total Organic Solids (TOS) of the phosphodiesterase I preparations and the phosphodiesterase I activities were determined for three different batches of the UF concentrate:

Calculation of the TOS					
Batch number	Activity (5'FDU/g)	Ash (%)	Water (%)	TOS (%)	5'FDU/mg TOS
4100026801	4394	0.74	89.4	9.9	44.56
413006501	7390	0.31	81.9	17.8	41.52
413008601	6410	0.28	82.2	17.5	36.63
MEAN	6065	0.44	84.5	15.1	40.90

The TOS values of the final, formulated enzyme preparations can be calculated on basis of the above values. For example, a standardized enzyme preparation containing 5000 5'FDU/g will contain 122 mg TOS/g.

5.2. General Production Controls and Specifications

Quality standards require a strictly controlled fermentation process. Enzyme fermentation experience in the DSM factory in Seclin, France, has resulted in a solidly established Good Food Manufacturing Practice within the framework of a certified ISO system (ISO 9001:2008 and FSSC 22000:2011).

5.2.1. Technical Measures

The batches of primary seed material are prepared, preserved and stored in such a way that contamination and degeneration is avoided and genetic stability is secured. The vials are clearly labeled and strict aseptic techniques are applied during the recovery of the culture.

Only sterilized raw materials are used to prepare the nutrient medium for the fermentation.

The fermentor is a contained system. Prior to inoculation, the fermentor is cleaned, rinsed and sterilized. Membrane valves, air filters and seals are regularly checked, cleaned and replaced if necessary. Only sterilized air is used in the fermentation. The sterilized nutrient medium and the complete biomass broth are transferred aseptically to the main fermentor. The methods used effectively prevent microbial contamination during fermentation. The preparation of sterile media and the cleaning of the equipment are described in Quality Assurance documents and are strictly followed.

Microbial contamination is prevented during downstream processing by several germ reduction filtrations. The filters are thoroughly cleaned before each production run.

5.2.2. Control Measures

After preparation of a new batch of primary seed material, samples are checked for identity, viability and microbial purity. If these parameters are correct, the strain is tested for production capacity. Only if the productivity and the product quality meet the required standards will the new batch of primary seed material be accepted for further production runs. Each time a vial from such a certified batch of primary seed material is used for production, the viability, purity and identity of the strain is checked.

The raw materials used for the fermentation and recovery of the product are suited for the intended use leading to the required safety status of the product. The raw materials meet predefined quality standards that are controlled by the Quality Assurance Department of DSM. The raw materials used for the formulation are of food grade quality.

At regular intervals during the seed fermentation, samples are taken aseptically for analysis of pH, and microbiological quality in the laboratory.

During the main fermentation, the dissolved oxygen content, pH, temperature, viscosity and microbial quality are monitored. If microbial controls show that significant contamination has occurred, the fermentation will be discontinued.

Also during downstream processing, samples are taken and checked for the level of microbial contamination. If these checks show that significant contamination has occurred, the downstream processing is discontinued.

The finished product is subjected to extensive controls and complies with JECFA and FCC specifications. See Section 4.6: Quality Control of Finished Product.

6. APPLICATION

6.1. Mode of Action

Phosphodiesterase I cleaves 5'-nucleotides (5'AMP, 5'GMP, 5'CMP and 5'UMP) from oligonucleotides originating from RNA. It hydrolyses both ribonucleotides and deoxyribonucleotides, and has low activity towards polynucleotides. 5'-Nucleotides like 5'GMP and 5'IMP are known to exhibit flavour enhancing properties in food. The enzyme phophodiesterase I liberates 5'GMP from the natural occurring yeast RNA. Yeast RNA is also hydrolysed by endogenous enzymes; however, the released 3'-nucleotides do not have flavour enhancing properties. The production of yeast extract or yeast autolysate in presence of phosphodiesterase I thus results in a yeast extract or autolysate with enhanced flavour properties when compared to yeast extract or autolysate prepared without phosphodiesterase I (Steensma, A. *et al.*, 2004, Burdock, G.A. *et al.*, 2000).

6.2. Application

The phosphodiesterase I described in this dossier is applied specifically for the production of yeast extract or yeast autolysate with taste enhancing properties and improved overall savory taste and mouth feel.

The yeast extracts or autolysates produced with phosphodiesterase I are intended to be used in a wide range of savory products, such as soups and sauces, snacks[5], processed cheese, dressings and spreads, flavours and seasonings.

6.3. Use Levels

Generally, enzyme preparations are used in "quantum satis," or the amount which is needed. Phosphodiesterase I can be used on yeast autolysates during the production of yeast extracts or autolyzed yeasts. The average dosage of the enzyme depends on the process conditions and on the desired properties of the yeast product.

A typical use level would be 10,000 -250,000 5'FDU per kg yeast dry matter or 1.6 – 41 g of phosphodiesterase I preparation described in this dossier per kg yeast dry matter.

6.4. Enzyme Residues in the Final Food

The enzyme phosphodiesterase I naturally occurs in human, animals, plants as well as microorganisms. Literature describes its presence in humans, common beets, mung beans, carrots and barley rootlets, bovine, and many microorganisms such as *Lactobacillus acidophilus* and Penicillium sp. (Schomburg, D., 2013, Benaiges, M.D. *et al.*, 1989). It may therefore be concluded that the enzyme phosphodiesterase I is a common constituent of various foods.

The substrates for phosphodiesterase I are 3'-hydroxy termini of 3'-hydroxy-terminated oligonucleotides originating from RNA. Oligonucleotides are small RNA molecules consisting of a chain of linked nucleotides. Phosphodiesterase I successively releases 5'-nucleotides (5'AMP, 5'GMP, 5'CMP and 5'UMP) from the oligonucleotides. These free nucleotides can be found in naturally occurring RNA in all vegetable and animal products, such as barley, soya, maize, and fish meal (Sauer, N. and Bauer, E., 2011), and occurs in relatively high amounts in milk (Kobata, A. *et al.*, 1967, Sauer, N. and Bauer, E., 2011, Schlimme, E. *et al.*, 2000). Also yeast, and consequently processed yeast, naturally contains nucleotides (Sijben, J. *et al.*, 1998, Tibbets, G.W., 2007). The use of phosphodiesterase I merely increases the nucleotide content. There is increasing evidence that dietary nucleotides play a

[5] Examples: crackers, popcorn, pretzels, corn chips, tortilla chips, potato chips.

beneficial role in human health (Sauer, N. and Bauer, E., 2011, Schlimme, E. *et al.*, 2000). Consequently, adverse effects on nutrients are not to be expected.

It can thus be concluded that the substrate occurs in many food products from animal as well as plant origin, and the enzymatic conversion by phosphodiesterase I creates reaction products which are common constituents of the human diet. Hence, there is no basis to believe that the use of phosphodiesterase I will have a significant effect on processed foods or on the human body.

In all applications, after the conversion of the yeast oligonucleotides, the yeast extract or autolysate is heated by UHT (135 ºC). As mentioned in Section 3.3, the enzyme starts to be inactivated at temperatures of 65°C and higher. The UHT step is carried out to completely inactivate the enzyme so that no enzyme activity remains in the starch product. Therefore, no enzyme activity is present in the finished product.

Based on the information given in Sections 1.3.2 and 5.1, the following calculations can be made:

Final food	Enzyme use levels in food ingredient [5'FDU/kg yeast dm]	Maximal amount of ingredient in final food [%][6]	Residual amount of (denatured) enzyme in final food [5'FDU/kg]	Amount of TOS in final food [mg TOS/kg]
Soups and sauces, snacks[7], processed cheese, dressings and spreads, flavours and seasonings.	10,000-250,000	2	200-5000	4.9-122

6.4.1. Possible Effects on Nutrients

Given the very small residual amount of (denatured) enzyme that will be found in the final food, no relevant nutritional effects are foreseen. Nevertheless, according to the explanation given in 6.4, any nutritional effects that would possibly be expected to occur would be no different than an effect that would occur within nature.

7. SAFETY EVALUATION

7.1. Safety of the Production Strain

7.2. Nature of Pathogenicity and Virulence, Infectivity, Toxicity and Vectors of Disease Transmission

The safety of the production organism is paramount to assessing the probable degree of safety for enzyme preparations to be used in food production. According to the IFBC, food or food ingredients are safe to consume if they have been produced according to current Good Manufacturing Practices, from a nontoxigenic and nonpathogenic organism (Coulston, F. and Kolbye, A.C., 1990). A nontoxigenic organism is defined as "one which does not produce injurious substances at levels that are detectable or demonstrably harmful under ordinary conditions of use or exposure" and a nonpathogenic organism

[6] Ratio based on 'worst case' figure obtained from Eurasyp (European Association for Specialty Yeast products)

[7] Examples: crackers, popcorn, pretzels, corn chips, tortilla chips, potato chips.

as “one that is very unlikely to produce disease under ordinary circumstances” (Pariza, M.W. and Foster, E.M., 1983).

Representatives of the fungus *Leptographium procerum*, present in many strain collections, have been isolated from wood pulps or infected pine-trees. The fungus commonly occurs on the surface of pine-wood and appears to be associated with wood-infesting insects, mainly *Coleoptera* such as bark beetles and weevils. There is evidence from the literature that the wild *Leptographium procerum* strains found in nature are weakly phytopathogenic (Wingfield, M.J., 1985). There even is evidence that the fungus is only infectious when the trees are in stressed or damaged conditions (Klepzig, K.D. *et al.*, 1996, Lewis, K.J. and Alexander, S.A., 1985). In terms analogous with human pathogens, *Leptographium procerum* may be termed an opportunistic phytopathogenic fungus.

Leptographium procerum does not appear on the official EC lists of pathogens in Annex III of Directive 2000/54/EC on the protection of workers from risks related to exposure to biological agents at work (EU, 2000a). In the official lists of Council Directive 2000/29/EC (EU, 2000b) and the list of the European and Mediterranean Plant Protection Organization (EPPO, 2013), the fungus *Leptographium procerum* or one of its synonyms is not mentioned as a plant pathogen. Similarly, *Leptographium procerum* is not mentioned on the list of pathogens on the Belgian Biosafety Server (Belgium, 2010) and of the Dutch Commission on Genetic Modification (Netherlands, 2011). Finally, the eight strains of *Leptographium procerum* deposited in the renowned ATCC Culture Collection are all classified as Biosafety Level 1 (ATCC, 2013).

Moreover, literature reveals no evidence of the fungus being a pathogen for animals or humans (see Annex 7.1.A for the results of a literature search). As a consequence, it can be concluded that *Leptographium procerum* can be regarded as non-pathogenic.

The DSM Gist *Leptographium procerum* strain has a history of safe use of more than three decades in both laboratory and large-scale manufacturing environments (Noordam, B. and Meijer, F.R., 2007). This has never led to any pathogenic symptoms for plants, animals or humans. As a consequence, it can be concluded that *Leptographium procerum* can be regarded as non-pathogenic.

The DSM Gist *Leptographium procerum* strain has been tested to determine if it is able to produce any known toxins under conditions which are known to induce toxin production in general. These test showed that the strain does not produce any known toxins under these conditions (see Annex 2.11.A). Also in samples of the enzyme before chromatography and of the final phosphodiesterase I preparation, no known mycotoxins were found (see Annex 2.11.B).

The fact that the DSM Gist *Leptographium procerum* strain also does not produce any unknown toxins has been confirmed by toxicological tests presented in Section IV of this dossier.

Based on the above, it can be concluded that the phosphodiesterase I producing *Leptographium procerum* strain is non-toxicogenic.

7.3. Safety of the phosphodiesterase I enzyme

Consumer safety of enzyme preparations is determined usually by three variables: the producing organism, the raw materials used in the production process and the production process itself. In certain cases the enzyme might be of concern as well.

The safety of the production process is embedded in current Good Manufacturing Practice (cGMP) and Hazard Analysis of Critical Control Points (HACCP). The raw materials used for the production of phosphodiesterase I meet predefined quality standards that are controlled by the Quality Assurance Department of DSM (see also 5.2).

The enzyme activity of phosphodiesterase I produced by *Leptographium procerum* with IUBMB number 3.1.4.1 is listed in the Pariza and Johnson (Pariza, M.W. and Johnson, E.A., 2001) paper under "Enzymes used in food processing today." The phosphodiesterase I produced by *Leptographium procerum* was evaluated for its safety by Steensma et al. (Steensma, A. *et al.*, 2004)(see also annex 7.2.A), and it was concluded that this enzyme is safe for food production.

As described in 7.1, phosphodiesterase I produced by *Leptographium procerum* has already been used for food production for several decades. In China, phosphodiesterase I (nuclease) from *Penicillium citrinum* is an approved additive (GB 2760-2011, see annex 7.2.B).

Since it is generally accepted that the commercial enzyme preparation phosphodiesterase I of *Leptographium procerum* is not toxic, and since it is a natural constituent of many organisms, including microorganisms, plants and animals consumed as food (see Section 6.4), it is not expected that phosphodiesterase I would have any toxic properties.

The enzyme preparation phosphodiesterase I derived from *Leptographium procerum* strain (abbreviated as FDA) was evaluated according the Pariza & Johnson Decision Tree. The decision tree is based on the safety evaluation methodology published by Pariza and Foster in 1983, which was extended by the IFBC into the decision tree format and published in 1990. In 2001, Pariza and Johnson published an update. DSM's decision tree analysis, based on the most recent update of the decision tree, has been published by Steensma et al (see annex 7.2.A). According to the decision tree, the enzyme phosphodiesterase I derived from *Leptographium procerum* was accepted for use in food processing.

7.3.1. Allergenicity

As described in the reference given above, many of the naturally occurring foods containing phosphodiesterase I are part of the human diet. The given foods are not on the list of major food allergens of the Food Allergen Labeling and Consumer Protection Act (FALPA, 2004, 21 USC 343). Since the phosphodiesterase I produced in *Leptographium procerum* is a normal protein (composed of natural amino acids), it will be digested in the human gastrointestinal tract just as any other food protein/enzyme would.

As proteins, enzymes have the potential to cause allergic responses. Although virtually all allergens are proteins, it is noteworthy that only a small percentage of all dietary proteins are food allergens. Enzymes have a long history of safe use in food. The unique role of enzymes in food processing is as a catalyst. Due to the specific nature of enzymes, only small amounts are required to make desired modifications to the property of a food. Moreover, residual enzyme still present in the final food will be subjected to digestion in the gastro-intestinal system. Because exposure to enzymes used as processing aids in food is very low, even if they were potentially allergenic by the oral route, the likelihood of allergic sensitization of consumers to these proteins is virtually zero.

The allergenicity potential of enzymes was studied by Bindslev-Jensen et al. (Bindslev-Jensen, C. *et al.*, 2006) and reported in the publication: "Investigation on possible allergenicity of 19 different commercial enzymes used in the food industry". The investigation comprised enzymes produced by wild-type and strains, as well as protein engineered variants. The study population was comprised of 400 patients with a diagnosed allergy to inhalation allergens, food allergens, or bee or wasp allergies. It was concluded from this study that ingestion of food enzymes in general is not likely to be a concern with regard to food allergy.

Previously, a Working Group of the AMFEP (Association of Manufacturers and Formulators of Enzyme Products) on Consumer Allergy Risk from Enzyme Residues in Food performed an in-depth analysis of the allergenicity of enzyme products (Dauvrin, T. *et al.*, 1998). The overall conclusion is that there is no indication that enzyme residues in foods may represent an unacceptable risk for consumers. Thus,

there are no scientific indications that small amounts of enzymes in food can sensitize or induce allergic reactions in consumers.

The amino-acid sequence for the phosphodiesterase I enzyme protein has not been determined. Therefore, no homology search could be performed against food allergen databases.

With regard to allergenicity of the fermentation media, DSM has concluded that the data that it has and the public data and information allow it to conclude that there is no published or unpublished data that suggest there is an allergen causing protein from the fermentation media in the finished enzyme product. To reach that conclusion, DSM relies on:

1. The Enzyme Technical Association in 2004 conducted a survey of its members, and collected information on the possible presence of protein from the fermentation media in the final enzyme product. ETA provided the supporting data and information to FDA in a letter in 2005, and sent an accompanying public statement which is posted on ETA's website. The statement concludes that no allergens protein from the fermentation medium has been found in the finished enzyme, and states that regulatory bodies in both the EU and Japan have concluded that enzyme preparations do not pose an allergen risk that would require allergen labeling on the final product. Further, ETA points out that the typical manufacturing process of enzyme preparations includes a step to separate the biomass and fermentation media from the enzyme. This step ensures the enzyme product purity and stability, and would likely remove most proteins present in the fermentation media. A copy of the public statement from the ETA website is included in Annex 7.3.1.A.

2. In addition, the Food Allergy Research and Resource Program (FARRP) issued a paper in August of 2013 which concluded that because of the nature of enzymes as catalysts, they are used in very small amounts and the fermentation media is consumed during the enzymatic process. It is clear that any *de minimis* amount of fermentation media protein that survived the fermentation process will not cause a significant public health risk to the consumer. FARRP also underscores the fact that the proteins would likely be removed during the filtration of the enzyme product, as discussed by ETA. Further, FARRP indicates that there is no reliable assay that could be used to detect the presence of most allergen proteins in the final enzyme products, as the proteins would likely be degraded fragments that would not reach levels of quantitation available with current commercial ELISA assays. The full August 2013 statement, provided as an attachment, clearly concludes that that any protein allergen present in the final enzyme product would not be present at a level that requires labeling or present at a level that raises a public health concern (Annex 7.3.1.B).

3. In addition, DSM has data from a study where wheat derived carbohydrates were used during fermentation, and an analysis after the fermentation shows the absence of gluten despite a detection limit of 10 parts per million (ppm). Finally, soy flour was used as a fermentation media, and post-fermentation analysis of the enzyme product revealed that no soy residue was present, with a level of detection of 0.5 ppm.

Finally, it is our understanding that a search of the scientific literature will not result in a reported allergic reaction from an enzyme caused by the fermentation medium. The ETA has conducted similar literature searches in the past, with no findings of allergic reactions due to fermentation media. The fermentation media as noted above is consumed in the process, and is removed with subsequent purification and filtration steps used in the enzyme production process. There is no evidence to support that a level of protein from the fermentation media exists in the final enzyme product which would cause an allergic reaction.

7.3.2. Leading Publications on the Safety of Phosphodiesterase I Enzymes or Enzymes that are Closely Related

The safety of the production organism is the main point of attention when assessing the safety of enzymes used in food processing. In this case, the production organism, *Leptographium procerum*, has been demonstrated to be nonpathogenic and nontoxigenic under the fermentation conditions used and any food ingredient (enzyme) from that organism will exhibit the same safety properties if manufactured under current Good Manufacturing Practices ("cGMPs"). Pariza and Foster (1983) noted that a non-pathogenic organism is very unlikely to produce a disease under ordinary circumstances. In their publication, *Leptographium procerum* is included in the authors' listing of the organisms being commonly used in the industry (Pariza and Johnson, 2001).

Moreover, phosphodiesterase I has a long history of safe use in yeast autolysis applications (Steensma, A. *et al.*, 2004), it naturally occurs in common beet, mung beans, carrots, and has a long history of human consumption.

An enzyme preparation with a very comparable type of action is the commercial product RP-1 containing endonucleolytic 5' phosphodiesterase activity that is produced by *Penicillium citrinum*. Similar to phosphodiesterase I described in this dossier, this enzyme is used to hydrolyse RNA into 5' nucleotides. This 5' phosphodiesterase has been evaluated for its safety by both Burdock et al. (Burdock, G.A. *et al.*, 2000) and Kondo et al. (Kondo, M. *et al.*, 2001). The *Penicillium citrinum* 5' phosphodiesterase did not display mutagenic activity, or clastogenic activity. Additionally, in 35-days and 13-weeks subchronic oral toxicity studies in rats the NOAEL was determined to be 500 and 319 mg /kg bw/day respectively. Consequently, phosphodiesterase I from *Penicillium citrinum* was considered as safe for the use as food enzyme. The safety studies described in Section 7.5 of this dossier support the fact that the *Leptographium procerum* enzyme concerned in this dossier does not result in any toxic effects.

These arguments show that the use of the phosphodiesterase I in food applications is unlikely to result in any adverse effects in humans.

7.3.3. Substantial Equivalence

Several expert groups have discussed the concept of substantial equivalence in relation to food safety assessment of enzymes. Essentially, all these groups conclude that if a food ingredient is substantially equivalent to an existing food ingredient known to be safe, then no further safety tests other than those for the existing ingredient are necessary. This concept is also accepted by the FDA, which in addition has evaluated the safety aspects of differences in glycosylation but concluded that chemical modification or site-directed mutagenesis would not raise safety concerns and the proteins are still considered substantially equivalent.
The following arguments describe why DSM considers its phosphodiesterase I as substantially equivalent to known enzymes:

a. Phosphodiesterase I activity from *Leptographium procerum* is substantially equivalent to phosphodiesterase I enzymes naturally present in common beet, barley, carrot, pig and bovine that are part of the human diet: all these enzymes catalyze the same reaction (EC.3.1.4.1).

b. Phosphodiesterase I from *Leptographium procerum* is substantially equivalent to 5' phosphodiesterase from *Penicillium citrinum* that is used for the same food application: Both enzymes hydrolyze yeast RNA into 5' nucleotides. 5' phosphodiesterase from *Penicillium citrinum* was considered to be safe for use as food enzyme (Kondo, M. *et al.*, 2001, Burdock, G.A. *et al.*, 2000)(see Chinese approval in annex 7.2.B).

7.4. Safety of the Manufacturing Process

Phosphodiesterase I meets the general and additional requirements for enzyme preparations as outlined in the monograph on Enzyme Preparations in the Food Chemicals Codex. As described in Section 4, the phosphodiesterase I preparation is produced in accordance with current good manufacturing practices, using ingredients that are acceptable for general use in foods, under conditions that ensure a controlled fermentation and are subject to testing to assure the enzyme product meets the stated specification. These methods are based on generally available and accepted methods used for the production of microbial enzymes.

7.5. Safety Studies

This section describes the studies performed to evaluate the safety of DSM's phosphodiesterase I preparations produced with the recombinant *Leptographium procerum*. All safety studies were performed according to internationally accepted guidelines (OECD) and are in compliance with the principles of Good Laboratory Practice (GLP) according to the FDA/OECD.

7.5.1. Summary of Safety Studies

The following studies were performed with the phosphodiesterase I enzyme preparation enzyme preparation:

- Ames test
- Chromosome aberration test
- 90-day oral toxicity study in rats

The safety studies for the *Leptographium procerum* phosphodiesterase I were performed with the same representative batch of unformulated UF concentrate, batch number 4100026801, referred to as 'tox-batch', containing 4394 5'FDU/g and 9.86% TOS.

7.5.2. Results of the Mutagenicity Texts

<u>Ames test</u>
A bacterial reverse mutation test was performed with the tox-batch at Notox, the Netherlands, in order to assess its mutagenic activity in four selected strains of *Salmonella typhimurium*, TA 1535, TA 1537, TA 98 and TA 100, as well as in the *Escherichia coli* mutant WP2 *uvrA*. All were tested in both the absence and presence of a metabolic activation system (S9-mix) with concentrations of the tox-batch up to 5000 µg TOS/plate. The study was conducted in accordance with OECD guideline no. 471, Genetic toxicology: Bacterial Reverse Mutation Test, adopted 21st July 1997.

The bacterial background lawn was not reduced at any of the concentrations tested, and no biologically relevant decrease in the number of revertants was observed.
The tox-batch did not induce a significant dose-related increase in the number of revertant (His+) colonies in each of the four tester strains (TA1535, TA1537, TA98 and TA100) and in the number of revertant (Trp+) colonies in tester strain WP2uvrA both in the absence and presence of S9-metabolic activation. These results were confirmed in an independently repeated experiment. In this study, the negative and strain-specific positive control values were within the laboratory historical control data ranges, indicating that the test conditions were adequate and that the metabolic activation system functioned properly.

Based on the results of this study, it is concluded that the tox-batch is not mutagenic in the *Salmonella typhimurium* reverse mutation assay and in the *Escherichia coli* reverse mutation assay.

<u>Chromosomal aberration test</u>

A chromosomal aberration test *in vitro* was performed with the tox-batch at Notox, the Netherlands, in order to assess its ability to induce structural chromosomal aberrations in cultured human lymphocytes in the absence and presence of a metabolic activation system. The study was conducted in accordance with OECD guideline 473, Genetic toxicology: *In vitro* Mammalian Chromosome Aberration Test, adopted 21st July 1997.

The possible clastogenicity of the enzyme product was tested in two independent experiments. In the first cytogenetic assay, cells were treated with the tox-batch up to 5000 µg TOS/ml for 3 hours in the presence and the absence of 1.8% (v/v) S9-mix. The harvesting time of the cells was 24 hours after treatment. This is the highest concentration that should be tested according to the guidelines.

In the second cytogenetic assay, the tox-batch was tested up to 5000 µg TOS/ml for a 24 h continuous exposure time with a 24 h fixation time, and up to 4000 µg TOS/ml for a 48 h continuous exposure time with a 48 h fixation time in the absence of S9-mix. In the presence of S9-mix the test substance was also tested up to 5000 µg TOS/ml for a 3 h exposure time with a 48 h fixation time.

The incidence of structural chromosomal aberrations, found in the negative (vehicle) controls, was within the historical range. The positive control substances mitomycin C (in the absence of the S9-mix) and cyclophosphamide (in the presence of the S9-mix) induced the expected increases in the incidence of structural chromosomal aberrations, indicating that the test conditions were adequate and that the metabolic activation system functioned properly.

The tox-batch did not induce a statistically significant or biologically relevant increase in the number of cells with chromosome aberrations in the absence and presence of S9-mix in either of the two independently repeated experiments.

No biologically relevant effects of the tox-batch on the number of polyploid cells and cells with endo-reduplicated chromosomes were observed both in the absence and presence of S9-mix. Therefore, it can be concluded that the tox-batch does not disturb mitotic processes and cell cycle progression and does not induce numerical chromosome aberrations under the experimental conditions described.

It was therefore concluded that the tox-batch is not clastogenic to cultured human lymphocytes under the conditions employed in this study.

<u>90-day oral toxicity</u>
A sub-chronic (90 day) oral toxicity study with the tox-batch was conducted at Advinus, India, in order to assess the systemic toxicity potential of the test item when administered orally by gavage to rats. The study was conducted in accordance with OECD guideline 408, Repeated Dose 90-day Oral Toxicity Study in Rodents, adopted 21st September 1998.

The study comprised four groups of 10 male and 10 female Wistar rats: one control group and three test groups (low, mid, and high dose). For a period of 90 consecutive days, the tox-batch was administered by oral gavage to the rats at doses of 100 mg (low-dose), 300 mg (mid-dose) and 1000 mg (high-dose) of TOS (Total Organic Solids)/kg body weight. The animals in the control group received vehicle (MilliQ water) only, all groups were dosed at a volume of 10.4 ml/kg body weight/day. The low, mid, and high dose correspond with respectively 1043 mg, 3128 mg, and 10428 mg enzyme preparation/kg body weight/day. These dosages were based on a 14-days dose range finding study.

The following parameters were evaluated in all animals:
- General clinical observations
- Neurobehavioral testing (detailed clinical observations, FOB and motor activity)
- Ophthalmoscopic examination
- Body weight
- Food consumption
- Haematology

- Clinical chemistry
- Urinalysis
- Organ weights of principal organs
- Macroscopic examination
- Histopathology of organs (control and high-dose group)
- Histopathology of all lesions

Results

There were no pre-terminal deaths, nor any treatment-related clinical signs observed. General health, growth, body weight and feed consumption were unaffected by the administration of the test substance. No treatment-related changes were observed in the functional observation tests, ophthalmoscopic examination, haematology, clinical chemistry, urinalysis, organ weights and organ weight ratios. Gross and histopathology examination did not reveal any treatment related changes.

In view of the results described above, as no changes of toxicological significance were noted among the animals that received doses up to 1000 mg TOS/kg body weight/day or 10428 mg tox-batch/kg body weight/day, this dose level is considered to be the No Observed Adverse Effect Level (NOAEL) in Wistar rats, under the test conditions employed.

7.5.3. Conclusion

Summarizing the results obtained from the several toxicity studies the following conclusions can be drawn:

- The tox-batch did not show any mutagenic or clastogenic activity under the given test conditions;
- Based on the results of the sub-chronic oral toxicity study the No Observed Adverse Effect Level (NOAEL) of the tox-batch is 10428 mg/kg body weight/day, corresponding with 1000 mg TOS/kg body weight/day or 40900 5'FDU/kg body weight/day, which is the highest dose level tested for both sexes.
- Taking together the results of these safety studies and those published in literature (Steensma), phosphodiesterase I produced with *Leptographium procerum* is considered safe for human consumption.

7.6. Estimates of Human Consumption and Safety Margin

The human consumption of phosphodiesterase I from *Leptographium procerum* was estimated using two approaches.

7.6.1. Estimated Daily Intake (based on US survey)

Yeast autolysate and yeast extract, the raw material processed by phosphodiesterase I from *Leptographium procerum*, are used in a wide variety of applications (see 6.2). However, an attempt is made to estimate the human consumption of this enzyme preparation on the basis of the information given in Section 6.4:

Final food	Residual amount of (denatured) enzyme in final food (5'FDU/kg)	Amount of TOS in final food (mg TOS/kg)	90th percentile intake level (g food/person/day)[8]	Estimated Daily Intake of (denatured) enzyme[9] (5'FDU/kg bw/day)	Estimated Daily Intake of TOS[4] (mg TOS/kg bw/day)
Soups[10]	200-5000	4.9-122	144	0.48-12	0.012-0.29
Sauces[11]	200-5000	4.9-122	28	0.09-2.3	0.002-0.057
Processed cheese[12]	200-5000	4.9-122	32	0.11-2.7	0.003-0.065
Flavours, seasonings[13]	200-5000	4.9-122	220	0.73-18.3	0.018-0.45
Snacks[14]	200-5000	4.9-122	70	0.23-5.8	0.006-0.14
Total					**0.041-1.002**

7.6.2. Theoretical Maximum Daily Intake (based on "Budget method")

Due to the wide variety of applications of yeast autolysate and yeast extract (see 6.2), the raw material processed by phosphodiesterase I from *Leptographium procerum*, the most appropriate way to estimate the human consumption in the case of food enzymes is using the so-called Budget Method (Hansen, S., 1966, Douglass, J.S. *et al.*, 1997). This method enables calculating a Theoretical Maximum Daily Intake (TMDI) based on conservative assumptions regarding physiological requirements for energy from food, and the energy density of food rather than on food consumption survey data. This method

[8] Intake levels of soups, sauces, processed cheese, flavours and seasonings, and snacks based on (Wilson, J.W. *et al.*, 1997). 90th percentile is approximately 2 times the intake level (CFSAN / Office of Food Additive Safety, 2006).

[9] Calculated for a person weighing 60 kg.

[10] Includes the following categories: "tomatoes" and "other vegetables". "Noodle and rice soups" are already included in the category "mixtures mainly grain, see Flavours and seasonings.

[11] Correspond to the category "fats and oils".

[12] Included in the category "cheese".

[13] Includes the following category: "mixtures mainly grains".

[14] Includes the following categories: "crackers, popcorn, pretzels, corn chips" and "fried white potatoes".

has previously been used in the GRAS notification procedure, as it was incorporated into GRN 201, which received a "No Questions" letter from the FDA.

The Budget Method was originally developed for determining food additive use limits, and is known to result in conservative estimations of the daily intake. It is based on assumptions regarding physiological requirements for energy from food, and on the density of food, rather than on food consumption survey data. Some assumptions are taken into consideration. The maximum energy intake over the course of a lifetime is 50 kcal/kg bw/day, equivalent to 25 g food/kg bw/day. 50% of goods consumed have been processed using phosphodiesterase I from *Leptographium procerum* as processing aids, therefore the maximum daily consumption of processed food will be 12.5 g/kg bw/day.

On the basis of this assumption of a maximum daily consumption of processed food of 12.5 g/kg bw/day, and the information given in Section 6.4, the following theoretical maximum daily intake can be calculated:

Final food	**Residual amount of (denatured) enzyme in final food (5'FDU/kg)**	**Amount of TOS in final food (mg TOS/kg)**	**Theoretical Maximum Daily Intake of (denatured) enzyme (5'FDU/kg bw/day)**	**Theoretical Maximum Daily Intake of TOS (mg TOS/kg bw/day)**
Soups and sauces, snacks, processed cheese, dressings and spreads, flavours and seasonings	200-5000	4.9-122	2.5-62.5	**0.061-1.53**

The Margin of Safety for human consumption can be calculated by dividing the NOAEL by either the Estimated Daily Intake (EDI) or the Total Theoretical Maximal Daily Intake (TMDI). As was shown in Section7.5, the 90-day oral toxicity study showed a NOAEL of 10428 mg tox-batch/kg body weight/day, corresponding with 1000 mg TOS/kg bw/day. Consequently, the Margin of Safety lies between 650 and 24,390.

8. GRAS Conclusion

As documented in this notification, the strain *Leptographium procerum* has been evaluated on its safety (see also 7.1), and it was concluded that the strain is non-pathogenic and non-toxicogenic. The safety studies described in Section 7.5 of this dossier support the fact that the produced enzyme does not exhibit any toxic effects.

In general, enzymes (proteins) are unlikely to produce toxic effects after oral exposure, since they are built from naturally occurring amino acids (normal food constituents) and are degraded in the gastrointestinal tract. Furthermore, only the denatured enzyme protein is present in the intended food applications, as the enzyme is already inactivated during the manufacturing process of the yeast autolysate or yeast extract (see **Error! Reference source not found.**6.4.1). Consequently, the enzyme is not expected to exert any further activity in the gastrointestinal tract. Should residual enzyme activity exist, this activity would not be expected to have any toxicological or nutritional effect, as the amounts added to the food applications are too small to have an effect on the carbohydrate digestion in the gastrointestinal system.

Based on the results of the safety evaluation and review of the published literature, it can be concluded that phosphodiesterase I is safe for its intended use in producing yeast extracts or yeast autolysates with taste enhancing properties. Toxicity evaluation did not raise any concern and the estimated safety margins are high. This is further supported by the long history of safe use of

Leptographium procerum as production organism for food enzymes and the natural occurrence of phosphodiesterase I in plants and animals consumed as foods. Furthermore, the conclusions are supported by several studies reported in literature. Based upon these factors, as well as upon the standardized production of the enzyme preparations, it is DSM's conclusion that the phosphodiesterase I preparation from *Leptographium procerum* is GRAS for its intended use.

9. LIST OF ANNEXES

2.2.A	Strain identification FDA
2.11.A	Mycotoxins analysis *Leptographium procerum #00046864*
2.11.B	Mycotoxins analysis in fermentation broth of *Leptographium procerum #00060090*
3.3.A	Enzyme activity assay for phosphodiesterase I #00060083
4.1.A	Flow diagram of manufacturing process phosphodiesterase I
4.2.A	FDA letter on approved antifoams and flocculants #00044855
7.1.A	Literature search on *Leptographium procerum*
7.2.A	Safety evaluation of phosphodiesterase derived from *Leptographium procerum*
7.2.B	Approval phosphodiesterase I China
7.3.1.A	Enzyme Technical Association Statement Regarding Allergenicity from Fermentation Media Raw Materials
7.3.1.B	FARRP Statement Regarding Allergens from Fermentation Media Raw Materials

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Annex 2.2.A

CULTURES
SCHIMMELS

CENTRAALBUREAU VOOR SCHIMMELCULTURES

Postal address:
P.O. Box 273,
3740 AG BAARN
The Netherlands

Visiting address:
Oosterstraat 1,
3742 SK BAARN

Tel.: 02154 - 11841
Fax: 02154 - 16142
Datanet-1: 1210085

Uw/Your Ref:
Onze/Our Ref: HS/WS/269 d.d. 13.06.89
Det.: 153 RAS/as

De heer H. Spierenburg
Research & Development
Gist Brocades
Postbus 1
2600 MA DELFT

Archief
Res. & Dev.
24 JUL 1989
G9429

BAARN, 21.07.1989

DETERMINATIE SERVICE

Spie

1x

Hieronder vindt U de uitslag van het door U gevraagde onderzoek.

DS 14595 *Verticicladiella procera* Kendrick = FDA-1

Dr. Gams, die deze schimmel heeft gedetermineerd zou deze stam gaarne in de kollektie willen opnemen. Is dit mogelijk?

Tevens treft U de rekening aan voor dit onderzoek.

Met vriendelijke groeten,

(b) (6)

Dr. R.A. Samson

CENTRAALBUREAU VOOR SCHIMMELCULTURES

Your Ref: HS/WS/269 d.d.13.06.89
Id.: 153 RAS/as

De heer H. Spierenburg
Research & Development
Gist Brocades
Postbus 1
2600 MA DELFT

F

Baarn, 21.07.1989

IDENTIFICATION SERVICE

Below, you will find the results of the research you have requested.

DS 14595 *Verticicladiella procera* Kendrick = FDA-1

Dr. Gams, who identified the strain, would like to include this strain in the collection. Would that be possible?

You will also find the bill for this study.

With kind regards,

Dr. R.A. Samson

Annex 2.11.A

CENTRAALBUREAU VOOR SCHIMMELCULTURES
Institute of the Royal Netherlands Academy of Arts and Sciences

Report

Analyses of

four samples of 5'Phosphodiesterase

for

toxic metabolite production

Centraalbureau voor Schimmelcultures,
P.O.Box 273, 3740 AG BAARN

Aim of the investigation

Fungi are known to produce many secondary metabolites. Some of these metabolites are considered mycotoxins which are significant for food. The aim of this investigation was to analyse extracts of four fermentation mashes for toxic metabolites.

Extracting of the samples were performed at the Centraalbureau voor Schimmelcultures in Baarn. The extracts were sent to Prof. Jens C. Frisvad (Technical University of Denmark, Lyngby), who analysed the metabolites and compared them with all important toxins which may be significant in food.

Methods

The following samples were examined:

5'Phosphodiesterase Fin de Fermentation FDA 7101 08-07-97
5'Phosphodiesterase Cté UF avant dilution FDA 7101 11-07-97
5'Phosphodiesterase Fin de Chromatographie FDA 7101 19-07-97
5'Phosphodiesterase Fin de Chromatographie FDA 7101 24-07-97

For metabolite analysis, the samples were extracted by the method described in Frisvad and Thrane (1987) and analysed by high performance liquid chromatography (HPLC) with diode array detection (DAD) (Frisvad and Thrane, 1993).

The metabolites found were compared to a spectral UV library made from authentic standards run at the same conditions (the maximal similarity was a match of 1000), and retention indices were compared with those of standards.

Results

All samples contained several metabolites (see enclosed HPLC spectra) but no compounds which could be identified as mycotoxins.

Conclusions

When compared to a spectral UV library made from authentic standards of all important fungal toxins which may be of significance in food, no important mycotoxins were found.

None of the metabolites found are considered significant mycotoxins (Cole and Cox, 1981; Smith and Moss, 1985).

References

Cole, R.J. & Cox, R.H. (1981). Handbook of toxic fungal metabolites. Academic press, New York.

Frisvad, J.C. & Thrane, U. 1993. Liquid column chromatography of mycotoxins. In: Betina, V. (ed.): Chromatography of mycotoxins. Techniques and applications. Journal of Chromatography Library 54: 253-372. Elsevier, Amsterdam.

Smith, J.E. & Moss, M.O. (1985). Mycotoxins. Formation, analysis and significance. John Wiley & Sons, Chichester.

(b) (6)

Baarn, 17-11-1997

Dr. R.A. Samson

Annex 2.11.B

CENTRAALBUREAU VOOR SCHIMMELCULTURES
Institute of the Royal Netherlands Academy of Arts and Sciences

Report

Analyses of

four samples of 5'Phosphodiesterase

for

toxic metabolite production

Centraalbureau voor Schimmelcultures,
P.O.Box 273, 3740 AG BAARN

Aim of the investigation

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5'Phosphodiesterase Fin de Chromatographie FDA 7101 19-07-97
5'Phosphodiesterase Fin de Chromatographie FDA 7101 24-07-97

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Results

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Conclusions

When compared to a spectral UV library made from authentic standards of all important fungal toxins which may be of significance in food, no important mycotoxins were found.

None of the metabolites found are considered significant mycotoxins (Cole and Cox, 1981; Smith and Moss, 1985).

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Frisvad, J.C. & Thrane, U. 1993. Liquid column chromatography of mycotoxins. In: Betina, V. (ed.): Chromatography of mycotoxins. Techniques and applications. Journal of Chromatography Library **54**: 253-372. Elsevier, Amsterdam.

Smith, J.E. & Moss, M.O. (1985). Mycotoxins. Formation, analysis and significance. John Wiley & Sons, Chichester.

(b) (6)

Baarn, 17-11-1997

Dr. R.A. Samson

Annex 3.3.A

Analysis Service and solutions	METHOD OF ANALYSIS	No : 2484 Version : 3 Page : 1 of 16

Title:
Spectrophotometric determination of 5'-phosphodiesterase (5'FDase) activity with guanosine 2'&3'-monophosphate as substrate (relative method, Konelab analyzer).

Product:	Validated method	Date of issue:
Fermentation broth Filtrate DSP samples ccUF Finished product	YES	22 NOV. 2011

AUTHORIZATION

Compiled by	A. Vriend	(b) (6) [redacted] Date: 21-11-2011
Approved by expert	J. Godefrooij	(b) (6) [redacted] Date: 14-11-2011
Approved by QA/QC Analysis	M.M. Immerzeel	(b) (6) [redacted] Date: 22-11-2011
Approved by Team Lead Service Lab Delft	M. Steenbeek	(b) (6) [redacted] Date: 17-11-2011
Approved by external QA/QC (if applicable)	n.a.	Date:
Approved by external QA/QC (if applicable)	n.a.	Date:

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Title:
Spectrophotometric determination of 5'-phosphodiesterase (5'FDase) activity with guanosine 2'&3'-monophosphate as substrate (relative method, Konelab analyzer).

1 SAFETY AND ENVIRONMENT

Restrictions for working with chemicals and ML-I samples are mentioned in the work instructions concerning management, storage and use of chemicals, the handling of dangerous substances and standard rules for ML-I laboratories. These restrictions are also applicable for material that has been in contact with ML-I samples.

When working with strong acids, bases, carcinogenic matters and toxic matters etc. take all necessary precautions.

When working with highly concentrated enzyme preparations take all necessary precautions. Avoid inhalation of dust and / or prolonged contact with unprotected skin.

2 PRINCIPLE

2.1 Application

This method is applicable for the determination of 5-phosphodiesterase activity in fermentation samples (broth, supernatant, filtrate), downstream processing samples (Ultra Filtrate, powder, granulate) and formulated finished products.

2.2 Description of the method

Enzymatic activity of 5'-phosphodiesterase is measured by examination of quantitative release of phosphate. Action of the enzyme on 3'-GMP substrate results in release of guanosine and phosphate. The incubation and enzymatic activity is stopped by adding molybdate/vanadate reagent, resulting (in the presence of released phosphate) in formation of a yellow coloured vanadomolybdo-phosphor complex. Absorbance of the yellow colour is measured at a wavelength of 405 nm and is a direct measure for the 5'-phosphodiesterase activity. Described method is executed using a Konelab analyser.
Underlying method of analysis is a so-called relative method. Examined results are correlated to a 5'-phosphodiesterase preparation (standard curve) with an officially assigned activity, enabling calculation of 5'FDase activity in sample preparations.

2.3 Unit definition

The activity is expressed in **5'FDU** (5'-phosphodiesterase unit). One 5'FDU is defined as the amount of enzyme that liberates 1 micro mol phosphate per minute under the conditions of the test described in method of analysis 60756 (pH 5.6, 65°C).

2.4 Measuring range

The measuring range of this method is 0.20 to 1.60 5'FDU per mL to be incubated.

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2.5 Summary of the validation report

In table 1 a summary of results (versus acceptance criteria) is listed, gathered during validation and transfer of method of analysis B1981. Further details can be found in validation report VR61981.01 and method transfer report MOR61981.01 part 1 & 2.

Table 1: Experimental results of validation and method transfer versus acceptance criteria.

Validated	Sample discription	repeatability		Intermediate precision		
		Acceptance criteria	Results	Acceptance criteria	Results	Results
		RSD $_{within\ day}$	RSD $_{within\ day}$	RSD $_{between\ days}$	RSD $_{between\ days}$	RSD $_{duplicate}$
T2	Broth OP6075	< 3%	0.6	< 3%	1.1	1.2
T2	Filtr 5140F1 F+L	< 2%	0.9	< 2%	1.9	2.0
T2	Filtr 5140F1		1.5		0.8	1.4
AV/T1	ccUF V208		1.0 / 2.6		1.2 / 1.6	1.5 / 2.4
AV/T1	ccUF V404		1.3 / 1.9		0.8 / 0.8	1.3 / 1.6
T2	End-product AG1540	< 3%	0.9	< 4%	2.8	2.9
AV/T1	End-product R6923		3.1 / 2.3		2.8 / 1.6	3.6 / 2.3
AV/T1	End-product R6772		1.2 / 2.6		4.1 / 2.2	4.1 / 2.9
AV/T1/T2	Control C2	< 2%	1.0 / 1.2 / 0.3	< 2%	1.2 / 1.2 / 2.6	1.2 / 1.5 – 2.7

validated	Transfer parameters	Acceptance criteria	result	accepted
AV	Accuracy (confidence limits)	After standard addition: 110> recovery>90%	96.4 – 104.4%	Yes
AV	Linearity (best fit model)	Range 0.20 – 1.60 5'FDU/mL	2nd order polynomial	Yes
AV	Robustness	Record	satisfying	Yes
AV	System precision	Record	1.14%	Yes
AV	Limit of detection	NI	NI	NI
AV	Limit of quantitation	NI	NI	NI
AV	Selectivity / specificity	NI	NI	NI

T1 = Method transfer part I
T2 = Method transfer part 2
AV = Assay Validation
NI = Not Investigated

3 APPARATUS AND CONDITIONS

3.1 Apparatus

- Balance, accurately to 0.0001 g : Mettler AE 240
- Balance, accurately to 0.001 g : Mettler PM 400
- Balance, accurately to 0.01 g : Sartorius model 2004 MP
- Diluter, provided with 0.5 and 5.0 mL cylinders : Hamilton, microlabs
- pH Meter : Radiometer PHM 83

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- Vortex mixer	: Genie-2, Scientific Industries
- Folded filters	: 595½ , S&S
- Magnetic stirrer	: Variomag, Multipoint HP15
- Magnetic stirrers	: IKA labortechnik, MINI MR
- Disposable glass reagent tubes	: Corning
- Clinical analyzer	: Konelab Arena 30 analyzer, Thermo
- Konelab sample cups	: Contents 1.5 mL
- Konelab reagent vessels	: 20 mL

Or equivalent equipment.

3.2 Conditions

Not applicable.

4 MATERIALS

4.1 Chemicals

- Acetic acid 100%, p.a.	: Merck 1.00063
- Sodium acetate 3 H_2O, p.a.	: Merck 1.06267
- Zinc sulphate 7 H_2O, p.a.	: Merck 1.08883
- Guanosine 2'-&3'-monophosphate	: Sigma G8002
- Aerosol 22	: Sigma A9753
- Sodium hydroxide pellets, pure	: Merck 1.06462
- Ammoniumheptamolybdate 4 H_2O, p.a.	: Riedel de Haen 31402
- Ammoniummonovanadate, p.a.	: Merck 1.01226
- Ammonia 25%, p.a.	: Merck 1.05432
- Nitric acid 65%, p.a.	: Merck 1.00456

Or equivalent quality.

4.2 References, standards and controls

Standard:
Use a standard preparation with an officially assigned 5'-FDase activity. The activity is expressed in 5FDU/g. The stock of the FDase standard and amounts for daily use are stored in the freezer.

Control:
Use a control preparation with an officially assigned 5'-FDase activity. The activity is expressed in 5FDU/g. The stock of the FDase control and amounts for daily use are stored in the freezer.

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4.3 Reagents

- Water:
Ultra high quality water, conductivity ≤ 0.10 µS.cm.

- Zinc sulphate acetate buffer 0.1 mol/L, pH 5.60:
Consecutively dissolve in a 1 L volumetric flask containing approximately 800 mL water 0.60 g 100% acetic acid and 12.25 g sodium acetate.$3H_2O$ and 0.288 g zinc sulphate.$7H_2O$. Make up to volume with water and mix. Check the pH of the buffer; pH should be within the range 5.55 – 5.65.

- Substrate (5FDU-sub):
Dissolve 0.1816 g guanosine 2'&3'-monophosphate in a 50 mL volumetric flask containing approximately 40 mL zinc sulphate acetate buffer 0.1 mol/L, pH 5.60. If necessary adjust the pH to 5.60 +/- 0.05 by adding acetic acid 0.1 mol/L or sodium hydroxide 0.1 mol/L. Make up to volume with zinc sulphate acetate buffer 0.1 mol/L, pH 5.60 and mix. Always use a freshly prepared solution. Transfer the substrate solution into a 20 mL Konelab reagent vessel.

- Aerosol 22 100 g/L:
Dissolve 10.0 g Aerosol 22 in a 100 mL volumetric flask containing approximately 60 mL water. Make up to volume with water and mix. This solution may be kept for 14 days at room temperature.

- Zinc sulphate acetate buffer/Aerosol:
Add 2.0 mL Aerosol 22 100 g/L to 1000 mL zinc sulphate/acetate buffer, pH 5.60 and mix.

- Diluted nitric acid solution (23%):
While stirring continuously, carefully add 70 mL nitric acid 65% to 130 mL water.

- Stock solution Ammoniummonovanadate 2.35 g/L:
Completely dissolve 2.35 g ammoniummonovanadate in a 1 L glass beaker containing approximately 400 mL water of 60°C. Allow to cool to room temperature during continuous stirring for 2 hours to ensure complete solution and prevent precipitate formation in next step.
While stirring, slowly add 20 mL diluted nitric acid solution and cool to room temperature. Quantitatively transfer this solution to a 1 L volumetric flask with water. Make up to volume with water and mix. This solution may be kept, shielded from light, at room temperature for 4 weeks.

- Stock solution ammoniumheptamolybdate 100 g/L:
Dissolve 100 g ammoniumheptamolybdate.$4H_2O$ in a 1 L volumetric flask containing approximately 800 mL water. Add 10 mL ammonia 25%, make up to volume with water and mix.

This solution may be kept, shielded from light, at room temperature for 4 weeks.

- Colour/stop mix (5FDU-stop):
Consecutively transfer to a 100 mL volumetric flask; 25.0 mL ammoniumheptamolybdate stock solution, 25.0 mL ammoniummonovanadate stock solution and 16.0 mL nitric acid 65%. Cool to room temperature, make up to volume with water and mix. Always use a freshly prepared solution. Transfer the substrate solution into a 20 mL Konelab reagent vessel.

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5 PROCEDURE

5.1 Preparation

Not applicable.

5.2 Pre-treatment reference

Not applicable.

5.3 Pre-treatment standard

Before use allow the standard to obtain room temperature. Weigh, accurately to within 0.1 mg an amount of standard corresponding to 5000 5'FDU in a 50 mL volumetric flask. Suspend in approximately 40 mL zinc sulphate/acetate buffer pH 5.60, make up to volume with buffer and mix. Dissolve by stirring on a magnetic stirrer for 20 minutes. Using the dilutor, dilute this solution according to the table below with zinc sulphate acetate buffer, pH 5.60 in reagent tubes and mix. Perform the final dilution with zinc sulphate acetate/aerosol buffer. Store all diluted solutions in a bath with melting ice until starting the incubation.

encoding	activity [5FDU/mL]	standard solution [mL]	buffer to be added [mL	diluted standard solution [mL]	buffer/aerosol to be added [mL]	total dilution factor
S1	0.20	0.500	4.500	0.100	4.900	25000
S2	0.40	0.500	4.500	0.200	4.800	12500
S3	0.60	0.500	4.500	0.300	4.700	8333
S4	1.00	0.500	4.500	0.500	4.500	5000
S5	1.43	0.500	3.000	0.500	4.500	3500
S6	1.67	0.500	2.500	0.500	4.500	3000

5.4 Pretreatment control

Allow the control sample to attain room temperature before use. Weigh from the control sample in duplicate, accurately to within 1 mg amounts of control sample corresponding to approximately 5 000 5FDU in 50 mL volumetric flasks. Suspend in approximately 40 ml zinc sulphate acetate buffer pH 5.60, make up to volume with the same buffer and mix. Dissolve by stirring on a magnetic stirrer for 20 minutes. Using the dilutor, dilute 0.300 mL of these solutions with 4.700 mL zinc sulphate acetate buffer, pH 5.60 in reagent tubes and mix. Dilute 0.500 mL of these diluted solutions with 4.500 mL zinc sulphate acetate/aerosol buffer in reagent tubes and mix. Store all diluted solutions in a bath with melting ice until starting the incubation.

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5.5 Pretreatment samples

5.5.1 Samples to be diluted:
Dilute sample solutions with zinc sulphate acetate buffer pH 5.60 to obtain final sample dilutions containing between 0.20 and 1.60 5FDU/mL (e.g. according to the dilution table in annex 4). Prepare the final sample dilutions using zinc sulphate acetate/aerosol buffer solution. Store all diluted solutions in a bath with melting ice until starting the incubation.

5.5.2 Samples to be weighed:
Weigh, accurately to 1 mg, approximately 1 g sample in a volumetric flask. Dissolve in buffer pH 5.60, make up to volume with the same buffer and mix. Dilute these sample solutions with zinc sulphate acetate buffer pH 5.60 to obtain final sample dilutions containing between 0.20 and 1.60 5FDU/mL (e.g. according to the dilution table in annex 4). Prepare the final sample dilutions using zinc sulphate acetate/aerosol buffer solution. Store all diluted solutions in a bath with melting ice until starting the incubation.

5.5.3 Fermentation samples:
Weigh approximately 2 g sample, accurately to within 1 mg in a 100 mL volumetric flask. Dissolve in approximately 80 mL zinc sulphate acetate buffer pH 5.60, make up to volume with same buffer and mix. Filter the solution over a S&S filter. Dilute the filtrate with zinc sulphate acetate buffer pH 5.60 to a final dilution of approximately 0.6 5FDU/mL. Prepare the final sample dilutions using zinc sulphate acetate/aerosol buffer solution. Store all diluted solutions in a bath with melting ice until starting the incubation.

5.5.4 Liquid concentrate:
In duplicate weigh approximately 1 g sample, accurately to within 1 mg in a 100 mL volumetric flasks. Dissolve in approximately 80 mL zinc sulphate acetate buffer pH 5.60, make up to volume with same buffer and mix. Dilute 0.100 mL of these sample solutions with 4.900 mL zinc sulphate acetate/aerosol buffer in reagent tubes and mix. Store all diluted solutions in a bath with melting ice until starting the incubation.

5.6 Preparation measurement

Introduce approximately 1 mL of the final dilutions into sample cups and place them in the Konelab sample segments.

5.7 Measurement

Using the Konelab Arena 30 analyzer:
- Log in the Konelab software as user "ara" and fill in the password.

- A yellow flagged message stating "start up needed" is visible in the main screen. Press F1-startup to perform start up operations and press OK.
- When start up is completed, check the water blanks by pressing F8, F2, F8 and F1, check water blank. Press F5 to show all tested wavelengths. If outliers are seen (above 2 mA), then repeat the water blank by pressing F1 twice.

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Title:
Spectrophotometric determination of 5'-phosphodiesterase (5'FDase) activity with guanosine 2'&3'-monophosphate as substrate (relative method, Konelab analyzer).

Requesting samples:
- Press the Samples button on top of the main screen.
- Press F8-more
- Press F4-batch entry
-Type the sample name using the SLD-number (format: SLD-01234) and press Enter.
- Select the used segments and used positions (see par. 5.6) from the pull-down menus.
- Select the test to run
B2484S for running samples
B2484B for running sample blanks
- Press F2-Save changes
- Introduce the sample segments in the Konelab one by one by opening and closing the sample segment door. The Konelab automatically detects which segments are inserted.

Inserting reagents:
- Press the Reagents button on the top of the main screen.
- Press on a free reagent position number
- Press F2-Insert reagent
- Select **5FDU-sub** (substrate) reagent from the list
- Press OK and follow on-screen instructions
- Repeat this procedure for stop reagent **5FDU-stop** (colour stop reagent).

Starting analysis:
- Because a relatively large amount of sample is incubated, the sample temperature has a big effect on the incubation temperature. For a reliable measurement it is therefore essential that all samples are at the same temperature at the start of each incubation. **To allow all samples to reach 10°C, do not start the analysis within 10 minutes after introducing the sample segments in the Konelab!**
- Go to the main screen and press the green start button, which is located on the keyboard at the right of the Home button. This is the only way the analyzer can be started.

Results:
- When analysis is complete, press the Results button on the top of the main screen
- Press F8-more, F4 to see all analysed samples
- Press F8-more twice, F1 to see details of a selected sample. Press F1 once more to turn details off.
- Press F5-Print results to print the results

Generating peak files:
- When the sample series is completely finished, go to the main screen and press F4-Reports and F8-more.
- Select items to report "samples" and "all" from the pull down menus
- Press F4-Results to file
- Select "one row per result" and press OK
- **Always** enter "results" as the filename

Subsequently use the available program to generate the actual peak file.

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Cleaning up:
- Remove the sample segments from the Konelab when your series is finished by pressing 'Sample disk' in the main screen
- Press one of the present segment numbers
- Press F3-remove segment and remove the segment from the Konelab.
- Press F6-remove all samples to clear the samples from the memory.
- Select all your other segments one by one from the pull down menu and repeat the two last steps until all segments are removed.
- Remove used reagents from the reagent tray by pressing Reagents on the main screen.
- Click on a reagent and then press F3-remove reagent and follow the on-screen instructions
- Repeat this until all reagents are removed.

Standby:
At the end of each series a Standby has to be done. The needles are then cleaned and the tension is taken off the moving parts.
- Press F2-Standby in the main screen and follow the on-screen instructions

6 CALCULATION

Carry out the calculation with the aid of the computer program available for this analysis. If this is not possible carry out the calculation as follows:

Prepare a calibration curve by plotting delta absorbance per minute (absorbance sample – absorbance blank) versus the exactly calculated activity in 5FDU per mL sample solution to be incubated of the diluted standard solutions (S1-S6). This calibration curve must be fitted according to a first degree function ($y = ax + b$, in which y = absorbance, x = activity in 5FDU/mL). This curve is used to determine the amount of activity in the unknown samples according to $x = (A - b)/a$ = 5FDU/mL.

Calculate the activity in the samples as follows:

Calculation samples per mL:

5FDU/mL obtained from calibration line x Df = 5FDU/mL

Or:

(5FDU/mL obtained from calibration line x Df) / W [g] = 5FDU/g

Where:

Df = Total dilution factor = Df x V = dilution factor x volume volumetric flask.
W = sample weight [g]

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7 ASSESSMENT

7.1 Requirements

- A (diluted) sample solution must have an activity fitted within the measuring-range.
- The level of each control value must fit in the range : $C_{assigned} \pm 3 \times SD_{overall}$
  ($C_{assigned}$ = Assigned control value; $SD_{overall}$ = overall standard deviation of the average control value calculated from past series).
  - The *relative (absolute) difference* in level between (duplicate) *control* values within a daily series is not allowed to exceed a value of $2.8 \times RSD_{within\ day}$.
  (Relative absolute difference in control values = $(|\text{control value 1} - \text{control value 2}| / \text{Average control value}) \times 100\%$;
  $RSD_{within\ day}$ = relative overall standard deviation "within a day" calculated from past series using control values e.g. as determined in validation of the method).
- The *relative (absolute) difference* in level between (duplicate) *sample* values is not allowed to exceed a value of $2.8 \times RSD_{within\ day}$.
  (Relative absolute difference in sample values = $(|\text{sample value 1} - \text{sample value 2}| / \text{Average sample value}) \times 100\%$;
  $RSD_{within\ day}$ = relative overall standard deviation "within a day" calculated from past sample series with a comparable type of matrix e.g. as determined in validation of the method).
- No more than one calibration point may deviate more than 5% from the calibration line.

The results of the control sample must be expressed as percentage of the assigned value.
The results of the control samples must be imported into the control charts available for this method of analysis. All results have to be evaluated.

Actions

- Repeat a sample analysis with an adjusted dilution when results (of the diluted sample) are out of the measuring range.
- Repeat analysis when the control values do not comply with the requirements.
- Repeat analysis of a sample exceeding the "difference of duplicate" requirement.
- Repeat the analysis if more than one calibration point deviates more than 5% from the calibration line.

7.2 Authorisation

After a training period by a laboratory technician authorised for this method, a technician will be authorised for this method when he/she succeeds on performing the test single-handedly, whereby the standards and selected samples meet the criteria mentioned above.

8 REFERENCES

- Validation report no. 61981 (version 1).
- Method transfer report part I and part II (MOR 61981, version 1.0).
- Memo ANA 2004-0126: "Development of a semi-automated assay for the examination of 5"-phosphodiesterase (5'FDase) activity.

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- Memo "Transfer of 5FDase assay (B1981 and CQA 4 090 00) from Cobas Mira to Konelab analyzer." by J. Godefrooij, dated May 7, 2007.

9 REMARKS

Not applicable.

10 ANNEXES

Annex 1: Konelab sample program B2484S
Annex 2: Konelab blank program B2484B
Annex 3: Example of a 5'FDase calibration line
Annex 4: Dilution tables

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Annex 1: Konelab 5FDU sample program

Test	B2484S				
Full name	sample test 5FDase				
Online name	B2484S	Test In Use			
Test type	Photometric		LOW	HIGH	
		Test limit	0.0000	*	abs
Result unit	abs	Initial absorbance	0.000	*	A
Number of Decim.	4	Dilution limit	*	*	abs
		Secondary dil 1+	0.0	0.0	
		Critical limit	*	*	abs
		Reflex test limit	*	*	abs
		Reflex test limit			
Acceptance	Automatic	Reference class	LOW	HIGH	In Use
Dilution 1+	0.0				
Sample type	Sample type 1	Correction factor	1.00		
		Correction bias	0.00	abs	
		Temperature	37.0	°C	
Calibration type	None				
Factor	1.00	Bias		0.00	
Bias correction in use	NO				
Manual QC in Use	NO	Routine QC in use		NO	
Blank	None				
		Fixed cuvette			
Reagent	5FDUsub	Volume (ul)		40	
Disp. With	Extra	Add. Volume (ul)		30	
wash reagent	None				
Incubation		Time (sec)		300	
Sample		Volume (ul)		35	
Disp. with	Extra	Add. Volume (ul)		40	
Dilution with	Water	Wash reagent		None	
Incubation		Time (sec)		600	
Reagent	5FDUstop	Volume (ul)		40	
Disp. with	Extra	Add. Volume (ul)		30	
Wash reagent	None				
Incubation		Time (sec)		250	
Measurement	End point				
Wavelength (nm)	405 nm	Side wavel. (nm)		None	
Meas. Type	Fixed timing				

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Annex 2: Konelab 5FDU blank program

Test	B2484B				
Full name	blank test 5FDase				
Online name	B2484B	Test In Use			
Test type	Photometric		LOW	HIGH	
		Test limit	0.0000	*	abs
Result unit	abs	Initial absorbance	0.000	*	A
Number of Decim.	4	Dilution limit	*	*	abs
		Secondary dil 1+	0.0	0.0	
		Critical limit	*	*	abs
		Reflex test limit	*	*	abs
		Reflex test limit			
Acceptance	Automatic	Reference class	LOW	HIGH	In Use
Dilution 1+	0.0				
Sample type	Sample type 1	Correction factor	1.00		
		Correction bias	0.00	abs	
		Temperature	37.0	°C	
Calibration type	None				
Factor	1.00	Bias		0.00	
Bias correction in use	NO				
Manual QC in Use	NO	Routine QC in use		NO	
Blank	None				
		Normal cuvette			
Reagent	5FDUsub	Volume (ul)		40	
Disp. With	Extra	Add. Volume (ul)		30	
wash reagent	None				
Reagent	5FDUstop	Volume (ul)		40	
Disp. with	Extra	Add. Volume (ul)		30	
Wash reagent	None				
Sample		Volume (ul)		35	
Disp. with	Extra	Add. Volume (ul)		40	
Dilution with	Water	Wash reagent		None	
Incubation		Time (sec)		200	
Measurement	End point				
Wavelength (nm)	405 nm	Side wavel. (nm)		None	
Meas. Type	Fixed timing				

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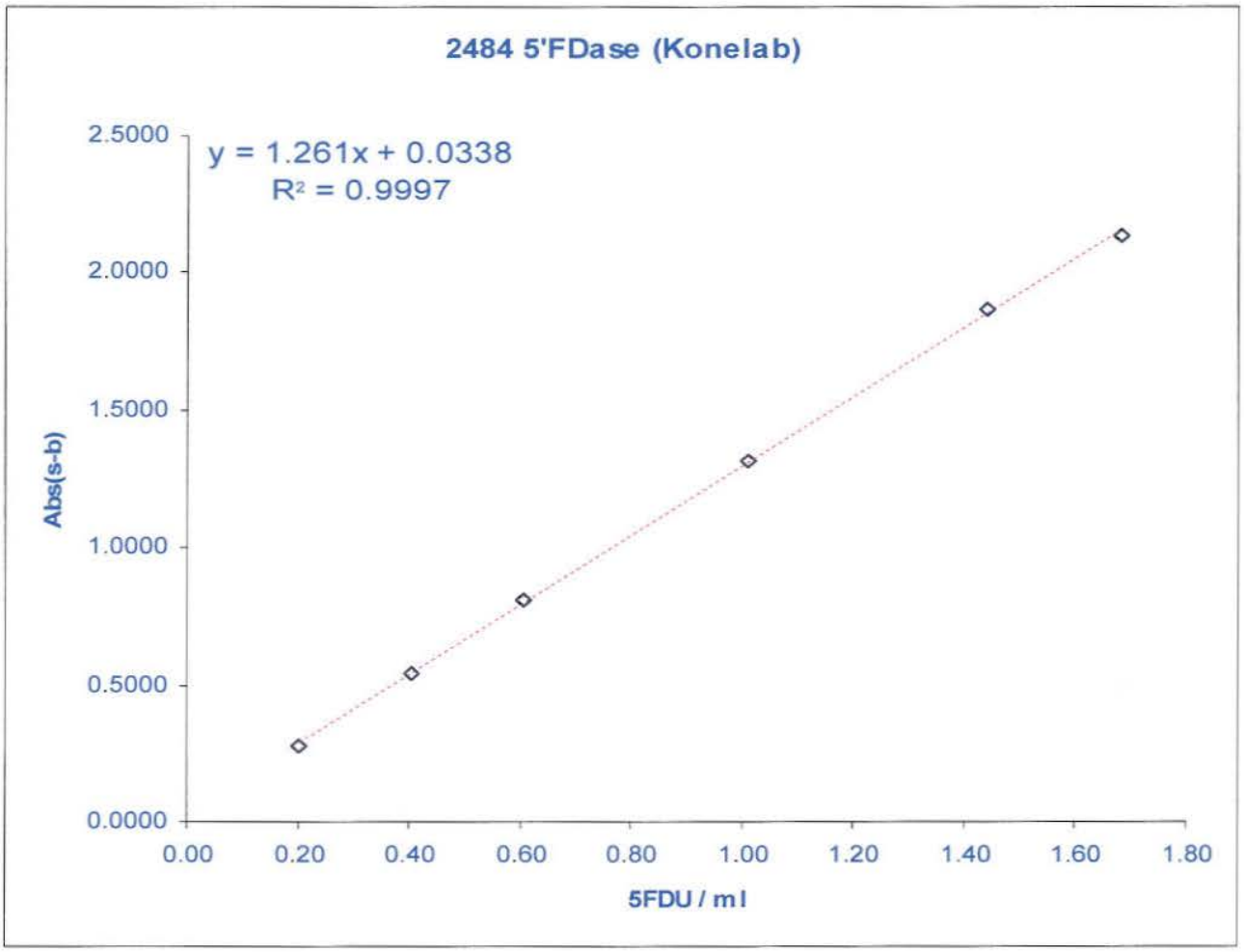
Annex 3: Example of the 5"FDase calibration line



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Annex 4: Dilution tables

Samples to be diluted

estimated activity [5FDU/mL]	sample [mL]	buffer to be added [mL]	diluted sample or sample solution [mL]	buffer to be added [mL]	diluted sample or sample solution [mL]	buffer / aerosol to be added [mL]	dilution factor
2 - 7					1.000	4.000	5
8 - 28					0.250	4.750	20
29 - 130			0.500	4.000	0.500	4.500	90
131 - 460			0.500	4.500	0.150	4.850	333.3
461 - 1750			0.100	4.900	0.200	4.800	1 250
1750 - 5600	0.500	4.500	0.500	3.500	0.100	4.900	4 000
5601 - 17500	0.500	4.500	0.250	4.750	0.100	4.900	10 000
17500 - 58000	0.200	4.800	0.150	4.850	0.100	4.900	41 667

Samples to be weighed

estimated activity [5FDU/mL]	sample [g]	buffer to be added [mL]	diluted sample solution [mL]	buffer to be added [mL]	diluted sample or sample solution [mL]	buffer / aerosol to be added [mL]	dilution factor
2 - 7	2	10.0 *					10
8 - 35	1	25.0 *					25
36 - 130	1.1	100.0 *					100
131 - 460	1.5	100.0			1.000	4.000	500
461 - 1650	1.7	100.0			0.250	4.750	2 000
1651 - 5800	1.2	100.0			0.100	4.900	5 000
5801 - 20600	1.7	100.0	1.000	4.000	0.100	4.900	25 000
20600 - 75000	1.9	100.0	0.250	4.800	0.100	4.900	100 000

*) dissolve iin buffer/aerosol

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Title:
Spectrophotometric determination of 5'-phosphodiesterase (5'FDase) activity with guanosine 2'&3'-monophosphate as substrate (relative method, Konelab analyzer).

HISTORY

Version	Description of the modification
1	First version. This method is based on the Seclin QC method CQA 4090 00 version 04, dated 20070711.
2	VtW-SLD-09-27 New lay out. Pre-treatment control sample adapted for new control preparation.
3	Unit definition corrected ("under conditions of the test" -> "under conditions of the test described in method of analysis 60756"). Ammoniummonovanadate to be stirred 2 hours before nitric acid addition to prevent precipitate formation. Pre-treatment standard adapted for new standard preparation. Typo corrected in 5.5.2 (pH 6.50 -> pH 5.60). Fit of calibration curve corrected to linear instead of second degree function.

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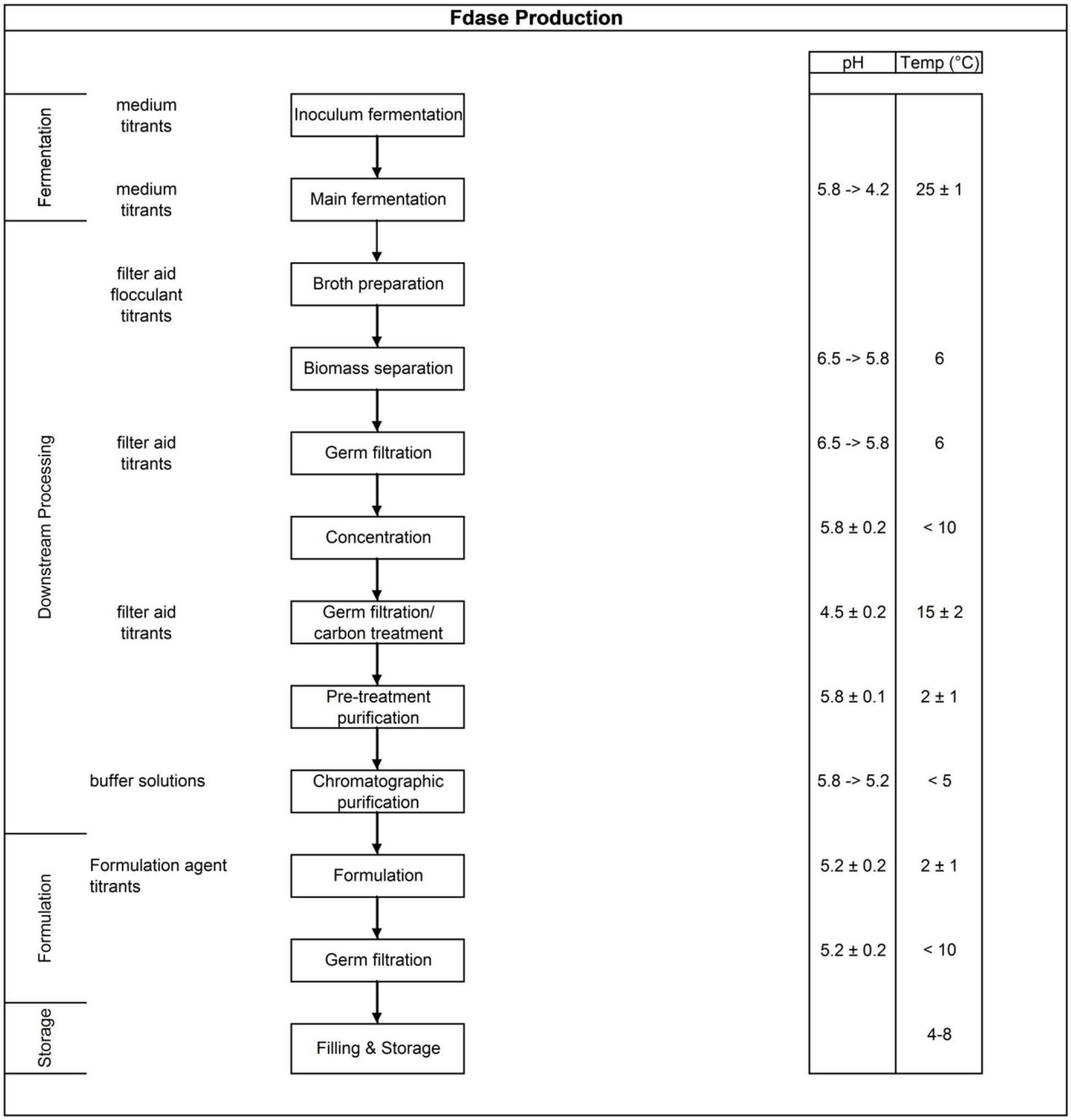
Annex 4.1.A

Annex 4.1.A
Flowsheet of manufacturing process of phospodiesterase I from *Leptographium procerum*



Annex 4.2.A

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Washington DC 20204

September 11, 2003

Mr. Gary Yingling
Kirkpatrick & Lockhart LLP
1800 Massachusetts Avenue, NW
Second Floor
Washington, DC 20036-1221

Dear Mr. Yingling:

You requested, on behalf of the Enzyme Technical Association, that OFAS review the use of certain defoaming and flocculating agents in the manufacture of enzyme preparations used in food. You provided information related to these compounds in your letters of December 20, 1996 (to Dr. Alan Rulis), 4-24-1998 (to Dr. Zofia Olempska-Beer), and 11-30-99 (to Dr. Zofia Olempska-Beer). You also arranged for a teleconference between ETA members and OFAS representatives, facilitated telephone contacts with technical experts from ETA member companies, and responded to numerous requests for clarification. We appreciate your and ETA's cooperation.

We reviewed the information on defoaming and flocculating agents that you submitted as well as the information provided in GRAS affirmation petitions and GRAS notices for enzyme preparations. The enclosed attachment provides a brief overview of our evaluation and itemizes the evaluated defoamers (Table 1) and flocculants (Table 2). We conclude that these compounds are used by enzyme manufacturers in accordance with the principles of good manufacturing practice (GMP).

Sincerely yours,

(b) (6)

Laura M. Tarantino, Ph.D.
Acting Director
Office of Food Additive Safety, HFS-200
Center for Food Safety and Applied Nutrition

Defoaming and Flocculating Agents Used in the Manufacture of Enzyme Preparations Used in Food

Enzyme Preparations

Most enzymes currently used in food are derived from microorganisms. The manufacturing process of such enzymes includes three major steps: fermentation, enzyme recovery, and enzyme formulation. The formulated products are generally referred to as enzyme preparations. In addition to the enzymes of interest, enzyme preparations contain added substances such as diluents, preservatives, and stabilizers. They may also contain metabolites derived from the production microorganism and the residues of substances used in the manufacturing process, such as components of the fermentation medium or defoaming and flocculating agents used during fermentation and recovery. When FDA reviews safety data on enzyme preparations, it considers all components of the preparation.

Defoaming Agents

Defoaming agents (defoamers) are used by enzyme manufacturers to reduce or prevent foaming during fermentation and recovery. They are formulated with ancillary ingredients such as surface-active agents or carriers. Defoamers currently used in the manufacture of food enzymes are listed in Table 1. The Table includes five major defoamers that are identified by a double asterisk and several compounds that are used either as secondary defoamers or ancillary ingredients in defoamer formulations.

The major defoamers are added to the fermentation broth at levels within the range of 0.05-1% on a weight basis. Some of these defoamers, for example, polyoxyethylene-polyoxypropylene block copolymer, may contain trace levels of ethylene oxide, propylene oxide, and 1,4-dioxane which are known to cause cancer in laboratory animals. The Office of Food Additive Safety (OFAS) has evaluated the use of defoamers listed in Table 1 and determined that human exposure to the residues of these defoamers in enzyme preparations does not present human safety concern.

Flocculating Agents

Flocculating agents (flocculants) are used in the enzyme recovery step to separate microbial cells and cell debris from the fermentation broth containing the dissolved enzyme. The flocculation typically consists of two steps - primary flocculation and secondary flocculation. In the primary flocculation, inorganic salts (such as calcium chloride or aluminum sulfate) or "low molecular weight" polymers (such as polyamines) are used to agglomerate the cellular debris. The primary flocculation is usually followed by the secondary flocculation in which "high molecular weight" polymers are used to aid the formation of larger agglomerates that are subsequently removed by centrifugation or filtration. The polymers used as flocculants can be either cationic or anionic. The cationic polymers are added to the fermentation broth at levels not higher than 1% on a

weight basis. The anionic polymers are used at levels at or below 0.025%.

The flocculants used in the manufacture of food enzymes are listed in Table 2. They include inorganic salts, polyamines, and polyacrylamides. Several of these compounds are regulated in 21 CFR either as food additives or GRAS substances. Certain polyamines may contain traces of epichlorohydrin and 1,3-dichloro-2-propanol. Polyacrylamides usually contain very low levels of acrylamide. These contaminants of polyamines and polyacrylamides are known to cause cancer in laboratory animals. OFAS has evaluated all polymers included in Table 2 and determined that human exposure to the residues of these flocculants in enzyme preparations does not present human safety concern.

Sources of Information on Defoamers and Flocculants

OFAS compiled data on defoamers and flocculants listed in Tables 1 and 2 using information voluntarily submitted by the Enzyme Technical Association. OFAS also relied on the information provided in GRAS affirmation petitions and GRAS notices for enzyme preparations. Other sources of information included published articles, computer searches, and Material Safety Data Sheets issued by manufacturers of defoamers and flocculants.

Table 1. Defoamers Used in the Manufacture of Food Enzymes

Compound	CAS Reg. No.	Supplemental Information
Polypropylene glycol**	25322-69-4	Average MW: 2000
Polyglycerol polyethylene-polypropylene glycol ether oleate**	78041-14-2	
Polyoxyethylene-polyoxypropylene block copolymer**	9003-11-6	Average MW: 2000
Polypropylene glycol monobutyl ether**	9003-13-8	
Polydimethylsiloxane**	63148-62-9 68083-18-1	
Silica	7631-86-9 63231-67-4	
Stearic acid	57-11-4	
Sorbitan sesquioleate	8007-43-0	
Glycerol monostearate	123-94-4	
Polysorbates (polyoxyethylene sorbitan fatty acid esters)		Polysorbate 60 (CAS No. 9005-67-8), Polysorbate 65 (CAS No. 9005-71-4), and polysorbate 80 (CAS No. 9005-65-6) are regulated as food additives and components of defoamer formulations
Rape oil mono- and diglycerides	93763-31-6	
White mineral oil	64742-47-8	

Table 2. Flocculants Used in the Manufacture of Food Enzymes

Compound	CAS Reg. No.	Supplemental Information
Dimethylamine-epichlorohydrin copolymer	25988-97-0	Cationic polyamine
Methylamine-epichlorohydrin copolymer	31568-35-1	Cationic polyamine
Dimethylamine-epichlorohydrin-ethylenediamine terpolymer	42751-79-1	Cationic polyamine
Polyacrylamide modified by condensation with formaldehyde and dimethylamine	67953-80-4	Cationic polyacrylamide
Acrylamide-acryloxyethyl-trimethyl-ammonium chloride copolymer	69418-26-4	Cationic polyacrylamide
Acrylamide-acrylic acid copolymer	25987-30-8 9003-06-9	Anionic polyacrylamide
Aluminum sulfate	10043-01-3	
Calcium chloride	10035-04-8 10043-52-4	

Annex 7.1.A

DSM
BRIGHT SCIENCE. BRIGHTER LIVING.

Memo

DSM Intellectual Properties

Date
November 5, 2013

From	To	cc
John Boonman	Piet van Dijck	R&D Archives

On the safety of *Leptographium*

For the production of 5'-phosphodiesterase (FDase) a strain of the fungus *Leptographium procerum*, also known as *Verticicladiella procera* is used, which was originally isolated from wood pulp. The ascomycete is associated with root-infesting beetles on pines and as such may cause blue stain in timber. Due to this preference it is predominantly found in mild to cold environments. The fungus is generally not considered to be a (plant) pathogen.
In this memo a comprehensive overview is presented on the literature on *Leptographium procerum sensu stricto*. In the first appendix the applied search strategy is outlined as well as the used sources as provided by the host STN. The metadata of resulting 168 papers are listed in appendix 2.

In a second research *Leptographium*, being the anamorphs of *Grosmannia* as well as related genera have more closely been examined on possible adverse effects including the production of potentially toxic compounds. In appendix 3 the search strategy is outlined as well as the sources used. The metadata of resulting 60 papers are listed in appendix 4.

Three potentially interesting publications are labeled in red. These are likely to be studied in more detail.

P.S. Please note that additional redundancies between appendix 2 and appendix 4 were removed manually, explaining why the numbering of the references is not continuous throughout.

Appendix 1. Search strategy on *Leptographium sensu stricto*

```
* * * * * * * * *   Welcome to STN International   * * * * * * * * * *

=> index all

98 FILES IN THE FILE LIST IN STNINDEX

=> s verticicladiella or leptographium


  59 FILES HAVE ONE OR MORE ANSWERS,   98 FILES SEARCHED IN STNINDEX

L1   QUE VERTICICLADIELLA OR LEPTOGRAPHIUM

=> d rank
F1        1574   GENBANK
F2         500   BIOSIS
F3         414   CABA
F4         352   SCISEARCH
F5         242   PQSCITECH
F6         196   AGRICOLA
F7         192   PASCAL
F8         161   ESBIOBASE
F9         113   CAPLUS
F10         95   USPATFULL
F11         87   PCTFULL
F12         50   MEDLINE
F13         45   COMPENDEX
F14         44   EMBASE
F15         44   EPFULL
F16         42   CANPATFULL
F17         38   CNFULL
F18         33   DISSABS
F19         31   INFULL
F20         29   CROPB
F21         29   PATDPAFULL
F22         28   BIOTECHNO
F23         26   JPFULL
F24         26   USPAT2
F25         22   TOXCENTER
F26         14   DGENE
F27         13   AUPATFULL
F28         12   CROPU
F29         11   GBFULL
F30         11   WPIDS
F31         11   WPINDEX
F32          7   NAPRALERT
F33          7   USGENE
F34          6   RDISCLOSURE
F35          5   FRFULL
F36          4   IFIALL
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F37          3   BIOTECHABS
F38          3   BIOTECHDS
F39          3   FROSTI
F40          3   FSTA
F41          3   PIRA
F42          2   ENCOMPPAT2
F43          2   ENERGY
F44          2   INPADOCDB
F45          2   INPAFAMDB
F46          2   TEMA
F47          1   CASREACT
F48          1   DDFU
F49          1   DPCI
F50          1   DRUGU
F51          1   EMBAL
F52          1   INSPEC
F53          1   JAPIO
F54          1   NTIS
F55          1   PATDPA
F56          1   UFORDAT
F57          1   ULIDAT
F58          1   USPATOLD
F59          1   WSCA

=> file f2-f9, f13-f14, f18, f20,f22

=> s leptographium procerum or verticicladiella procera

L2         530 LEPTOGRAPHIUM PROCERUM OR VERTICICLADIELLA PROCERA

=> duplicate

L3          204 DUPLICATE REMOVE L2 (326 DUPLICATES REMOVED)
```

Additional manual deduplication because of inaccurate technological deduplication. 168 unique papers remained.

Appendix 2. Papers on *Leptographium procerum* or *Verticicladiella procera*.

1. **Characterization of the mating-type genes in Leptographium procerum and Leptographium profanum**

T. A. Duong, Z. W. de Beer, B. D. Wingfield and M. J. Wingfield.

Affiliation: Department of Genetics, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria 0002, South Africa; Affiliation: Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria 0002, South Africa; Correspondence Address: Duong, T.A.; Department of Genetics, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria 0002, South Africa; email: tuan.duong@fabi.up.ac.za

Fungal Biol. 2013, 117 (6): 411-421

English

ABSTRACT Leptographium procerum and the closely related species Leptographium profanum, are ascomycetes associated with root-infesting beetles on pines and hardwood trees, respectively. Both species occur in North America where they are apparently native. L. procerum has also been found in Europe, China New Zealand, and South Africa where it has most probably been introduced. As is true for many other Leptographium species, sexual states have never been observed in L. procerum or L. profanum. The objectives of this study were to clone and characterize the mating type loci of these fungi, and to develop markers to determine the mating types of individual isolates. To achieve this, a partial sequence of MAT1-2-1 was amplified using degenerate primers targeting the high mobility group (HMG) sequence. A complete MAT1-2 idiomorph of L. profanum was subsequently obtained by screening a genomic library using the HMG sequence as a probe. Long range PCR was used to amplify the complete MAT1-1 idiomorph of L. profanum and both the MAT1-1 and MAT1-2 idiomorphs of L. procerum. Characterization of the MAT idiomorphs suggests that the MAT genes are fully functional and that individuals of both these species are self-sterile in nature with a heterothallic mating system. Mating type markers were developed and tested on a population of L. procerum isolates from the USA, the assumed center of origin for this species. The results suggest that cryptic sexual reproduction is occurring or has recently taken place within this population. © 2013 The British Mycological Society.

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2. **Blackstain root disease and other Leptographium diseases.**

Eckhardt, L. G. Editor: Gonthier,P. and G. Nicolotti.

Forest Health Dynamics Laboratory, School of Forestry and Wildlife Sciences, Auburn University, Auburn, AL 36849, USA. EMAIL: eckhalg@auburn.edu

Infectious forest diseases 2013, 283-297

English

3. **Assessing the virulence of ophiostomatoid fungi associated with the pine-infesting weevils to Scots pine Pinus sylvestris L. seedlings.**

R. Jankowiak.

Department of Forest Pathology, Agricultural University of Cracow, Al. 29 Listopada 46, 31-425 Cracow, Poland. EMAIL: rljankow@cyf-kr.edu.pl

Acta Agrobotanica 2013, 66 (2): 85-93

Polish

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4. **Diversity of ophiostomatoid fungi associated with the large pine weevil, Hylobius abietis, and infested Scots pine seedlings in Poland**

R. Jankowiak and P. Bilański.

Affiliation: Department of Forest Pathology, University of Agriculture in Kraków, Al. 29 Listopada 46, 31-425 Kraków, Poland; Affiliation: Department of Forest Protection, Forest Entomology and Climatology, University of Agriculture in Kraków, Al. 29 Listopada 46, 31-425 Kraków, Poland; Correspondence Address: Jankowiak, R.; Department of Forest Pathology, University of Agriculture in Kraków, Al. 29 Listopada 46, 31-425 Kraków, Poland; email: rljankow@cyf-kr.edu.pl

Ann.Forest Sci. 2013, 70 (4): 391-402

English

ABSTRACT Context: Bark beetles are known to be associated with fungi, especially the ophiostomatoid fungi. However, very little is known about role of pine weevils, e.g., Hylobius abietis, as a vector of these fungi in Europe. Aims: The aims of our study were to demonstrate the effectiveness of H. abietis as a vector of ophiostomatoid fungi in Poland and to identify these fungi in Scots pine seedlings damaged by weevil maturation feeding. Methods: Insects and damaged Scots pine seedlings were collected from 21 reforestation sites in Poland. The fungi were identified based on morphology, DNA sequence comparisons for two gene regions (ITS, β-tubulin) and phylogenetic analyses. Results: Sixteen of the ophiostomatoid species were isolated and identified. In all insect populations, Leptographium procerum was the most commonly isolated fungus (84 %). Ophiostoma quercus was also found at a relatively high frequency (16 %). Other ophiostomatoid fungi were found only rarely. Among these rarer fungi, four species, Leptographium lundbergii, Ophiostoma floccosum, Ophiostoma piliferum and Sporothrix inflata, were isolated above 3 %. L. procerum was isolated most frequently and was found in 88 % of the damaged seedlings. S. inflata was isolated from 26 %, while O. quercus occurred in 10 % of the seedlings. Conclusion: This study confirmed that L. procerum and O. quercus were common associates of H. abietis, while others species were found inconsistently and in low numbers, indicating causal associations. H. abietis also acted as an effective vector transmitting ophiostomatoid species, especially L. procerum and S. inflata, to Scots pine seedlings. © 2013 INRA and Springer-Verlag France.

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5. **Association of the pine-infesting Pissodes species with ophiostomatoid fungi in Poland**

R. Jankowiak and P. Bilański.

Affiliation: Department of Forest Pathology, University of Agriculture in Kraków, Kraków, Poland; Affiliation: Department of Forest Protection, Forest Entomology and Climatology, University of Agriculture in Kraków, Al. 29 Listopada 46, 31-425 Kraków, Poland; Correspondence Address: Jankowiak, R.; Department of Forest Pathology, University of Agriculture in Kraków, Kraków, Poland; email: rljankow@cyf-kr.edu.pl

Eur.J.For.Res. 2013, 132 (3): 523-534

English

ABSTRACT Bark weevils are consistently associated with various fungi. They act as effective vectors for root-rot fungi, the rust pathogen and the ophiostomatoid fungi. In comparison with bark beetles, the interaction between ophiostomatoid fungi and bark weevils has been poorly studied in Europe. This study aims to clarify the ties among ophiostomatoid fungi and their weevil vectors in Pinus sylvestris. Samples associated with three bark weevils, including Pissodes castaneus, P. piniphilus and P. pini were collected from seven pine stands in Poland. Fungi were isolated from laboratory-reared and field-collected beetles, larvae and galleries of weevils. Isolates were identified based on morphology, DNA sequence comparison for two gene regions (ITS, ß-tubulin) and phylogenetic analyses. Fourteen morphological and phylogenetic ophiostomatoid species were identified among the 1,219 isolates. These 14 species included 11 novel associations between fungi and bark weevils. The most commonly encountered fungal associates of bark Pissodes species were Leptographium procerum, Ophiostoma quercus and O. minus. The spectrum of fungal associates was similar in three bark weevil species, despite some differences between species. Except for P. castaneus, all species of bark weevil were frequently associated with ophiostomatoid fungi. Pissodes castaneus does appear to be a consistent vector of L. procerum and Sporothrix inflata while O. minus, O. quercus and O. cf. rectangulosporium were often found in association with P. piniphilus and P. pini. © 2013 Springer-Verlag Berlin Heidelberg.

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6. **ROOT-INHABITING BARK BEETLES (COLEOPTERA: CURCULIONIDAE) AND THEIR FUNGAL ASSOCIATES BREEDING IN DYING LOBLOLLY PINE IN ALABAMA.**

George Matusick, Roger D. Menard, Yuan Zeng and Lori G. Eckhardt.

Nature Conservancy, Chattahoochie Fall Line Project, Ft Benning, CA USA gmatusick@tnc.org

Fla.Entomol. 2013, 96 (1): 238-241

English

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7. **Large shift in symbiont assemblage in the invasive red turpentine beetle**

Stephen J. Taerum, Tuan A. Duong, Z. Wilhelm de Beer, Nancy Gillette, Jiang-Hua Sun, Donald R.

Owen and Michael J. Wingfield.

Department of Genetics, Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria, S. Afr.

PLoS One 2013, 8 (10): e78126

English

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8. **Root-colonizing ophiostomatoid fungi associated with dying and dead young Scots pine in Poland**

R. Jankowiak, P. Bilański, M. Kolařík and D. Wasiuta.

Affiliation: Department of Forest Pathology, University of Agriculture in Kraków al, 29 Listopada 46, Kraków 31-425, Poland; Affiliation: Department of Forest Protection, Forest Entomology and Climatology, University of Agriculture in Kraków, Kraków, Poland; Affiliation: Institute of Microbiology ASCR, Vídeňská, Praha, Czech Republic; Correspondence Address: Jankowiak, R.; Department of Forest Pathology, University of Agriculture in Kraków al, 29 Listopada 46, Kraków 31-425, Poland; email: rljankow@cyf-kr.edu.pl

For.Pathol. 2012, 42 (6): 492-500

English

ABSTRACT Ophiostomatoid fungi are carried by various bark beetles. However, very little is known about the role of these fungi in conifer roots. We studied ophiostomatoid fungi in roots of dying and dead Pinus sylvestris trees and tested the potential phytotoxicity of some isolates using a sensitive bioassay with Lepidium sativum in Poland. Fungi were identified based on their morphology and DNA sequencing. Three ophiostomatoid fungi, Leptographium procerum, Sporothrix inflata and Ophiostoma pallidulum, were isolated from the roots. The most abundant soil-borne fungus, S. inflata, and relatively rare O. pallidulum were isolated for the first time from roots of dying and dead pine trees. The frequency of S. inflata and O. pallidulum correlated with tree decline. The fungi were isolated more frequently from roots of dead than dying trees. Sporothrix inflata and O. pallidulum slightly reduced the stem and root growth of L. sativum. Leptographium procerum reduced more significantly root than stem growth. This species reduced root elongation 32-54% after 10-17 days of incubation. © 2012 Blackwell Verlag GmbH.

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9. **Grosmannia and leptographium spp. associated with conifer-infesting bark beetles in finland and russia, including leptographium taigense sp. nov.**

R. Linnakoski, Z. W. De Beer, T. A. Duong, P. Niemelä, A. Pappinen and M. J. Wingfield.

Affiliation: Section of Biodiversity and Environmental Science, Department of Biology, University of Turku, 20014 Turku, Finland; Affiliation: Faculty of Science and Forestry, School of Forest Sciences, University of Eastern Finland, P.O. Box 111, 80101 Joensuu, Finland; Affiliation: Department of

Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria 0002, South Africa; Affiliation: Department of Genetics, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria 0002, South Africa; Correspondence Address: Linnakoski, R.; Section of Biodiversity and Environmental Science, Department of Biology, University of Turku, 20014 Turku, Finland; email: riikka.linnakoski@utu.fi

Antonie Van Leeuwenhoek Int.J.Gen.Mol.Microbiol. 2012, 102 (2): 375-399

English

ABSTRACT Species of Grosmannia with Leptographium anamorphs include important forest pathogens and agents of blue stain in timber. They are commonly found in association with forest pests, such as bark beetles. During a survey of ophiostomatoid fungi in eastern parts of Finland and neighboring Russia, species belonging to the genus Grosmannia were isolated from 12 different bark beetle species infesting Picea abies and Pinus sylvestris, the most economically important conifers in the region. Identification of these fungi was based on morphology, DNA sequence comparisons for three gene regions and phylogenetic analyses. A total of ten taxa were identified. These belonged to six different species complexes in Grosmannia. The phylogenetic analyses provided an opportunity to redefine the G. galeiformis-, L. procerum-, L. lundbergii-, G. piceiperda-, G. olivacea-And G. penicillata-complexes, and to consider the species emerging from the survey within the context of these complexes. The species included G. galeiformis, G. olivacea, L. chlamydatum, L. lundbergii, L. truncatum and a novel taxon, described here as L. taigense sp. nov. In addition, species closely related to G. cucullata, G. olivaceapini comb. nov., G. piceiperda and L. procerum were isolated but their identity could not be resolved. The overall results indicate that the diversity of Grosmannia species in the boreal forests remains poorly understood and that further studies are needed to clarify the status of several species or species complexes. © Springer Science+Business Media B.V. 2012.

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10. **Root lesions in large loblolly pine (Pinus taeda L.) following inoculation with four root-inhabiting ophiostomatoid fungi**

G. Matusick, G. L. Somers and L. G. Eckhardt.

Affiliation: Centre of Excellence for Climate Change Woodland and Forest Health, School of Biological Sciences and Biotechnology, Murdoch University, Murdoch, WA 6150, Australia; Affiliation: School of Forestry and Wildlife Sciences, Auburn University, Auburn, AL, United States; Affiliation: Forest Health Dynamics Laboratory, School of Forestry and Wildlife Sciences, Auburn University, Auburn, AL, United States; Correspondence Address: Matusick, G.; Centre of Excellence for Climate Change Woodland and Forest Health, School of Biological Sciences and Biotechnology, Murdoch University, Murdoch, WA 6150, Australia; email: G.Matusick@murdoch.edu.au

For.Pathol. 2012, 42 (1): 37-43

English

ABSTRACT Loblolly pine decline, characterized by deteriorating root systems leading to shortening and thinning of foliage, has been observed throughout portions of the south-eastern United States. Several root-inhabiting ophiostomatoid fungi, including Leptographium procerum, Leptographium terebrantis, Leptographium serpens, and Grosmannia huntii are associated with

lateral root damage on declining loblolly pine. Trees of various ages were inoculated in primary lateral roots during fall (2006 and 2007) and spring (2007 and 2008). All fungi caused a darkened, resin-filled lesion on the surface of the phloem, extending into the xylem that was larger than that of controls. Only lesions associated with G. huntii infection were significantly larger in the spring season, compared with the fall. Grosmannia huntii was found to be the most virulent fungus, causing lesions that were longer, deeper and larger than all other fungal species during the spring and larger than L. terebrantis and L. procerum in the fall. Leptographium serpens was the second most virulent fungal species, causing lesions larger than L. procerum and L. terebrantis (with the exception of lesion depth) during both seasons. These tests indicate that G. huntii and L. serpens are significant root pathogens, capable of causing considerable damage, while L. terebrantis and L. procerum may be less virulent. Depending on the actions of their vectors, G. huntii and L. serpens may be responsible for significant root deterioration and tree disease. © 2011 Blackwell Verlag GmbH.

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11. **Effect of associated fungi on the immunocompetence of red turpentine beetle larvae, Dendroctonus valens (Coleoptera: Curculionidae: Scolytinae)**

Z. -H Shi, B. Wang, S. R. Clarke and J. -H Sun.

Affiliation: State Key Laboratory, Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, Beijing, China; Affiliation: USDA Forest Service FHP, Lufkin, TX, United States; Correspondence Address: Sun, J.-H.; State Key Laboratory, Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China; email: sunjh@ioz.ac.cn

Insect Sci. 2012, 19 (5): 579-584

English

ABSTRACT Dendroctonus-fungus symbioses are often considered as the ideal model systems to study the development and maintenance of ectosymbioses, and diverse interactions, including antagonism, commensalism and mutualism, have been documented between these organisms. The red turpentine beetle, Dendroctonus valens LeConte (Coleoptera: Curculionidae: Scolytinae) is a pine-killing invasive beetle in northern China. Fungi species Ophiostoma minus, Leptographium sinoprocerum, L. terebrantis and L. procerum were associated with this bark beetle. Antagonistic interactions between D. valens and its associated fungi, such as O. minus and L. sinoprocerum, have been demonstrated, but the underlying causes of this phenomenon are unknown. Here, we first found the two tested fungi species retarded the net weight gain of D. valens larvae after completing 3-day feeding on their media. Furthermore, we provide direct evidence indicating the effect of associated fungi on the immunocompetence of D. valens larvae to explain the documented antagonism. Our results showed that the activity of phenoloxidase and total phenoloxidase in D. valens larvae were significantly upregulated by two strains of associated fungi, O. minus and L. sinoprocerum as compared with the controls. The phenoloxidase ratio increased significantly in the larvae which had fed for 3 days on media inoculated with O. minus. Because insect immune defenses are costly to be deployed, these results could be explored as one of the underlying mechanisms of the documented antagonism. © 2012 Institute of Zoology, Chinese Academy of Sciences.

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12. **Mutual interactions between an invasive bark beetle and its associated fungi**

B. Wang, C. Salcedo, M. Lu and J. Sun.

Affiliation: State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, Beijing, 100101, China; Affiliation: Graduate School, Chinese Academy of Sciences, Beijing, 100049, China; Correspondence Address: Sun, J.; State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, Beijing, 100101, China; email: sunjh@ioz.ac.cn

Bull.Entomol.Res. 2012, 102 (1): 71-77

English

ABSTRACT Interactions between invasive insects and their fungal associates have important effects on the behavior, reproductive success, population dynamics and evolution of the organisms involved. The red turpentine beetle (RTB), Dendroctonus valens LeConte (Coleoptera: Scolytinae), an invasive forest pest in China, is closely associated with fungi. By carrying fungi on specialized structures in the exoskeleton, RTB inoculates fungi in the phloem of pines (when females dig galleries for egg laying and when males join them for mating). After eggs hatch, larvae gregariously feed on the phloem colonized by the fungi. We examined the effects of five isolates of RTB associated fungi (two from North America, Leptographium terebrantis and L. procerum, and three from China, Ophiostoma minus, L. sinoprocerum and L. procerum) on larval feeding activity, development and mortality. We also studied the effects of volatile chemicals produced in the beetle hindgut on fungal growth. Ophiostoma minus impaired feeding activity and reduced weight in RTB larvae. Leptographium sinoprocerum, L. terebrantis and L. procerum did not dramatically influence larval feeding and development compared to fungi-free controls. Larval mortality was not influenced by any of the tested fungi. Hindgut volatiles of RTB larvae, verbenol, myrtenol and myrtenal, inhibited growth rate of all the fungi. Our results not only show that D. valens associated fungus, O. minus, can be detrimental to its larvae; but, most importantly, they also show that these notorious beetles have an outstanding adaptive response evidenced by the ability to produce volatiles that inhibit growth of harmful fungus. © Copyright Cambridge University Press 2011.

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13. **Thinning affects on incidence of ophiostomatoid fungal species in decline-impacted Pinus taeda stands.**

Y. Zeng and L. G. Eckhardt.
PhytopathologyAPR 2012Annual Meeting of the American-Phytopathological-Society (APS) Southern-Division. Birmingham, AL, USA. February 05 -06, 2012. Amer Phytopathol Soc (APS), So Div
Auburn Univ, Sch Forestry and Wildlife Sci, Forest Hlth Dynam Lab, Auburn, AL 36849 USA
2012, 102 (4; Suppl. 2): 11

English

14. **Do novel genotypes drive the success of an invasive bark beetle-fungus complex? Implications for potential reinvasion**

M. Lu, M. J. Wingfield, N. Gillette and J. -H Sun.

Affiliation: State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China; Affiliation: Tree Protection Co-operation Programme, Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria 0002, South Africa; Affiliation: PSW Research Station, USDA Forest Service, Berkeley, CA 94701, United States; Correspondence Address: Sun, J.-H.; State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China; email: sunjh@ioz.ac.cn

Ecology 2011, 92 (11): 2013-2019

English

ABSTRACT Novel genotypes often arise during biological invasions, but their role in invasionsuccess has rarely been elucidated. Here we examined the population genetics and behavior of the fungus, Leptographium procerum, vectored by a highly invasive bark beetle, Dendroctonus valens, to determine whether genetic changes in the fungus contributed to the invasive success of the beetle-fungal complex in China. The fungus was introduced by the beetle from the United States to China, where we identified several novel genotypes using microsatellite markers. These novel genotypes were more pathogenic to Chinese host seedlings than were other genotypes and they also induced the release of higher amounts of 3-carene, the primary host attractant for the beetle vector, from inoculated seedlings. This evidence suggests a possible mechanism, based on the evolution of a novel genotype during the two or three decades since its introduction, for the success of the beetle-fungal complex in its introduced region. © 2011 by the Ecological Society of America.

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15. **Complex interactions among host pines and fungi vectored by an invasive bark beetle**

M. Lu, M. J. Wingfield, N. E. Gillette, S. R. Mori and J. -H Sun.

Affiliation: State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China; Affiliation: Tree Protection Co-operation Programme, Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria 0002, South Africa; Affiliation: PSW Research Station, USDA Forest Service, Berkeley, CA 94701, United States; Correspondence Address: Sun, J.-H.; State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China; email: sunjh@ioz.ac.cn

New Phytol. 2010, 187 (3): 859-866

English

ABSTRACT Recent studies have investigated the relationships between pairs or groups of exotic species to illustrate invasive mechanisms, but most have focused on interactions at a single trophic level. Here, we conducted pathogenicity tests, analyses of host volatiles and fungal growth tests to elucidate an intricate network of interactions between the host tree, the invasive red turpentine beetle and its fungal associates. Seedlings inoculated with two strains of Leptographium procerum isolated from Dendroctonus valens in China had significantly longer lesions and higher mortality rates than seedlings inoculated with other fungal isolates. These two strains of L. procerum were significantly more tolerant of 3-carene than all other fungi isolated there, and the infection of

Chinese pine (Pinus tabuliformis) seedlings by these two strains enhanced the production and release of 3-carene, the main attractant for D. valens, by the seedlings. Our results raise the possibility that interactions among the fungal associates of D. valens and their pine hosts in China may confer advantages to these strains of L. procerum and, by extension, to the beetles themselves. These interactions may therefore enhance invasion by the beetle-fungal complex. © The Authors (2010). Journal compilation © New Phytologist Trust (2010).

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16. **Pathogenicity and virulence of root-inhabiting ophiostomatoid fungi on Pinus species of the southeastern United States**

George Matusick III [Ph.D.] and Lori [advisor] Eckhardt.

Auburn University (0012)

Dissertation Abstracts International 2010, 71 (8B): 4557.

English

17. **Variation in virulence among four root-inhabiting Ophiostomatoid fungi on Pinus taeda L., P. palustris Mill, and P. elliottii Engelm. seedlings**

G. Matusick and L. G. Eckhardt.

Affiliation: Forest Health Dynamics Laboratory, School of Forestry and Wildlife Sciences, Auburn University, Auburn, AL 36849, United States; Correspondence Address: Matusick, G.; Forest Health Dynamics Laboratory, School of Forestry and Wildlife Sciences, Auburn University, Auburn, AL 36849, United States; email: matusge@auburn.edu

Can.J.Plant Pathol. 2010, 32 (3): 361-367

English

ABSTRACT Ophiostomatoid fungi have been implicated in root disease of pines in the southeastern United States. To understand more about their virulence, inoculation studies were conducted on loblolly (Pinus taeda), longleaf (Pinus palustris), and slash pine (Pinus elliotii). One-year-old bareroot seedlings, after being planted and established for 11 weeks, were wound-inoculated with one of four prominent North American ophiostomatoid fungal species. After three months, a darkened lesion, extending from the point of inoculation was observed for all species. Grosmannia huntii (L. huntii) caused the greatest lesion and occlusion length in loblolly pine and slash pine. Leptographium procerum and L terebrantis caused similar lesion and occlusion lengths and were the least virulent among the fungi tested. These studies indicate clear virulence differences among the four North American fungi. Grosmannia huntii, previously not known to be pathogenic, was observed causing significant damage compared with other well-known Leptographium species. Finally, lesion and tissue occlusion lengths were significantly smaller in longleaf pine for all fungal species when compared to loblolly and slash pine. © 2010 The Canadian Phytopathological Society.

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18. **The pathogenicity and virulence of four Ophiostomatoid fungi on young Longleaf pine trees**

G. Matusick and L. G. Eckhardt.

Affiliation: Forest Health Dynamics Laboratory, School of Forestry and Wildlife Sciences, Auburn University, Auburn, AL 36849, United States; Correspondence Address: Matusick, G.; Forest Health Dynamics Laboratory, School of Forestry and Wildlife Sciences, Auburn University, Auburn, AL 36849, United States; email: matusge@auburn.edu

Can.J.Plant Pathol. 2010, 32 (2): 170-176

English; French

ABSTRACT In southeastern USA, insect vectors transfer pathogenic ophiostomatoid fungi that cause disease in southern pines. During 2007 and 2008, potted longleaf pines (P. palustris Mill.), of similar ages ranging in height from 58 to 198 cm, were inoculated with the following fungi to assess their pathogenicity (and virulence): Grosmannia huntii, Leptographium procerum, L. serpens and L. terebrantis. Seventeen weeks after inoculation, L. terebrantis, L. serpens and G. huntii were found to cause significantly larger lesions and more sapwood discolouration than wounded uninoculated controls. Leptographium terebrantis caused significantly more sapwood discolouration than all other fungi. Despite significant sapwood occlusion after fungal inoculation, no reductions in needle water potentials were observed between treatments. All fungal species were successfully re-isolated from longleaf pine trees. © 2010 The Canadian Phytopathological Society.

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19. **Susceptibility of longleaf pine roots to infection and damage by four root-inhabiting ophiostomatoid fungi**

G. Matusick, L. G. Eckhardt and G. L. Somers.

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For.Ecol.Manage. 2010, 260 (12): 2189-2195

English

ABSTRACT Restoration of longleaf pine-dominated uplands is common on many public and private lands throughout the southeastern United States. The once dominant longleaf pine ecosystem is important to many now-threatened and endangered plant and animal species, and land managers are increasing efforts to reestablish this fire-dependent forest. Unfortunately, tree mortality in longleaf pine has been observed following attempts to re-introduce prescribed fire. Root-inhabiting ophiostomatoid fungi and their insect vectors have invaded roots of symptomatic longleaf pine. Although, the relationship between ophiostomatoid fungi and longleaf pine roots is poorly understood. In order to assess the pathogenicity and virulence of four ophiostomatoid fungi to longleaf pine, trees within two broad age classes (20-30 and 40-60 years) were used for root

inoculations during the fall of 2006 and 2007 along with the spring of 2007 and 2008. All fungal species consistently caused resin-filled, discolored lesions on the phloem surface extending to the xylem. The successful inoculation of healthy longleaf pine roots confirms the pathogenicity of Grosmannia huntii, Leptographium procerum, Leptographium serpens, and Leptographium terebrantis. G. huntii caused the largest lesions, including 22.20cm 2, 13.37cm 2, and 9.21cm 2 larger than L. procerum, L. terebrantis, and L. serpens respectively. In contrast, L. procerum caused significantly smaller lesions than all other fungi, including 8.65cm 2 smaller than L. terebrantis and 10.69cm 2 smaller than L. serpens. Restoration efforts of longleaf pine may be affected by fungal root infection in the future. Future studies should focus on the interactions between stress factors associated with longleaf pine to define more clearly the ecological role of root-inhabiting ophiostomatoid fungi in the ecosystem. © 2010 Elsevier B.V.

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20. **Barcoding and microcoding using "identiprimers" with Leptographium species**

Natalie R. van Zuydam, Dina Paciura, Karin Jacobs, Michael J. Wingfield, Martin P. A. Coetzee and Brenda D. Wingfield.

Department of Genetics, University of Pretoria, Pretoria, 0002, S. Afr.

Mycologia 2010, 102 (6): 1274-1287

English

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21. **Ecology of root-feeding beetles and their associated fungi on longleaf pine in georgia**

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Environ.Entomol. 2010, 39 (2): 415-423

English

ABSTRACT Root-feeding beetles, particularly of the curculionid subfamilies Scolytinae and Molytinae, are known to be effective vectors of Ophiostomatoid fungi. Infestation by these insects and subsequent infection by the Ophiostomatoid fungi may play an important role in accelerating symptom progression in pine declines. To examine the relationship between beetles and fungi in longleaf pine stands, root-feeding curculionids were collected in pitfall traps baited with ethanol and turpentine for 62 wk, and Ophiostomatoid fungi were isolated from their body surfaces. The most abundant root-feeding beetles captured were Hylastes tenuis, H. salebrosus, Pachyhbius picivorus, Hylobius pales, and Dendroctonus terebrans. The number of insects captured peaked in spring and fall, although peaks for different insect taxa did not coincide. The most frequently isolated fungi were Grosmannia huntii, Leptographium procerum, L. terebrantis, and L. serpens. Other Ophiostomatoid

fungi recovered included Ophiostoma spp. and Pesotum spp. Insect infestation data suggest that Hylastes spp. share an ecological niche, as do Hb. pales and P. picivorus, because the ratios of their fungal symbionts were similar. The fungi associated with D. terebrans suggest that it did not share habitat with the other principle vectors. © 2010 Entomological Society of America.

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22. **Effects of symbiotic bacteria and tree chemistry on the growth and reproduction of bark beetle fungal symbionts**

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Can.J.For.Res. 2009, 39 (6): 1133-1147

English

ABSTRACT Bark beetles are associated with diverse assemblages of microorganisms, many of which affect their interactions with host plants and natural enemies. We tested how bacterial associates of three bark beetles with various types of host relationships affect growth and reproduction of their symbiotic fungi. Fungi were exposed to volatiles from bacteria in an arena that imposed physical separation but shared airspace. We also exposed fungi to vapors of the host compound, α-pinene, and to combinations of bacterial volatiles and α-pinene. Bacterial volatiles commonly stimulated growth of Leptographium procerum (W.B. Kendr.) M.J. Wingf. and. Grosmannia clavigera (Rob.-Jeffr. & R.W. Davidson) Zipfel, Z.W. de Beer & Wingf., which are symbiotic fungi of Dendroctonus valens LeConte and Dendroctonus ponderosae Hopkins, respectively. They less commonly stimulated growth of Ophiostoma ips (Rumbold) Nannf., which is associated with Ips grandicollis Eichhoff. Some bacteria inhibited. L procerum, Ophiostoma montium (Rumbold) von Arx (another associate of D. ponderosae), and O. ips. Bacteria greatly stimulated spore production of symbionts of D. valens and D. ponderosae. α-Pinene strongly affected some of these relationships, causing amplification, reduction, or reversal of the interactions among the bacteria and fungi. Our results show that some bacteria associated with bark beetles directly affect fungal symbionts and interact with tree chemistry to affect fungal growth and sporulation. The strongest effects were induced by bacteria associated with beetles adapted to attacking living trees with vigorous defenses, and on fungal reproductive structures.

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23. **In vitro evaluation of combination of Trichoderma harzianum and chitosan for the control of sapstain fungi**

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Biol.Control 2009, 50 (3): 262-266

English

ABSTRACT In vitro assays were undertaken to evaluate the control of two sapstain fungi, Leptographium procerum and Sphaeropsis sapinea by a combination of chitosan or chitosan oligomer and an albino strain of Trichoderma harzianum. Spore germination and hyphal growth of the test fungi were assessed on media amended with chitosan or chitosan oligomer with and without T. harzianum using either simultaneous inoculation with test fungus or inoculation 1, 2, or 3 days after pre-infection with test fungus. There was no mycelial growth of the test fungi regardless of chitosan concentrations used when either L. procerum or S. sapinea was simultaneously inoculated with T. harzianum. However, the dose-response of chitosan or chitosan oligomer on the test fungi was apparent when T. harzianum was not simultaneously inoculated with test fungus but introduced later. There was a greater growth reduction at higher concentrations (0.075-0.1% v/v) of chitosan, and overall chitosan oligomer was more effective than chitosan aqueous solution. Chitosan alone was able to restrict or delay the germination of spores but the combination of chitosan and T. harzianum inhibited spore germination and hence colony formation of test fungi regardless of time delay. © 2009 Elsevier Inc. All rights reserved.

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24. **Effects of oleoresins and monoterpenes on in vitro growth of fungi associated with pine decline in the Southern United States**

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For.Pathol. 2009, 39 (3): 157-167

English

ABSTRACT Summary As a means of exploring pine resistance to root disease and declines, the effects of host plant secondary metabolites on the growth of root colonizing fungi associated with three diseases/declines of southern pines - loblolly pine decline, littleleaf disease and annosum root rot were tested. The associated fungi -Leptographium huntii, L. serpens, L. terebrantis, L. procerum, Heterobasidion annosum and Phytophthora cinnamomi- were grown in saturated atmospheres or in direct contact with, pure monoterpenes and crude oleoresin collected from the four southern pines (Pinus taeda, P. eschinata, P. palustris and P. elliotti) for 7 day. Fungal growth was measured at 3, 5 and 7 day. Root-infecting fungi differed significantly in sensitivity to crude oleoresin and pure monoterpenes. All fungi tested were inhibited, to some extent, by the resins tested. H. annosum and

P. cinnamomi were strongly inhibited by all the monoterpenes tested. The ophiostomatoid fungi were significantly less affected by the compounds tested. L. huntii and L. serpens were less inhibited by monoterpenes than either L. terebrantis or L. procerum. These fungal growth studies show that the kind and amount of secondary metabolite produced by the host plant have a profound effect on tree pathogens. Alterations of tree physiology may have implications for defenses against tree pathogens as well as to the ecology and management of forest ecosystems. Difference in incidence of root disease observed in the field may be explained by the ability of the fungus to tolerate these host defense mechanisms. © 2008 Blackwell Verlag, Berlin.

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25. **Forest disease records on eastern white pine in Atlantic Canada: 1950 to 1996**

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For.Chron. 2009, 85 (4): 604-608

English

ABSTRACT Between 1936 and 1996, the Forest Insect and Disease Survey (FIDS) of the Canadian Forest Service of Natural ResourcesCanada surveyed eastern white pine (Pinus strobus L.) throughout Atlantic Canada. During those years, FIDS reported its insect and disease findings in a wide variety of regional and national reports and in the scientific literature. The National Forest Health Database contains these electronic records and indicates the distribution and relative importance of forest diseases and fungi for many tree species in Atlantic Canada. This paper is a frequency analysis of the fungal diseases recorded from the Atlantic provinces in the National Forest Health Database and reported in the various Maritime, Newfoundland and national FIDS reports. The significant eastern white pine diseases (e.g., white pine blister rust - Cronartium ribicola J.C. Fisch.) that are established in Atlantic Canada as well as other diseases that are found elsewhere in Canada, but not yet in the Atlantic provinces are discussed. Occurrence data for 40 of the less frequently collected fungalspecies are presented in a summary table.

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26. **Ophiostomatoid fungi associated with the invasive pine-infesting bark beetle, Dendroctonus valens, in China.**

Min Lu, X. D. Zhou, Z. W. De Beer, M. J. Wingfield and J. -H Sun.

Chinese Acad Sci, Inst Zool, State Key Lab Integrated Management Pest Insects, Beijing, Peoples R China sunjh@ioz.ac.cn

Fungal Diversity, 2009, SEP 30 (38): 133-145

English

27. **Ophiostomatoid fungi (Ascomycota) associated with Pinus tabuliformis infested by Dendroctonus valens (Coleoptera) in northern China and an assessment of their pathogenicity on mature trees**

Q. Lu, C. Decock, X. Y. Zhang and H. Maraite.

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Antonie Van Leeuwenhoek Int.J.Gen.Mol.Microbiol. 2009, 96 (3): 275-293

English

ABSTRACT Dendroctonus valens is an invasive pest in coniferous forests of northern China. It was suspected of being responsible for the death of more than three million Pinus tabuliformis trees. The present study sought to identify the ophiostomatoid fungi associated with D. valens in northern China and understand the possible role of these fungi in the pine decline. On the basis of morphology, physiology, mating compatibility and phylogenetic analyses of multiple DNA sequences, seven species of ophiostomatoid fungi were isolated from and around D. valens galleries: Leptographium alethinum, Grosmannia koreana (teleomorph of L. koreanum), L. procerum, L. sinoprocerum, L. truncatum, Pesotum aureum and P. pini. All have been recorded for the first time in China. Among them, the occurrence of the dominant species L. procerum is positively linked to attack intensities of D. valens. The pathogenicity of four species (L. koreanum, L. procerum, L. sinoprocerum and L. truncatum) was tested on mature P. tabuliformis trees by stem inoculation. All inoculated strains caused significant necrotic lesions on the inner bark. However, L. koreanum and L. truncatum induced more extensive lesions than L. procerum and L. sinoprocerum. Their association with D. valens and the P. tabuliformis decline is discussed. © Springer Science+Business Media B.V. 2009.

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28. **Evolution of rDNA ITS1 and ITS2 sequences and RNA secondary structures within members of the fungal genera Grosmannia and Leptographium.**

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Fungal Genetics and Biology 2009, 46 (11): 855-867

English

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29. **Genes within Genes: Multiple LAGLIDADG Homing Endonucleases Target the Ribosomal Protein S3 Gene Encoded within an rnl Group I Intron of Ophiostoma and Related Taxa**

J. Sethuraman, A. Majer, N. C. Friedrich, D. R. Edgell and G. Hausner.

Department of Microbiology, University of Manitoba, Winnipeg, MB, Can.

Mol.Biol.Evol. 2009, 26 (10): 2299-2315

English

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30. **Biology and ecology of root-feeding beetles and ophiostomatoid fungi in sandhills longleaf pine stands**

James Warren [Ph D.]. Zanzot, Lori G. [advisor] Eckhardt and Scott A. [advisor] Enebak.

Auburn University (0012)

Dissertation Abstracts International 2009, 70 (12B): 7287

English

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31. **Assessment of longleaf pine on high-risk and low-risk loblolly pine decline sites at Fort Benning, Georgia.**

J. W. Zanzot and L. G. Eckhardt.
PhytopathologyJUN 2009Annual Meeting of the American-Phytopathology-Society. Portland, OR, USA. August 01 -05, 2009. Amer Phytopathol Soc
Auburn Univ, Auburn, AL 36849 USA
2009, 99 (6; Suppl. S): S148

English

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32. **Leptographium sinoprocerum sp. nov., an undescribed species associated with Pinus tabuliformis-Dendroctonus valens in northern China**

Q. Lu, C. Decock, X. Y. Zhang and H. Maraite.

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Institute of Forest Ecology, Environment and Protection, Chinese Academy of Forestry, Beijing, 100091, China; email: xyzhang@caf.ac.cn

Mycologia 2008, 100 (2): 275-290

English

ABSTRACT During a study of ophiostomatoid fungi associated with the invasive pest Dendroctonus valens in the Pinus tabuliformis ecosystem in northern China, a multigenic (ITS2-LSU, b-tubulin and EF1-a) phylogenetic analysis and examination of morphological features revealed in addition to Leptographium procerum the occurrence of an undescribed species. The new species, Leptographium sinoprocerum, belongs to the L. procerum-L. profanum clade. Both L. procerum and L. sinoprocerum are similar to each other and occur sympatrically in the ecosystem studied. Nevertheless L. sinoprocerum can be distinguished from L. procerum by shorter conidiophore stipes arising from both submerged and aerial hyphae, slightly more oblong conidia, a granular ornamentation on the submerged hyphae and dark olivaceous colonies on MEA. © 2008 by The Mycological Society of America.

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33. **Progress in study on the fungi associated with Dendroctonus valens.**

LueQuan, Zhang XingYao, Yang ZhongQi, H. Maraite, Yin DeShan, Ren XiaoHong, Q. Lue, X. Y. Zhang, Z. Q. Yang, D. S. Yin and X. H. Ren.

Research Institute of Forest Ecology, Environment and Protection, CAF, Key Lab of Forest Ecology and Protection of SFA, Beijing 100091, China.

Scientia Silvae Sinicae 2008, 44 (2): 134-142

English

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34. **In-vitro antifungal activity of chilli extracts in combination with Lactobacillus casei against common sapstain fungi**

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Int.Biodeterior.Biodegrad. 2008, 62 (4): 364-367

English

ABSTRACT The efficacy of chilli juice and/or chilli (capsicum) extract oleoresins as antisapstain agents was evaluated against two common wood-discolouring fungi, Sphaeropsis sapinea and Leptographium procerum. Possible synergy between chilli juice and Lactobacillus casei as

antisapstain agents was also assessed. Both the chilli juice and the capsicum oleoresin showed moderate antifungal activity. No growth of the test fungi was observed on plates amended with 50% chilli juice after 3 weeks of incubation. In the presence of 0.1% oleoresins, fungal biomass was reduced by more than half when compared with controls. The synergy between chilli and L. casei was apparent; the combination chilli/L. casei treatment system afforded much better inhibition than chilli or L. casei alone. In the presence of 25% chilli juice with L. casei the growth of test fungi was stopped. © 2008 Elsevier Ltd. All rights reserved.

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35. **Leptographium bhutanense sp. nov., associated with the root collar weevil Hylobitelus chenkupdorjii on Pinus wallichiana in Bhutan**

X. D. Zhou, K. Jacobs, T. Kirisits, D. B. Chhetri and M. J. Wingfield.

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Persoonia: Mol.Phylogeny Evol.Fungi 2008, 21 1-8

English

ABSTRACT Leptographium spp. are commonly associated with bark beetles and weevils (Coleoptera: Curculionidae), and some are important tree pathogens. In a recent survey of diseases and insect pests of conifer trees in Bhutan, the root collar weevil, Hylobitelus chenkupdorjii was found girdling young Himalayan blue pine (Pinus wallichiana) trees in Central Bhutan. Intensive wood staining and a Leptographium sp. were associated with damage by this insect. The fungus was also isolated from individuals of H. chenkupdorjii. It was tentatively identified based on morphology and then compared with other Leptographium spp. using DNA sequences for three gene regions. Morphological characteristics showed that the Leptographium sp. from H. chenkupdorjii is similar to, but distinct from L. procerum and L. profanum. DNA sequence comparisons revealed that the isolates from Bhutan resided in a distinct well-supported clade and confirmed that they represent an undescribed taxon for which the name Leptographium bhutanense sp. nov. is provided. © 2008 Nationaal Herbarium Nederland Centraalbureau voor Schimmelcultures.

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36. **Insect-fungal complex associated with loblolly pine decline in central Alabama**

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Affiliation: USDA, United States Forest Service, Forest Health Protection, Pineville, LA 71360, United States; Correspondence Address: Eckhardt, L.G.; School of Forestry and Wildlife Sciences, Auburn University, AL 36849, United States; email: eckhalg@auburn.edu

For.Sci. 2007, 53 (1): 84-92

English

ABSTRACT Loblolly pine decline, characterized by an expanding area of declining and dead trees, is becoming increasingly prevalent in loblolly pine (Pinus taeda L.) forests in central Alabama. A 3-year study was conducted to determine the fungal, root, and lower stem-infesting insect, and/or soil parameters associated with this decline. Hylastes salebrosus, Hylastes tenuis, Pachylobius picivorus, and Hylobius pales were significantly more abundant in declining plots than in asymptomatic plots. Root- and lower stem-infesting insects consistently carried Leptographium terebrantis, L. procerum, and L. serpens. Sampled roots had high levels of root damage, mortality, and staining typically associated with Leptographium species. Root damage and mortality preceded aboveground symptoms of short chlorotic needles, sparse crowns, reduced radial growth, and tree mortality. A sequence of biotic and abiotic factors is proposed as the cause of loblolly pine decline complex.
Copyright © 2007 by the Society of American Foresters.

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37. **Contribution to pathogenicity of three blue-stain fungi associated with the pine sawyer beetle (Monochamus galloprovincialis) (Coleoptera: Cerambycidae) to Scots pine in Poland.**

R. Jankowiak, R. Rossa and P. Bilanski.

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Phytopathologia Polonica 2007, (46): 37-46

Polish

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38. **Fungi associated with Tomicus piniperda in Poland and assessment of their virulence using Scots pine seedlings**

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Affiliation: Agriculture University of Cracow, Department of Forest Pathology, Al. 29 Listopada 46, 31-425 Cracow, Poland; Correspondence Address: Jankowiak, R.; Agriculture University of Cracow, Department of Forest Pathology, Al. 29 Listopada 46, 31-425 Cracow, Poland; email: rljankow@cyf-kr.edu.pl

Ann.Forest Sci. 2006, 63 (7): 801-808

English

ABSTRACT The species composition and virulence of fungi associated with Tomicus piniperda were studied at eight locations in Poland. The fungi were isolated from phloem and sapwood samples taken from insect galleries and then identified. The virulence of the most common ophiostomatoid species was evaluated through inoculations using two-year-old Scots pine seedlings. A great diversity of fungi were found associated with T. piniperda, including 4 837 cultures and 67 species. The most important groups of fungi were the ophiostomatoids and moulds, including mainly Penicillium, Trichoderma and Mucor species. Among ophiostomatoid fungi, Ophiostoma minus and O. piceae dominated, with a frequency of occurrence of 32.4 and 11.5% of inspected galleries, respectively. Occasionally isolated species included Leptographium lundbergii, L. procerum, L. wingfieldii, Graphium pycnocephalum and Graphium sp. 'W'. In general, the frequency of the ophiostomatoid species was highly variable among locations. Leptographium wingfieldii and O. minus, were the only species capable of killing whole plants and penetrated deeper into the sapwood than other species (87-100% mortality during the 11 week incubation period). Other fungi, including O. piliferum, O. piceae and L. procerum, were considerably less virulent. © INRA, EDP Sciences, 2006.

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39. **The early stages of fungal succession in Pinus sylvestris phloem and sapwood infested by Tomicus piniperda.**

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Dendrobiology 2006, 56 27-36

English

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40. **Effects of fire and mechanical wounding on Pinus resinosa resin defenses, beetle attacks, and pathogens.**

Maria J. Lombardero, Matthew P. Ayres and Bruce D. Ayres.

Univ Santiago, Dept Prod Vegetal, EPS Campus Lugo, Lugo 27002, Spain Flombard@lugo.usc.es

For.Ecol.Manage. 2006, 225 (1-3): 349-358

English

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41. **Survey of Ophiostomataceae associated with Hylurgus ligniperda (Curculionidae: Scolytinae) in New Zealand**

S. D. Reay, J. M. Thwaites and R. L. Farrell.

Department of Biological Sciences, The University of Waikato, Private Bag 3105, Hamilton,New Zealand EMAIL: s.d.reay@xtra.co.nz

N. Z. Entomol. 2006, 29 21-26

English

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42. **Wood protection. Bioactive research at Ensis.**

Tripti Singh, C. Chittenden, Adya Singh, T. Singh and A. Singh.

Wood Processing Newsletter 2006, (38): 1-2

English

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43. **Fungi vectored by the introduced bark beetle Tomicus piniperda in Ontario, Canada, and comments on the taxonomy of Leptographium lundbergii, Leptographium terebrantis, Leptographium truncatum, and Leptographium wingfieldii**

G. Hausner, M. Iranpour, J. -J Kim, C. Breuil, C. N. Davis, E. A. Gibb, J. Reid, P. C. Loewen and A. A. Hopkin.

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Can.J.Bot. 2005, 83 (10): 1222-1237

English

ABSTRACT Fungi isolated from Tomicus piniperda (L.) galleries in infected trap logs, standing trees, and directly from insects were identified using morphological features and molecular data obtained from the mitochondrial and nuclear DNA region. Identified strains represented Leptographium wingfieldii Morelet, Leptographium procerum (Kendr.) Wingf., Leptographium lundbergii Lag. & Melin sensu Jacobs & Wingfield, Ophiostoma ips (Rumb.) Nannf., Ophiostoma minus (Hedg.) H. & P. Syd., and Sphaeropsis sapinea sensu lato. Leptographium wingfieldii is believed to be a potentially pathogenic introduced fungus, but sequence data suggest a possible connection between it and the teleomorph of Ophiostoma aureum (Robinson-Jeffrey & Davids.) T.C. Harrington (reported from British Columbia and the western United States). Our data also show that the ex-type culture of Leptographium terebrantis Barras & Perry, a species very similar morphologically to L. wingfieldii, also grouped with L. wingfieldii. We also identified strains of

Leptographium truncatum (Wingf. & Marasas) Wingf.; this species has been synonymized with L. lundbergii, but our data indicate that these are distinct species, and therefore, the name L. truncatum should be reinstated. We also report the extended presence of L. procerum in Ontario. Previously viewed as a "southern" species frequently associated with pine-root decline diseases, it has been infrequently reported from New York state and but once each from Ontario and Quebec. © 2005 NRC.

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44. **Ophiostomatoid fungi isolated from Pinus radiata logs imported from New Zealand to Korea**

G. -H Kim, J. -J Kim, W. L. Young and C. Breuil.

Affiliation: Division of Environmental Science and Ecological Engineering, Korea University, Seoul 136-701, South Korea; Affiliation: Department of Wood Science, University of British Columbia, Vancouver, BC V6T 1Z4, Canada; Correspondence Address: Kim, J.-J.; Department of Wood Science, University of British Columbia, Vancouver, BC V6T 1Z4, Canada; email: jaekim@interchange.ubc.ca

Can.J.Bot. 2005, 83 (3): 272-278

English

ABSTRACT Ophiostomatoid fungi discolor lumber, logs, and tree sapwood. Stained wood has a lower market value and can be refused by importing customers because such products can potentially carry pathogenic fungi. Little information is available on the ophiostomatoid fungi that colonize Pinus radiata D. Don (radiata pine) logs imported from New Zealand into Korea. In this work, we attempted to identify the native and non-native fungi colonizing wood imported into Korea. At least 12 species of ophiostomatoid fungi, including an unknown Ophiostoma sp. A, were identified among the fungi-staining radiata pine. They were Leptographium procerum (Kendr.) Wingf., Leptographium bistatum Kim & Kim, Ophiostoma floccosum Mathiesen, Ophiostoma huntii (Rob.) Hoog & Scheff., Ophiostoma ips (Rumbold) Nannf., Ophiostoma nigrocarpum (David.) Hoog, Ophiostoma piceae (Münch) H. & P. Sydow, Ophiostoma piliferum (Fries) H. & P. Sydow, Ophiostoma quercus (Georgév.) Nannf., Ophiostoma radiaticola Kim et al., and Ophiostoma setosum Uzunovic et al. Of these species, O. floccosum was the dominant species in both logs and boards. We confirmed that many of the sapstain species that we isolated have been previously reported in Korea. However, four species, O. radiatcola, O. setosum, O. huntii, and O. nigrocarpum have not been reported previously in Korea. We also found the new species, L. bistatum, along with an unknown Ophiostoma sp. A. © 2005 NRC Canada.

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45. **A new Leptographium species associated with Tomicus piniperda infesting pine logs in Korea**

Jae-Jin Kim, Young Woon Lim, Colette Breuil, Michael J. Wingfield, Xu Dong Zhou and Gyu-Hyeok Kim.

Department of Wood Science, University of British Columbia, Vancouver, V6T 1Z4, Can.

Mycol.Res. 2005, 109 (3): 275-284

English

46. **Frequency of blue staining fungi isolated from pine trees of Experimental Forests in Kangwon National University and its resistance to fungicide, Woodguard.**

N. Pashenova, Lee JongKyu, Cho NamSeok, J. K. Lee and N. S. Cho.

Department of Physical and Chemical Biology and Biotechnology of Woody Plants, V.N. Sukachev Institute of Forest SB RAS, Krasnoyarsk, Russia. EMAIL: nscho@chungbuk.ac.kr

Mokchae Konghak = Journal of the Korean Wood Science and Technology 2005, 33 (2): 56-64,-64,

English

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47. **A survey of Ophiostoma species vectored by Hylastes ater to pine seedlings in New Zealand**

S. D. Reay, J. M. Thwaites and R. L. Farrell.

Affiliation: Department of Biological Sciences, University of Waikato, Private Bag 3105, Hamilton, New Zealand; Correspondence Address: Reay, S.D.; Department of Biological Sciences, University of Waikato, Private Bag 3105, Hamilton, New Zealand; email: sreay@clear.net.nz

For.Pathol. 2005, 35 (2): 105-113

English

ABSTRACT Ecological studies in New Zealand require consideration of the impact of exotic (introduced) organisms from disparate environments. A survey of Ophiostoma species isolated and identified from Pinus radiata stumps, the bark beetle Hylastes ater collected from these stumps and from P. radiata seedlings following sublethal damage by H. ater, was undertaken in 10 reforestation sites in New Zealand. Nine Ophiostoma species were isolated and identified in this survey. Ophiostoma galeiformis and Ophiostoma huntii were isolated most frequently from all the substrates sampled. Ophiostoma floccosum, Ophiostoma setosum, Leptographium procerum and Leptographium truncatum were also isolated from stumps, damaged seedlings and beetles. Ophiostoma quercus was only isolated from stumps and beetles and Ophiostoma ips only from beetles. A strong relationship between the severity of H. ater feeding damage, and the presence and number of species of Ophiostoma was found. Eight Ophiostoma species were transmitted by H. ater to P. radiata seedlings in laboratory transmission experiments. From the results of this field survey and the laboratory experiments we confirm that H. ater is a vector of Ophiostoma species to P. radiata seedlings in New Zealand. © 2005 Blackwell Verlag.

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48. **Erratum: Safety evaluation of phosphodiesterase derived from Leptographium procerum (Food and Chemical Toxicology (2004) 42 (935-944) DOI: 10.1016/j.fct.2004.01.017)**

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Food Chem.Toxicol. 2005, 43 (10): 1567

English

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49. **Survey of potential sapstain fungi on Pinus radiata in New Zealand**

J. M. Thwaites, R. L. Farrell, S. M. Duncan, S. D. Reay, R. A. Blanchette, E. Hadar, Y. Hadar, T. C. Harrington and D. McNew.

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New Zealand J.Bot. 2005, 43 (3): 653-663

English

ABSTRACT A nationwide survey of New Zealand sapstain fungi on Pinus radiata was undertaken between 1996 and 1998 with collections of 1958 samples of material from 869 sites in the North and South Islands. Material was collected from mills, ports, forest plantations of native, exotic, or P. radiata, nurseries, farms, and urban areas. Material collected included branches, twigs, needles or leaves, cones, logs, wood chips, timber, and veneer. From these collections, 2154 potential sapstain fungi, representing 14 known species plus a number of unidentified species, were isolated. The predominant sapstain fungi isolated were Sphaeropsis sapinea, Ophiostoma ips, O. floccosum, O. piliferum, Leptographium procerum, and O. querci. S. sapinea was isolated from all material sampled including collections from the forest floor (including branches, twigs, needles, leaves, cones, and logs) as well as from logs and timber. In contrast Ophiostoma species were mainly found on logs, timber, and wood chips. © The Royal Society of New Zealand 2005.

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50. **Advances in understanding bioactivity of chitosan and chitosan oligomers against selected wood-inhabiting fungi**

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Zealand; email: kirk.torr@scionresearch.com

Holzforschung 2005, 59 (5): 559-567

English

ABSTRACT Nitrous acid deaminative depolymerisation was used to prepare three chitosan oligomer (CO) mixtures from highmolecular weight chitosan. These mixtures of chitosan oligosaccharides were analysed by electrospray ionisation mass spectroscopy, potentiometric titration and gel permeation chromatography. A method based on potentiometric titration of the amino groups of the oligomers gave an average degree of polymerisation (DP) for the three preparations of 5 (CODP5), 9 (CODP9) and 14 (CODP14). Chitosan acetate and the chitosan oligomer mixtures were assayed against Leptographium procerum, Sphaeropsis sapinea and Trichoderma harzianum on nutrient media. Leptographium procerum and S. sapinea growth was prevented by chitosan acetate and chitosan oligomers at concentrations of 0.3-0.4% (w/v), whereas T. harzianum was able to overcome the fungistatic action of these compounds. The oligomer preparation CODP14 exhibited superior specific activity to both CODP5 and chitosan acetate, suggesting an optimum molecular weight for bioactivity. All oligomer preparations were more effective at pH 4 than at pH 6. This result, in combination with the inactivity of N-acetylated CODP14, indicated that amino group protonation was an important factor for fungistatic activity. The CODP14 preparation was reduced with sodium borohydride and fractionated by alkali precipitation and ion exchange chromatography. Bioassays of these fractions pointed towards DP and degree of deacetylation (DD) as key factors in chito-oligosaccharide bioactivity. Conversely, the terminal aldehyde groups generated by depolymerisation did not contribute to the activity observed. Copyright © by Walter de Gruyter.

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51. **Pathogenicity of Leptographium species associated with loblolly pine decline**

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Affiliation: Dept. Plant Pathol. Crop Physiol., LA Agricultural Experiment Station, LA State Univ. Agricultural Center, Baton Rouge, LA 70803, United States; Affiliation: United States Dept. of Agricultural, United States Forest Service, Southern Research Station, Pineville, LA 71360, United States; Correspondence Address: Eckhardt, L.G.; Dept. Plant Pathol. Crop Physiol., LA Agricultural Experiment Station, LA State Univ. Agricultural Center, Baton Rouge, LA 70803, United States; email: LeptoGirl03@aol.com

Plant Dis. 2004, 88 (11): 1174-1178

English

ABSTRACT Freshly lifted seedlings and 21-year-old trees of loblolly pine were wound-inoculated with Leptographium species recovered from the soil and/or roots of trees with loblolly decline symptoms in central Alabama. Seedlings inoculated with L. procerum in the greenhouse produced significantly fewer root initials and a smaller root mass than control seedlings. Vertical lesions produced in seedlings by L. serpens and L. terebrantis were significantly longer than in controls. Lesions produced in mature trees by L. serpens and L. lundbergii were significantly longer than in controls. Of the fungi tested, L. serpens, L. terebrantis, and L. lundbergii were the most aggressive and may pose the greatest threat to loblolly pines.

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52. **Safety evaluation of phosphodiesterase derived from Leptographium procerum**

A. Steensma, P. W. M. Van Dijck and R. A. Hempenius.

Affiliation: DSM Food Specialties B.V., PO Box 1, 2600 MA, Delft, Netherlands; Correspondence Address: Hempenius, R.A.; DSM Food Specialties B.V., PO Box 1, 2600 MA, Delft, Netherlands; email: rixta.hempenius@dsm.com

Food Chem.Toxicol. 2004, 42 (6): 935-944

English

ABSTRACT 5'-Phosphodiesterase is produced by fermentation of the fungus Leptographium procerum and is used to hydrolyse yeast RNA to produce flavour enhancers. To establish the safety in use of this enzyme preparation a number of studies have been performed: analysis for the potential of the production strain to produce toxic secondary metabolites, 28-days oral toxicity study of the preparation in the rat, bacterial mutation assay and in vitro mammalian chromosome aberration test in human lymphocytes. The production strain did not produce any secondary metabolites that may be of significance in food. Administration of dosage levels of 1250, 2500 and 5000 mg/kg body weight/day to rats for 28 day did not result in any toxicological significant changes. The enzyme preparation showed no mutagenic activity in the bacterial mutation assay and no clastogenic potency in an in vitro test. These results together with existing knowledge of the production organism and the chemical and microbiological characterisation of the enzyme preparation lead to the conclusion that the enzyme preparation containing 5'-phosphodiesterase activity from Leptographium procerum can safely be used for the production of flavour enhancers from bakers yeast at the anticipated intake levels for these uses. © 2004 Elsevier Ltd. All rights reserved.

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53. **Fungi associated with the European pine shoot beetle, Tomicus piniperda, in southern Ontario.**

G. Hausner, C. N. Davis, E. A. Gibb, J. Reid and A. A. Hopkin.
Canadian Journal of Plant PathologyDecember 2003
Department of Microbiology, University of Manitoba, Winnipeg, MB, R3T 2N2, Canada

Abstr, Annual Meeting of the Canadian Phytopathological Society 2003, 25 (4): 425

English

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54. **Albino strains of Ophiostoma species for biological control of sapstaining fungi**

B. W. Held, J. M. Thwaites, R. L. Farrell and R. A. Blanchette.

Affiliation: Department of Plant Pathology, University of Minnesota, 1991 Upper Buford Circle, St.

Paul, MN 55108-6030, United States; Affiliation: Department of Biological Sciences, University of Waikato, Hamilton 2001, New Zealand; Correspondence Address: Blanchette, R.A.; Department of Plant Pathology, University of Minnesota, 1991 Upper Buford Circle, St. Paul, MN 55108-6030, United States; email: robertb@umn.edu

Holzforschung 2003, 57 (3): 237-242

English

ABSTRACT Albino strains of Ophiostoma floccosum, O. piceae and O. pluriannulatum were selected and screened for biological control of sapstaining fungi on New Zealand radiata pine (Pinus radiata). Albino strains were obtained through matings and single ascospore isolations from cultures of prevalent species in New Zealand. These strains do not synthesize the melanin-like hyphal pigments of common sapstaining fungi. Additional mating studies were also carried out to obtain isolates of O. piceae and O. floccosum that lacked pigmented fruiting structures. Several albino isolates of O. piceae with colorless synnemata and isolates of O. floccosum with little to no synnemata were obtained. Biological control potential of the albino isolates was evaluated in the laboratory by challenging them on wood chips with fungi that cause extensive sapstain in pine, Leptographium procerum, Ophiostoma piliferum and Sphaeropsis sapinea. Many albino isolates of O. floccosum, O. piceae and O. pluriannulatum were effective in stopping the challenge fungi from staining wood chips and were fast growing and colorless when grown unchallenged on wood chips. Selected albino isolates of O. pluriannulatum were used in two field trials in New Zealand to control sapstain. Several strains were found to significantly reduce dark sapstain as compared to the untreated control logs.

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55. **Cellular response of loblolly pine to wound inoculation with bark beetle-associated fungi and chitosan.**

K. D. Klepzig and C. H. Walkinshaw.

Southern Research Station, USDA Forest Service, Pineville, LA 71360, USA.

Research Paper - Southern Research Station, USDA Forest Service 2003, (SRS-30): 9

English

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56. **Effects of New Zealand sapstaining fungi on structural integrity of unseasoned radiata pine**

A. Schirp, R. L. Farrell and B. Kreber.

Affiliation: Department of Biological Sciences, University of Waikato, Hamilton, New Zealand; Affiliation: Forest Research, Rotorua, New Zealand; Affiliation: Wood Mat. and Engineering Laboratory, Washington State University, Pullman, WA, United States; Correspondence Address: Schirp, A.; Wood Mat. and Engineering Laboratory, Washington State University, Pullman, WA, United States; email: schirp@wsu.edu

Holz Roh Werkst 2003, 61 (5): 369-376

English

ABSTRACT Four sets of green, side-matched specimens of radiata pine (Pinns radiata D. Don) sapwood were incubated with individual isolates of Ophiostoma floccosum, O. pluriannulatum, O. ips, O. piceae, Leptographium procerum and Sphaeropsis sapinea for 8 or 16 weeks, along with non-inoculated control samples. Three decay fungi, Gloeophyllum trabeum, Phlebiopsis gigantea and Schizophyllum commune, were included in the tests to serve as references for weight and toughness loss. Overall toughness and dry weight were not significantly ($p<0.05$) different between wood specimens inoculated with sapstaining fungi and the controls, except for O. ips #308 causing 18% toughness loss, but not weight loss, in one out of three experiments. In contrast, G. trabeum reduced toughness by 61% and caused a weight loss of 8% whereas S. commune and P. gigantea produced toughness losses of 32% and 16%, respectively, without any significant weight losses. None of the sapstaining fungi tested degraded lignin or structural carbohydrates in radiata pine sapwood after 16 weeks' incubation. Of the isolates tested for their ability to degrade extractives O. floccosum was most effective (54% reduction) and O. pluriannulatum least (0% reduction) effective.

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57. **Bioactivity of intra and extracellular substances from cyanobacteria and lactic acid bacteria on "wood blue stain" fungi**

G. Zulpa, M. C. Zaccaro, F. Boccazzi, J. L. Parada and M. Storni.

Affiliation: Depto. de Biodiversidad y Biol. Exp., Lab. Fisiol. Veg. Biol. C., Int. Güiraldes 2620 (C1428EHA), Ciudad de Buenos Aires, Argentina; Correspondence Address: Zulpa, G.; Depto. de Biodiversidad y Biol. Exp., Lab. Fisiol. Veg. Biol. C., Int. Güiraldes 2620 (C1428EHA), Ciudad de Buenos Aires, Argentina; email: cyanob@bg.fcen.uba.ar

Biol.Control 2003, 27 (3): 345-348

English

ABSTRACT Cyanobacteria (photoautotrophic prokariota) have potential for the control of pathogenic bacteria and fungi. The effect of intra and extracellular products from cyanobacterial strains on the growth of fungi isolated from "wood blue stain," was tested. Extracellular products were obtained by concentration and sterilization of the culture medium where cyanobacteria were grown. Cyanobacterial substances promoted or inhibited fungal growth according to the fungal and cyanobacterial strains tested. Extracellular products from Nostoc muscorum 79a and the methanolic extract from Microchaete tenera 84b biomass inhibited growth of Sphaeropsis sapinea 2157 (64.7 and 775.6%, respectively). Extracellular products of Nostoc piscinale 59 and biomass methanolic extract from N. muscorum 79a produced the highest growth promotion of Trichoderma boningii 452 (105.0%) and T. viride 993 (136.7%). Extracellular products of the heterotrophic lactic acid bacterium Streptococcus termophilus were also tested and strongly inhibited (64-92%) all the fungal strains. The tested fungi have different sensitivity to the bioactive substances present in the biomass and/or the culture medium of the studied cyanobacteria and lactic acid bacterium. N. muscorum 79a, M. tenera 84b, and S. termophilus have potential to control the wood blue stain fungi by a friendly environmental alternative. © 2003 Elsevier Science (USA). All rights reserved.

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58. **Association of declining red pine stands with reduced populations of bark beetle predators, seasonal increases in root colonizing insects, and incidence of root pathogens**

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For.Ecol.Manage. 2002, 164 (1-3): 221-236

English

ABSTRACT Declining red pine, Pinus resinosa (Aitman), stands in the Great Lakes region appear to arise from a complex of biotic and abiotic factors. We monitored stem and root colonizing beetles in declining and healthy plantations in Wisconsin from 1997 to 1999. We also conducted systematic field excavations and laboratory isolations of various root pathogens. Multiple funnel traps were baited with the synthetic aggregation pheromones of the two most common bark beetle (Coleoptera: Scolytidae) species in the region, Ips pini (Say) and Ips grandicollis (Eichhoff). Lower-stem flight traps were baited with 1:1 (+)-α-pinene:75% ethanol, and pitfall traps were baited with 1:1 (-)-α-pinene:75% ethanol. Healthy stands had higher populations of predators, particularly Thanasimus dubius (F.) (Coleoptera: Cleridae), Platysoma cylindrica (Paykull) (Coleoptera: Histeridae), and Platysoma parallelum Say than declining stands. Seasonal abundance patterns of predators and Ips also varied between declining and healthy stands. Declining stands had higher numbers of Ips and lower numbers of predators early in the season, whereas healthy stands had higher predator populations in the early season. Declining stands had more lower stem infesting bark beetles Dendroctonus valens LeConte (Scolytidae) and weevils (Curculionidae) Pissodes spp. Surprisingly, healthy stands had more root weevils, Hylobius pales (Herbst) and Hylobius radicis Buchanan, and root bark beetle Hylastes porculus Erichson, over the entire season. However, spatial by temporal patterns again were important. Populations of root colonizing insects were higher in healthy stands early in the season, but higher in declining stands throughout much of the ovipositional period. These results suggest dispersal patterns and overwintering behaviors are important in the interactions among herbivores, predators, and host plant condition in Red Pine Decline. The principal fungi isolated from roots were Leptographium procerum (Kendr.) Wingfield and Leptographium terebrantis Barras & Perry. These were more prevalent in declining than healthy stands. Staining fungi were more frequently isolated from roots of trees at the pocket margin than from trees in the asymptomatic portion of declining stands. Heterobasidion annosum (Fr.) Bref. was not isolated from root samples or stem disks, despite 40% recovery in positive controls using the latter method. Likewise, Armillaria spp. were not associated with declining plantations. Our results further support the view that forest declines are due to complex interactions among multiple biotic and abiotic stresses, and exhibit particular spatial and temporal patterns. © 2002 Elsevier Science B.V. All rights reserved.

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59. **Three new species of Leptographium from pine**

K. Jacobs, M. J. Wingfield, A. Uzunovic and S. Frisullo.

Affiliation: Department of Microbiology, Forestry and Agric. Biotech. Inst., Pretoria 0002, South Africa; Correspondence Address: Jacobs, K.; Department of Microbiology, Forestry and Agric. Biotech. Inst., Pretoria 0002, South Africa; email: jacobsk@em.agr.ca

Mycol.Res. 2001, 105 (4): 490-499

English

ABSTRACT Leptographium species are common inhabitants of fresh conifer logs and lumber that are known for their ability to cause blue-stain and, in some cases, their association with disease. L. procerum has been associated with a root disease although controversy surrounds its role in tree death. During the course of the past two decades, a relatively large number of isolates tentatively identified as L. procerum have been collected in various parts of the world. Some of these display morphological characters unlike those of L. procerum s. str. and this has prompted us to re-examine them. Four groups of morphologically distinct isolates were identified, of which L. procerum s. str. represented one. The remaining isolates of Leptographium are newly described as L. alethinum, L. pityophilum and L. euphyes spp. nov.

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60. **Ophiostomatoid fungi associated with three pine-infesting bark beetles in South Africa.**

Xu Dong Zhou, Z. Wilhelm de Beer, Brenda D. Wingfield and Michael J. Wingfield.

Tree Pathology Co-operative Programme (TPCP), Department of Genetics, University of Pretoria, Pretoria, 0002, South Africa

Sydowia 2001, 53 (2): 290-300

English

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61. **Influence of procerum root disease on the water relations of eastern white pine (Pinus strobus L.).**

J. R. Butnor, J. R. Seiler and J. A. Gray.

Southern Research Station, USDA Forest Service, 3041 Cornwallis Road, Research Triangle Park, NC 27709, USA.

Journal of Sustainable Forestry 2000, 10 (1/2): 95-105

English

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62. **On the phylogeny of members of Ceratocystis s.s. and Ophiostoma that possess different anamorphic states, with emphasis on the anamorph genus Leptographium, based on partial ribosomal DNA sequences**

G. Hausner, J. Reid and G. R. Klassen.

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Can.J.Bot. 2000, 78 (7): 903-916

English

ABSTRACT Phylogenetic analysis of partial ribosomal DNA sequences was undertaken to assess the phylogenetic distribution of ophiostomatoid anamorphs with an emphasis on members of Leptographium and the generic limits of Ceratocystis sensu lato Ell. and Halst. Molecular data identified one group of Ophiostoma species with anamorphs assignable to Pesotum and Leptographium that appears to be monophyletic. This study also showed that, based on molecular characters, Pesotum pini (Hutchison and Reid) Okada and all Leptographium strains tested, including L. terebrantis Barras and Perry, L. lundbergii Lag. and Melin, and L. procerum (Kendr.) Wingf., could be placed among Ophiostoma species. With regard to the anamorphs of Ophiostoma, we determined that the separation of Leptographium from Pesotum, although convenient, is artificial. The partial ribosomal DNA sequences also confirm that Europhium should be considered a synonym of Ophiostoma. The phylogenetic position of species of Ceratocystis sensu lato with Chalara-like, Knoxdaviesia, and Gabarnaudia anamorphs is also discussed.

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63. **Effect of methylene bisthiocyanate vapour on selected wood deteriorating fungi.**

Bernhard Kreber.

Forest Research, Rotorua, New Zealand

Material und Organismen (Berlin) 2000, 33 (3): 187-199

English

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64. **Ophiostoma and Leptographium species isolated from the stained sapwoods of Pinus species.**

Eun Sung Oh and Jong Kyu Lee.

Department of Forest Resources Protection, Kangwon National University, Chuncheon, South Korea

Mycobiology 2000, 28 (4): 204

English

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65. **Capability of staining fungi to cause structural changes in New Zealand radiata pine: toughness testing and enzyme production.**

A. Schirp, R. L. Farrell and B. Kreber.

Department of Biological Sciences, University of Waikato, Private Bag 3105, Hamilton, New Zealand.

Maderas: Ciencia y Technologia 2000, 2 119-129

English

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66. **Root-infecting fungi associated with a decline of longleaf pine in the southeastern United States**

W. J. Otrosina, D. Bannwart and R. W. Roncadori.

Affiliation: USDA Forest Service, Southern Research Station, Tree Root Biology, 320 Green Street, Athens, GA 30602, United States; Affiliation: University of Georgia, Department of Plant Pathology, Athens, GA 30602, United States; Correspondence Address: Otrosina, W.J.; USDA Forest Service, Southern Research Station, Tree Root Biology, 320 Green Street, Athens, GA 30602, United States; email: otrosina@negia.net

Plant Soil 1999, 217 (1-2): 145-150

English

ABSTRACT A 35-year-old longleaf pine stand exhibited trees in various stages of decline. A study was conducted to determine root-infecting fungi and other abnormalities associated with varying degrees of crown symptoms. A four-class crown symptom rating system was devised according to ascending symptom severity. Leptographium procerum and L. terebrantis were significantly associated with increasing crown symptom severity. Heterobasidion annosum was also isolated in higher frequency as crown symptoms increased. Also, evidence of insects on roots increased as did amount of resinosis observed. Edaphic and silvicultural factors may interact with these pathogens and insects to pose a pathological limitation on longer-term management objectives. Further research is needed to determine relationships among various edaphic, silvicultural, and biological factors associated with the decline syndrome on this site.

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67. **Localisation of fungal hyphae in wood using immunofluorescence labelling and confocal laser scanning microscopy**

Y. Xiao, B. Kreber and C. Breuil.

Affiliation: Manufacturing Technologies, Sapstain Control, Forest Research, Rotorua, New Zealand; Affiliation: Forest Products Biotechnology, Fac. Forest., Univ. Brit. Columbia, Vancouver, BC, Canada; Correspondence Address: Xiao, Y.; Manufacturing Technologies, Sapstain Control, Forest Research, Rotorua, New Zealand

Int.Biodeterior.Biodegrad. 1999, 44 (4): 185-190

English

ABSTRACT This study explored the feasibility of using immunofluorescence labelling in conjunction with confocal laser scanning microscopy (CLSM) for detection of common fungal colonisers of unseasoned radiata pine in New Zealand. Wood sections infected with Ophiostoma piceae were treated with monoclonal antibody IF3 (1), and then Oregon green 514 goat anti-mouse IgG, a fluorescent secondary antibody. Additional wood sections infected with other Ophiostoma spp., Sphaeropsis sapinea. Leptographium procerum, Trichoderma sp. and Phlebiopsis gigantea were treated similarly to determine whether the antibody was specific to O. piceae or was recognising other fungal species. Sections were examined using phase contrast and fluorescence light microscopy prior to CLSM. Immunolabelled fungal hyphae showed relatively weak fluorescence compared to the strong autofluorescence of wood cell walls and extractives. Labelled hyphae of O. piceae were detected in wood using CLSM but not with ordinary fluorescence microscopy. This is because CLSM has stronger illumination power and superior imaging ability compared with ordinary fluorescence microscopy. The monoclonal antibody did not cross-react with the other Ophiostoma species. However, non-specific antibody binding was observed with L. procerum and Trichoderma species. Furthermore, cell walls of L. procerum showed strong autofluorescence with optical properties similar to wood extractives when examined prior to incubation with the monoclonal and secondary antibody, therefore cross-reactivity tests were inconclusive for Leptographium and Trichoderma species. The current investigation demonstrated that CLSM provides possibilities for future investigations on in situ interactions of common radiata pine fungal colonisers, with one another and with wood. (C) 2000 Published by Elsevier Science Ltd.

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68. **Pathogenicity of three blue-stain fungi on four eastern United States pine species.**

D. L. Bannwart, W. J. Otrosina and R. W. Roncadori.
PhytopathologyNovember 8-12, 1998.
Univ. Georgia, Athens, GA 30602, USA

Abstr. Annual Meeting of the American Phytopathological Society 1998, 88 (9 SUPPL.): S5

English

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69. **Blue-stain fungi associated with decline of longleaf pine.**

D. L. Bannwart, W. J. Otrosina and R. W. Roncadori.
PhytopathologyNovember 8-12, 1998
Univ. Georgia, Athens, GA, USA

Abstr.Annual Meeting of the American Phytopathological Society. 1998, 88 (9 SUPPL.): S5

English

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70. **Causes of sapstain in New Zealand.**

R. L. Farrell, S. M. Duncan, A. P. Ram, S. J. Kay, E. Hadar, Y. Hadar, R. A. Blanchette, T. C. Harrington and D. Editor: Kreber McNew B.

Department of Biological Sciences, University of Waikato, Hamilton, New Zealand.

FRI Bulletin 1997, (204): 25-29

English

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71. **Biological control of sapstain in New Zealand.**

Kay, S. J. Editor: Kreber,B.

Natural Systems Group, Horticulture and Food Research Institute of New Zealand Ltd., Ruakura Research Centre, Ruakura Road, Private Bag 3123, Hamilton, New Zealand.

FRI Bulletin 1997, (204): 75-79

English

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72. **Blue-stain fungi associated with roots of southern pine trees attacked by the Southern Pine Beetle, Dendroctonus frontalis**

W. J. Otrosina, N. J. Hess, S. J. Zarnoch, T. J. Perry and J. P. Jones.

Affiliation: Supervisory Res. Plant Pathologist, USDA Forest Service, Southern Research Station, Athens, GA 30602, United States; Affiliation: Plant Pathologist, USDA Forest Service, Forest Health Staff, Pineville, LA 71360, United States; Affiliation: USDA Forest Service, Southern Research Station, Asheville, NC 2880, United States; Affiliation: USDA Forest Service, Southern Research Station, 2500 Shreveport Highway, Pineville, LA 71360, United States; Affiliation: Department of Plant Pathology, Louisiana State University, Baton Rouge 70893, United States; Correspondence Address: Otrosina, W.J.; USDA Forest Service, Southern Research Station, Athens, GA 30602, United States; email: otrosina@negia.net

Plant Dis. 1997, 81 (8): 942-945

English

ABSTRACT Forty paired plots were established from eastern Texas to Alabama to study root-infecting, bluestain fungi in southern pine stands undergoing southern pine beetle (SPB) attack. Woody roots were sampled in plots undergoing recent or current attack by the SPB. Comparisons were made between occurrence of Leptographium spp. and related fungi and data on various characteristics of natural stands and plantations studied. Three fungal species, L. terebrantis, L. procerum, and Ophiostoma ips, along with unidentified Leptographium and Graphium species, were isolated from sampled roots. L. terebrantis was isolated more frequently from SPB-attacked plots (P < 0.001) than was either L. procerum or O. ips. More blue-stain fungal species and related genera were isolated from SPB-attacked plots than from control plots (P < 0.001). This also was true for combined isolation percentages of L. terebrantis, L. procerum, and O. ips (P = 0.03). Presence of blue-stain fungi also was associated with higher stand basal area in the control plots (P = 0.045). Isolation frequencies of O. ips and L. procerum, along with the combination of these funsal species with L. terebrantis, were logistically related to increasing stand basal area in the control plots (P = 0.02, 0.02, and 0.01, respectively). No logistic relationship was found for frequency of any of the three blue-stain species with respect to basal area in SPB-attacked plots. These results suggest blue stain fungi are important in the dynamics of susceptibility of southern pines to SPB attack.

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73. **Combined chemical defenses against an insect-fungal complex**

K. D. Klepzig, E. B. Smalley and K. F. Raffa.

Affiliation: Department of Plant Pathology, University of Wisconsin-Madison, Russell Laboratories, 1630 Linden Drive, Madison, WI 53706, United States; Affiliation: Department of Entomology, University of Wisconsin-Madison, Russell Laboratories, 1630 Linden Drive, Madison, WI 53706, United States; Affiliation: USDA Forest Service, 2500 Shreveport Highway, Pineville, LA 71360, United States; Correspondence Address: Klepzig, K.D.; USDA Forest Service, 2500 Shreveport Highway, Pineville, LA 71360, United States

J.Chem.Ecol. 1996, 22 (8): 1367-1388

English

ABSTRACT This study considered how host plant allelochemicals may contribute to defense against insects and fungi that jointly colonize the subcortical tissues of trees, the relative roles of constitutive and inducible chemistry in these defenses, and how the actions of two different feeding guilds might be interrelated. Our model consisted of the coniferous tree Pinus resinosa, the root and lower stem-colonizing beetles Hylastes porculus and Dendroctonus valens, and their associated fungi Leptographium procerum and L. terebrantis, and the stem-colonizing bark beetle Ips pini and its associated fungus Ophiostoma ips. In a novel bioassay, extracts from reaction tissue elicted from wound inoculation with L. terebrantis were more repellent to bettles than were similar extracts from constitutive or mechanically wounded tissue. The effect on beetle behaviour was more pronounced in nonpolar extracts, which contain mostly monoterpenes, than in polar extracts, which contain mostly phenolics. Synthetic monoterpines at concentrations present in the various tissues exerted similar effects and were likewise repellent in dose- response experiments. Growth of L. procerum and L. terebrantis was inhibited by polar extracts from constitutive and reaction tissue. Inhibition was higher in wounded than control tissue, but the inhibition response did not vary with the type of

wounding. Synthetic monoterpenes strongly inhibited spore germination and mycelial growth of both fungi. Colonization of red pine roots by Leptographium spp. altered the subsequent effects of extracts of stem phloem tissue on L. pini. These effects varied with host condition. Beetles preferred extracts from constitutive stem phloem tissue of healthy trees to that of root-diseased trees. However, extracts from reaction tissues of healthy trees were more repellent to L. pini than were the reaction tissues of root-diseased trees. The implications of these results to plant defense against insect fungal complexes and interactions among different feeding guilds are discussed.

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74. **Interactions of ecologically similar saprogenic fungi with healthy and abiotically stressed conifers**

K. D. Klepzig, E. B. Smalley and K. F. Raffa.

Affiliation: Department of Plant Pathology, University of Wisconsin-Madison, Madison, WI 53706, United States; Affiliation: Department of Entomology, University of Wisconsin-Madison, Madison, WI 53706, United States; Affiliation: USDA Forest Service, 2500 Shreveport Highway, Pineville, LA 71360, United States; Correspondence Address: Klepzig, K.D.; USDA Forest Service, 2500 Shreveport Highway, Pineville, LA 71360, United States

For.Ecol.Manage. 1996, 86 (1-3): 163-169

English

ABSTRACT Saprogenic fungi are distinguished in part by their inability to cause disease in healthy hosts, and relatively high ability to kill or accelerate the decline of stressed hosts. We sought to determine the degree to which Leptographium terebrantis and L. procerum, ecologically similar, ophiostomatoid fungi, are saprogenic. In inoculation experiments, Leptographium terebrantis was better able to colonize roots of both mature and seedling Pinus resinosa than was L. procerum. In addition, L. terebrantis colonized roots of shade-stressed seedlings to a significantly greater extent than it colonized roots of non-stressed seedlings. L. procerum exhibited no such trend. Due to its greater virulence within stressed than healthy hosts and related traits, L. terebrantis was judged to have a relatively high degree of competitive saprogenic ability as compared with L. procerum. This study indicates the biologically important differences which may occur between closely related, ecologically similar fungi and may have implications to studies of declines and diseases with which L. terebrantis and L. procerum have been associated.

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75. **Evaluation of natural products as antifeedants for the pales weevil (Coleoptera: Curculionidae) and as fungitoxins for Leptographium procerum**

S. M. Salom, J. A. Gray, A. Randall Alford, M. Mulesky, C. J. Fettig and S. A. Woods.

Affiliation: Department of Entomology, Virginia Polytech. Inst. State Univ., Blacksburg, VA 24061-0219, United States; Affiliation: Dept. Plant Pathol., Physiol., W., Virginia Polytech. Inst. State Univ., Blacksburg, VA 24061-0331, United States; Affiliation: Dept. Appl. Ecol. and Environ. Sci., University of Maine, Orono, ME 04469-5772, United States; Correspondence Address: Salom, S.M.; Department of Entomology, Virginia Polytechnic Institute, State University, Blacksburg, VA 24061-0219, United States

J.Entomol.Sci. 1996, 31 (4): 453-465

English

ABSTRACT Four natural plant compounds (limonin, S (+) and R (-) carvone, and cucurbitacin) and one insect pheromone (verbenone) were evaluated for antifeedant activity against the pales weevil, Hylobius pales (Herbst), on Pinus strobus seedlings and for toxic activity against the pathogenic fungus, Leptographium procerum (Kendrick) Wingfield, which is vectored by H. pales to P. strobus. All compounds demonstrated significant antifeedant activity in a choice test on treated pine seedlings, but none completely eliminated feeding. Only cucurbitacin elicited a linear dose-response relationship, with significant activity occurring at concentrations as low as 0.10 µg/ml. The other compounds significantly reduced feeding at concentrations as low as 1 µg/ml (the lowest concentration at which they were tested). Total feeding activity was unaffected for all but one treatment (S (+) carvone at 1 µg/ml) when compared with feeding on the untreated control seedlings. It is, therefore, unlikely that the compounds in this study were toxic to the weevils during the 2 d evaluation period. In the fungitoxin test, all compounds except cucurbitacin suppressed germination of L. procerum spores. R (-) carvone was the most effective, allowing only 5% germination at 1 µg/ml, compared to 96% germination in the water solvent.

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76. **Leptographium root infections of pines in Florida.**

E. L. Barnard and J. R. Meeker.

FDACS, Divisions of Forestry and Plant Industry, P.O. Box 147100, Gainesville, FL 32614-7100, USA.

Plant Pathology Circular (Gainesville) 1995, (369): 3

English

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77. **Influence of tracheid conductivity and soil moisture on procerum root disease expression in Pinus strobus.**

J. R. Butnor, J. A. Gray and J. R. Seiler.
PhytopathologyAugust 12-16, 1995
VPI SU, Blacksburg, VA 24061-0330, USA

Abstr. Annual Meeting of the American Phytopathological Association 1995, 85 (10)

English

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78. **Leptographium procerum in field-grown Pinus strobus one and two years following artificial inoculation.**

J. A. Gray and S. M. Salom.
PhytopathologyAugust 12-16, 1995
VPI and State Univ., Blacksburg, VA 24061-0330, USA

Abstr. Annual Meeting of the American Phytopathological Association 1995, 85 (10)

English

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79. **Leptographium procerum in field-grown Pinus strobus one and two years following artificial inoculation**

J. A. Gray and S. M. Salom.
12-16 Jul 1995Pittsburgh, PA (USA),
VPI&SU, Blacksburg, VA 24061-0330, USA

Abstr.1995 Annual American Phytopathological Society Annual meeting 1995, (214)

English

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80. **Dendroctonus valens and Hylastes porculus (Coleoptera: Scolytidae): Vectors of pathogenic fungi (Ophiostomatales) associated with red pine decline disease.**

Kier D. Klepzig, Eugene B. Smalley and Kenneth F. Raffa.

Dep. Plant Pathol., Univ. Wisconsin-Madison, Madison, WI 53706, USA

Great Lakes Entomol. 1995, 28 (1): 81-87

English

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81. **Relationships between blue-stain fungi in roots of loblolly pine and southern pine beetle attack.**

William J. Otrosina, Nolan Hess, John P. Jones, Stanley J. Zarnoch and Thelma J. Perry.
PhytopathologyAugust 12-16, 1995
USDA Forest Serv., Inst. Tree Root Biol., 320 Green St., Athens, GA 30602, USA

Abstr. Annual Meeting of the American Phytopathological Association 1995, 85 (10)

English

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82. **PROCERUM ROOT DISEASE PHYSIOLOGY AND DISEASE INTERACTIONS WITH OZONE (ROOT DISEASE, PINUS STROBUS, LEPTOGRAPHIUM PROCERUM)**

JODI ANN [PH D.]. CARLSON and S. A. [advisor] ALEXANDER.

VIRGINIA POLYTECHNIC INSTITUTE AND STATE UNIVERSITY

Dissertation Abstracts International 1994, 55 (3B): 653

English

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83. **Short-term vector control fails to prevent procerum root disease in white pine.**

J. A. Carlson and S. A. Alexander.
PhytopathologyAugust 6-10, 1994
Dep. Plant Pathol. Physiol. Weed Sci., VPI and SU, Blacksburg, VA 24061-0330, USA

Abstr.Annual Meeting of the American Phytopathological Society 1994, 84 (10)

English

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84. **Xylophagous weevils of softwood trees: Possible vectors of phytopathogenic fungi.**

J. Levieux, D. Piou and P. Cassier.

Stn. Zool. Forest., INRA, F-45160 Olivet, France

Revue Forestiere Francaise (Nancy) 1994, 46 (4): 342-348

French

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85. **Association of phytopathogenic fungi for the Scots pine (Pinus sylvestris L.) with the European pine weevil Hylobius abietis (L.) (Col. Curculionidae)**

J. Levieux, D. Piou, P. Cassier, M. Andre and D. Guillaumin.

Affiliation: LA INRA,Univ.Orleans, 45067 Orleans, France; Correspondence Address: Levieux, J.France

Can.Entomol. 1994, 126 (4): 929-936

English

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86. **Root pathogens isolated from trees in forest health monitoring program surveys.**

M. Baldwin, J. A. Carlson and S. A. Alexander.
PhytopathologyNovember 6-10, 1993
Dep. Plant Pathol. Physiol. Weed Sci., VPI and SU, Blacksburg, VA 24061-0330, USA

Abstr. Joint Meeting of the American Phytopathological Society and the Society of Nematologists on Plant Pathology Beyond 2000 1993, 83 (12)

English

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87. **Possible role of Hylobius abietis (L) in the transport of Leptographium procerum (Kendr) Wingf and in the infection of Scots pine**

D. Piou.

Affiliation: ENGREF, Arboretum national des Barres, 45290 Bogent-sur-Vernisson, France.

Ann.Sci.For. 1993, 50 (3): 297-308

French

ABSTRACT Experiments suggest that L. procerum could increase the mortality rate of seedlings after partial feeding damage by the pine weevil. -from English summary

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88. **Root anatomy and microflora of pines attacked by the southern pine beetle.**

C. H. Walkinshaw, N. J. Hess and T. J. Perry.
PhytopathologyNovember 6-10, 1993
USDA Forest Serv., Box 5500, Pineville, LA 71361, USA

Abstr. Joint Meeting of the American Phytopathological Society and the Society of Nematologists on Plant Pathology Beyond 2000 1993, 83 (12)

English

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89. **Physiological effects of procerum root disease of eastern white pine Christmas trees.**

J. A. Carlson and S. A. Alexander.

Dep. Plant Pathol. Physiol. Weed Sci., VPI and SU, Blacksburg, Va. 24061-0330, USA

Phytopathology 1992, 82 (10): 1117 Meeting Info: Annual Meeting of the American Phytopathological Society, Portland, Oregon, USA, August 8

English

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90. **LEPTOGRAPHIUM.**
METHODS FOR RESEARCH ON SOILBORNE PHYTOPATHOGENIC FUNGI
author] HARRINGTON T C [Reprint.

DEP PLANT PATHOLOGY, IOWA STATE UNIV, AMES, IOWA 50011, USA
1992, 129-133

ENGLISH

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91. **Host stress and resistance of red pine to staining fungi (Leptographium spp.).**

K. D. Klepzig, E. B. Smalley and K. F. Raffa.
PhytopathologyAugust 8-12, 1992
Univ. Wisconsin-Madison, Madison, Wis. 53706, USA

Abstr. ANNUAL MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY 1992, 82 (10)

English

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92. **Verticillium wilt resistance in pistachio rootstock cultivars : assays and an assessment of two interspecific hybrids**

P. MORGAN D., L. EPSTEIN and L. FERGUSON.

Univ. California, dep. plant pathology, Berkeley CA 94720, United States

Plant Disease 1992, 76 (3): 310-313

English

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93. **Root- and stem-colonizing insects recovered from eastern white pines with procerum root disease**

R. J. Nevill and S. A. Alexander.

Affiliation: Dept. of Plant Pathology, Physiology & Weed Sci., Virginia Polytechnic Inst. & State Univ.,

Blacksburg, VA 24060-0330, USA

Canadian Journal of Forest Research 1992, 22 (11): 1712-1716

English

ABSTRACT Pinus strobus from SW Virginia Christmas tree plantations symptomatic of infection by Leptographium procerum were assigned to one of five symptom categories based on crown color and resin exudation at the root collar. Larvae of two weevil species, Hylobius pales and Pissodes nemorensis were recovered with L. procerum from 23, 17, and 21% of the trees in all symptom classes in 1988, 1989, and 1990, respectively. Pre-emergent adults of both weevil species contaminated with L. procerum were also recovered from excavated stems. Bark beetle genera (Scolytidae), including Pityogenes, Xyleborus, Orthotomicus, Ips and Pityophthorus, were recovered with L. procerum from 10, 11, and 8% of diseased trees with advanced symptoms in 1988, 1989, and 1990, respectively. The association of these insects, especially H. pales and P. nemorensis, with L. procerum throughout the decline of the host adds further evidence that they may act as vectors of the fungus. -from Authors

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94. **Distribution of Hylobius pales and Pissodes nemorensis (Coleoptera: Curculionidae) within Christmas tree plantations with procerum root disease**

R. J. Nevill and S. A. Alexander.

Affiliation: Dept Plant Pathology, Physiology and Weed Science, Virginia Poly Inst & State Univ, Blacksburg, VA 24060-0330, USA

Environ.Entomol. 1992, 21 (5): 1077-1085

English

ABSTRACT Procerum root disease caused by Leptographium procerum is the most serious disease problem facing Virginia Christmas tree growers producing eastern white pine Pinus strobus. Potential insect vectors were collected from plots containing baited pitfall traps. Insects recovered included two weevil species, Hylobius pales and Pissodes nemorensis, and bark beetles from the genera Ips, Pityophthorus, Pityogenes, Orthotomicus and Xyleborus. In plantations with procerum root disease, there were significantly more H. pales than P. nemorensis, and significantly more H. pales were contaminated with L. procerum. In plantations where procerum root disease was absent, there was no significant difference between the numbers of either weevil species or proportions contaminated with the fungus. Within the plots with procerum root disease, 27% of the trees developed symptoms of the disease during three years of observation, but no trees developed disease symptoms in the asymptomatic plots. -from Authors

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95. **Pathogenicity of three fungal associates of Hylobius pales and Pissodes nemorensis (Coleoptera: Curculionidae) to eastern white pine.**

R. J. Nevill and S. A. Alexander.

Dep. Plant Pathol. Physiol. Weed Sci., Va. Polytechnic Inst. State Univ., Blacksburg, Va. 24060-0330, USA

Canadian Journal of Forest Research 1992, 22 (9): 1438-1440

English

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96. **TRANSMISSION OF LEPTOGRAPHIUM - PROCERUM TO EASTERN WHITE PINE BY HYLOBIUS-PALES AND PISSODES-NEMORENSIS COLEOPTERA CURCULIONIDAE.**

R. J. NEVILL and S. A. ALEXANDER.

AUBURN UNIV SCH FOR, AUBURN UNIVERSITY, ALA 36849-5418

Plant Dis. 1992, 76 (3): 307-310

ENGLISH

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97. **Decline of white pine in Indiana.**

M. Putnam and G. Ruhl.

BP - Purdue University, Cooperative Extension Service 1992, 34 2

English

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98. **INCIDENCE OF HETEROBASIDION-ANNOSUM AND OTHER ROOT-INFECTING FUNGI IN RESIDUAL STUMPS AND ROOTS IN THINNED SLASH PINE PLANTATIONS IN FLORIDA USA.**

E. L. BARNARD, S. P. GILLY and W. N. DIXON.

INSECT DISEASE SECTION, BUREAU FOREST MANAGEMENT, FLORIDA DIV FORESTRY, FDACS, GAINESVILLE 32602, USA

Plant Dis. 1991, 75 (8): 823-828

ENGLISH

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99. **LEPTOGRAPHIUM - PROCERUM INFECTION OF PINE SEEDLINGS FOLLOWING OZONE EXPOSURE.**

CARLSON J A and ALEXANDER S A.
PhytopathologyAUGUST 17-21, 1991
DEP PLANT PATHOL, VPI AND SU, BLACKSBURG, VA 24061-0330, USA

Abstr. ANNUAL MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY, 1991, 81 (10)

ENGLISH

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100. **THE PINE SHOOT BEETLE TOMICUS-PINIPERDA AS A VECTOR OF BLUE STAIN FUNGI TO WINDBLOWN PINE.**

J. N. GIBBS and A. INMAN.

FORESTRY COMMISSION RES STATION, ALICE HOLT LODGE, WRECCLESHAM, FARNHAM, SURREY, GU10 4LH, ENGLAND, UK

Forestry (Oxford) 1991, 64 (3): 239-250

ENGLISH

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101. **Association of an insect-fungal complex with red pine decline in Wisconsin.**

K. D. Klepzig, K. F. Raffa and E. B. Smalley.

Dept. of Entomology, University of Wisconsin-Madison, WI 53706, USA.

Forest Science 1991, 37 (4): 1119-1139

English

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102. **Occurrence of Procerum root disease caused by Leptographium procerum on white pine in Connecticut.**

V. L. Smith.

Plant Pathology and Ecology, The Connecticut Agricultural Experiment Station, New Haven, CT 06504, USA.

Plant Disease 1991, 75 (4): 431

English

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103. **LEPTOGRAPHIUM AND GRAPHIUM-SPP ASSOCIATED WITH PINE-INFESTING BARK BEETLES IN ENGLAND.**

M. J. WINGFIELD and J. N. GIBBS.

DEP MICROBIOLOGY BIOCHEMISTRY, UNIV ORANGE FREE STATE, PO BOX 339, BLOEMFONTEIN 9300, SOUTH AFRICA

Mycol.Res. 1991, 95 (11): 1257-1260

ENGLISH

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104. **TRANSMISSION OF LEPTOGRAPHIUM - PROCERUM TO EASTERN WHITE PINE PINUS-STROBUS SEEDLINGS BY THE PALES WEEVIL HYLOBIUS-PALES.**

NEVILL R J and ALEXANDER S A.
PhytopathologyAUGUST 4-8, 1990
DEP PLANT PATHOL, PHYSIOL WEED SCI, VPI AND SU, BLACKSBURG, VA 24061-0330, USA

Abstr. 1990 ANNUAL MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY AND THE CANADIAN PHYTOPATHOLOGICAL SOCIETY 1990, 80 (10)

ENGLISH

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105. **THE ASSOCIATION AND TRANSMISSION OF LEPTOGRAPHIUM PROCERUM (KENDR.) WING., BY ROOT FEEDING INSECTS IN CHRISTMAS TREE PLANTATIONS**

NEVILL, RALPH JOHN LESLIE [PH.D.] and S. A. [advisor] ALEXANDER.

VIRGINIA POLYTECHNIC INSTITUTE AND STATE UNIVERSITY (0247)

Dissertation Abstracts International 1990, 52 (4B): 1805

English

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106. **ASSOCIATION OF ROOT FEEDING INSECTS WITH PROCERUM ROOT DISEASE IN CHRISTMAS TREE PLANTATIONS.**

NEVILL R J and ALEXANDER S A.
PhytopathologyAUGUST 20-24, 1989
DEP PLANT PATHOL, PHYS WEED SCI, VA POLYTECH INST STATE UNIV, BLACKSBURG, VA 24061,

USA

Abstr. ANNUAL MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY, 1989, 79 (10)

ENGLISH

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107. **Leptographium procerum as a pathogen of pines.**
Leptographium root diseases on conifers
S. A. Alexander, W. E. Horner and K. J. Lewis.

1988, 97-112

English

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108. **In vitro tests of antagonism against Heterobasidion annosum (Fr.) Bref.**

P. Capretti and L. Mugnai.

Ist. Patol. e Zool., For. e Agrar., Univ. Studi, Firenze, Italy

PHYTOPATHOL. MEDITERR. 1988, 28 (2): 150-154

English

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109. **LEPTOGRAPHIUM-SPP ASSOCIATED WITH ROOT DISEASE OF CONIFERS IN BRITISH COLUMBIA CANADA.**
LEPTOGRAPHIUM ROOT DISEASES ON CONIFERS
MORRISON D J and R. S. HUNT.

CANADIAN FORESTRY SERV, PACIFIC FORESTRY CENT, 506 W BURNSIDE ROAD, VICTORIA, BC CAN V8Z 1M5

1988, 81-96

ENGLISH

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110. **DIFFERENCES IN SYNCHRONIZATION OF STAGES OF CONIDIAL DEVELOPMENT IN LEPTOGRAPHIUM-SPP.**

P. S. VAN WYK, M. J. WINGFIELD and W. F. O. MARASAS.

DEP PLANT PATHOL, UNIV ORANGE FREE STATE, PO BOX 339, BLOEMFONTEIN 9300, S AFR

Transactions of the British Mycological Society 1988, 90 (3): 451-456

ENGLISH

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111. **LEPTOGRAPHIUM-SPP AS ROOT PATHOGENS OF CONIFERS AN INTERNATIONAL PERSPECTIVE.**

WINGFIELD M J, P. CAPRETTI and M. MACKENZIE.

PLANT PROTECTION RES INST, PRIVATE BAG X5017, STELLENBOSCH 7600, S AFR

1988

ENGLISH

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112. **ROLE OF BURSAPHELENCHUS-XYLOPHILUS IN THE MORTALITY OF SAND PINE IN A SEED ORCHARD IN CENTRAL GEORGIA USA.**

DWINELL L D.
Journal of NematologyJULY 19-22, 1987
USDA FOREST SERV, SOUTHEAST, FOR EXP STN, ATHENS, GA 30602, USA

Abstr, TWENTY-SIXTH ANNUAL MEETING OF THE SOCIETY OF NEMATOLOGISTS 1987, 19 (4)

ENGLISH

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113. **COLONIZATION PATTERNS OF VERTICICLADIELLA - PROCERA IN SCOTS AND EASTERN WHITE PINE AND ASSOCIATED RESIN-SOAKING REDUCED SAPWOOD MOISTURE CONTENT AND REDUCED NEEDLE WATER POTENTIAL.**

W. E. HORNER, S. A. ALEXANDER and K. J. LEWIS.

TULANE MED CENTER, 1500 PERDIDO ST, NEW ORLEANS, LA 70112, USA

Phytopathology 1987, 77 (4): 557-560

ENGLISH

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114. **DISTRIBUTION AND EFFICACY OF PROPAGULES OF VERTICICLADIELLA - PROCERA IN SOIL.**

K. J. LEWIS, S. A. ALEXANDER and W. E. HORNER.

MINISTRY FORESTS, PEST MANAGEMENT DIV, PRINCE GEORGE, BC, CAN

Phytopathology 1987, 77 (4): 552-556

ENGLISH

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115. **PATHOGENICITY OF BLUE-STAIN FUNGI ASSOCIATED WITH DENDROCTONUS-TEREBRANS.**

K. K. RANE and T. A. TATTAR.

DEP PLANT PATHOLOGY, UNIV MASSACHUSETTS, AMHERST 01003, USA

Plant Dis. 1987, 71 (10): 879-883

ENGLISH

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116. **Diseases of eastern white pine.**

C. S. Hodges.
Symposium on Eastern White Pine: Today and Tomorrow, June 12-14, 1985, Durham, New Hampshire.Durham, New Hampshire.
General technical report WO - U.S.Department of Agriculture, Forest Service 1986, (51): 93-98

English

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117. **QUALITATIVE DETERMINATION OF CELLULOSE IN THE CELL WALLS OF VERTICICLADIELLA - PROCERA .**

W. E. HORNER, S. A. ALEXANDER and M. M. JULIAN.

DEP PLANT PATHOL, PHYSIOL WEED SCI, VA POLYTECHNIC INST STATE UNIV, BLACKSBURG, VA 24061, USA

Mycologia 1986, 78 (2): 300-303

ENGLISH

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118. **ETIOLOGIC STUDIES OF VERTICICLADIELLA PROCERA KENDR. IN PINE CHRISTMAS TREES**

W. ELLIOTT [PH D.]. HORNER.

VIRGINIA POLYTECHNIC INSTITUTE AND STATE UNIVERSITY (0247)

Dissertation Abstracts International 1986, 47 (2B;): 457

English

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119. **INSECTS ASSOCIATED WITH THE TRANSMISSION OF VERTICICLADIELLA - PROCERA .**

K. J. LEWIS and S. A. ALEXANDER.

DEP PLANT PATHOLOGY, PHYSIOLGOY AND WEED SCI, VIRGINIA POLYTECHNIC INST AND STATE UNIV, BLACKSBURG, VA, USA 24061

Canadian Journal of Forest Research 1986, 16 (6): 1330-1333

ENGLISH

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120. **DIEBACK OF PINE ASSOCIATED WITH VERTICICLADIELLA-SPP.**

MORELET M.
Bulletin OEPPJUNE 4-6, 1985
LAB PATHOL FORESTIERE, CENT NATL RECHERCHES FORESTIERES, CHAMPENOUX, 54280 SEICHAMPS, FR

Abstr. EUROPEAN AND MEDITERRANEAN PLANT PROTECTION ORGANIZATION CONFERENCE ON PEST AND DISEASE PROBLEMS IN EUROPEAN FORESTS 1986, 16 (3)

FRENCH

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121. **PATHOGENICITY OF LEPTOGRAPHIUM - PROCERUM AND LEPTOGRAPHIUM-TEREBRANTIS ON PINUS-STROBUS SEEDLINGS AND ESTABLISHED TREES.**

M. J. WINGFIELD.

PLANT PROTECTION RES INST, PRIVATE BAG X5017, STELLENBOSCH 7600, SOUTH AFRICA

Eur.J.For.Pathol. 1986, 16 (5-6): 299-308

ENGLISH

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122. **PATHOGENIC FUNGI ASSOCIATED WITH SAND PINE PINUS-CLAUSA ROOT DISEASE IN FLORIDA USA.**

E. L. BERNARD, G. M. BLAKESLEE, J. T. ENGLISH and S. W. OAK.

DIV FORESTRY PLANT INDUSTRY, PO BOX 1269, GAINESVILLE, FL 32602, USA

Plant Dis. 1985, 69 (3): 196-199

ENGLISH

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123. **PERMEABILITY OF ASYMPTOMATIC RESIN-SOAKED AND VERTICICLADIELLA - PROCERA BLACK-STAINED PINE SAPWOOD.**

HORNER W E and ALEXANDER S A.
PhytopathologyAUG. 11-15, 1985
DEP OF PLANT PATHOL PHYSIOL AND WEED SCIENCE, VPI AND SU, BLACKSBURG, VA 24061, USA

Abstr, ANNUAL MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY 1985, 75 (11)

ENGLISH

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124. **HISTOPATHOLOGY OF VERTICICLADIELLA - PROCERA IN SCOTS PINE PINUS-SYLVESTRIS AND EASTERN WHITE PINE PINUS-STROBUS.**

HORNER W E and ALEXANDER S A.
PhytopathologyAUG. 11-15, 1985
DEPT PLANT PATHOLOGY PHYSIOL WEED SCI, VPI AND SU, BLACKSBURG, VA 24061, USA

Abstr, ANNUAL MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY, 1985, 75 (11)

ENGLISH

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125. **COLONIZATION PATTERNS OF VERTICICLADIELLA - PROCERA IN SCOTCH PINE PINUS-SYLVESTRIS AND EASTERN WHITE PINE PINUS-STROBUS.**

HORNER W E, LEWIS K J and ALEXANDER S A.
PhytopathologyMAR. 20-22, 1985
DEP PLANT PATHOLOGY, PHYSIOLOGY AND WEED SCI, VPI AND SU, BLACKSBURG, VA 24061, USA

Abstr.ANNUAL MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY (POTOMAC DIVISION) 1985, 75 (5)

ENGLISH

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126. **Histopathology of Verticicladiella procera in Scots and eastern white pine**

W. E. Horner and S. A. Alexander.
11-15 Aug 1985Reno, NV (USA)
Dep. Plant Pathol., Blacksburg, VA 24061, USA

Abstr. Conference: American Phytopathological Society 1985 Annual Meeting 1985

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127. **Permeability of asymptomatic, resin-soaked and Verticicladiella procera-black-stained pine sapwood**

W. E. Horner and S. A. Alexander.
11-15 Aug 1985Reno, NV (USA)
Dep. Plant Pathol., Physiol., Weed Sci., Blacksburg, VPI&SU, VA 24061, USA

Abstr. Conference: American Phytopathological Society 1985 Annual Meeting. 1985

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128. **GERMINABILITY OVER TIME OF VERTICICLADIELLA - PROCERA PROPAGULES IN ARTIFICIALLY INFESTED SOIL AND THEIR INABILITY TO CAUSE DISEASE.**

LEWIS K J and ALEXANDER S A.
PhytopathologyAUG. 11-15, 1985
DEPT PLANT PATHOLOGY, PHYSIOLOGY AND WEED SCIENCE, VPI AND SU, BLACKSBURG, VA 24061, USA

Abstr. ANNUAL MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY 1985, 75 (11)

ENGLISH

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129. **SOIL-BORNE PROPAGULES OF VERTICICLADIELLA - PROCERA THEIR DENSITY DISTRIBUTION AND ASSOCIATION WITH COLONIZED ROOTS OF CHRISTMAS TREES.**

LEWIS K J, HORNER W E and ALEXANDER S A.
PhytopathologyMAR. 20-22, 1985
DEP PLANT PATHOLOGY, PHYSIOLOGY AND WEED SCI, VPI AND SU, BLACKSBURG, VA 24061, USA

Abstr. ANNUAL MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY (POTOMAC DIVISION) 1985, 75 (5)

ENGLISH

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130. **Germinability over time of Verticicladiella procera propagules in artificially infested soil and their inability to cause disease**

K. J. Lewis and S. A. Alexander.
11-15 Aug 1985Reno, NV (USA)
Dep. Plant Pathol., Blacksburg, VA 24061, USA

Abstr. Conference: American Phytopathological Society 1985 Annual Meeting 1985

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131. **PHYSIOLOGICAL RESPONSES OF PINUS THUNBERGIANA FRANCO AND PINUS SYLVESTRIS L. TO DENDROCTONUS TEREBRANS OLIV. AND ITS ASSOCIATED BLUESTAIN FUNGI (BARK BEETLES)**

KAREN K. [PH D.]. RANE.

UNIVERSITY OF MASSACHUSETTS (0118)

Dissertation Abstracts International 1985, 46 (6B): 1773

English

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132. **INCIDENCE AND DEVELOPMENT OF VERTICICLADIELLA - PROCERA IN VIRGINIA USA CHRISTMAS TREE PLANTATIONS.**

A. L. LACKNER and S. A. ALEXANDER.

DEP PLANT PATHOL PHYSIOL, VA POLYTECHNIC INST STATE UNIV, BLACKSBURG 24061, USA

Plant Dis. 1984, 68 (3): 210-212

ENGLISH

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133. **Verticicladiella root disease.**

M. MacKenzie and M. Dick.

Forest Pathology in New Zealand, Forest Research Institute, New Zealand 1984, (6): 4

English

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134. **PATHOGENICITY OF 5 VERTICICLADIELLA SPECIES TO LODGEPOLE PINE PINUS-CONTORTA.**

C. L. BERTAGNOLE, J. Y. WOO and A. D. PARTRIDGE.

DEP FOREST RESOURCES, UNIV IDAHO, MOSCOW, ID, USA 83843

Canadian Journal of Botany 1983, 61 (7): 1861-1867

ENGLISH

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135. **PATHOGENICITY OF LEPTOGRAPHIUM AND VERTICICLADIELLA-SPP ISOLATED FROM ROOTS OF WESTERN NORTH AMERICAN CONIFERS.**

T. C. HARRINGTON and F. W. J. R. COBB.

DEP PLANT PATHOL, UNIV CALIFORNIA, BERKELEY 94720, USA

Phytopathology 1983, 73 (4): 596-599

ENGLISH

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136. **VERTICICLADIELLA - PROCERA ON PINUS-SYLVESTRIS CHRISTMAS TREES.**

HORNER W E and ALEXANDER S A.
PhytopathologyMAR. 23-25, 1983
DEP PLANT PATHOL PHYSIOL, VPI AND STATE UNIV, BLACKSBURG, VA 24061, USA

Abstr. ANNUAL MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY, (POTOMAC DIVISION), 1983, 73 (6)

ENGLISH

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138. **OCCURRENCE OF WHITE PINE ROOT DECLINE IN WEST-VIRGINIA USA.**

MEYER G J JR, QUINN D O and HINDAL D F.
PhytopathologyMAR. 23-25, 1983
WEST VA UNIV, MORGANTOWN, WV 26506, USA

Abstr. ANNUAL MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY, (POTOMAC DIVISION) 1983, 73 (6)

ENGLISH

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139. **Association of Verticicladiella procera and Leptographium terrebrantis with insects in the Lake States.**

M. J. Wingfield.

Affiliation: Dept of Plant Pathology, Univ. of Minnesota, 1519 Gortner Ave, St. Paul, MN, USA 55108.

Canadian Journal of Forest Research 1983, 13 (6): 1238-1245

English

ABSTRACT Verticicladiella procera was isolated from damage associated with Dendroctonus valens, Hylobius radicis, H. rhizophagus, H. pales and Pachylobius picivorus on pines in Minnesota, Wisconsin and Michigan. The fungus was also isolated from surface-sterilized adult D. valens, H. radicis, H. pales and P. picivorus. Leptographium terrebrantis was isolated from the galleries of D. valens on trees stressed by fire, flooding, windthrow, and tissue attacked by H. radicis or H. rhizophagus. Verticicladiella was not pathogenic on white pine Pinus strobus seedlings whereas L. terrebrantis killed 70% of the seedlings inoculated. Verticicladiella appears to be weakly pathogenic and is associated with primary (H. radicis, H. rhizophagus) and secondary (P. picivorus, D. valens, H. pales) forest insects in the north central USA. Leptographium terrebrantis was more virulent than V. procera and was associated with a secondary bark beetle which commonly attacks stressed trees. - Author

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140. **SOME VERTICICLADIELLA SPECIES INCLUDING VERTICICLADIELLA-TRUNCATA ASSOCIATED WITH ROOT DISEASES OF PINE IN NEW-ZEALAND AND SOUTH AFRICA.**

M. J. WINGFIELD and W. F. O. MARASAS.

DEP PLANT PATHOL, UNIV MINNESOTA, 1519 GORTNER AVE, ST PAUL, MINN 55108, USA

Transactions of the British Mycological Society 1983, 80 (2): 231-236

ENGLISH

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141. **FUNGI ASSOCIATED WITH ROOT DISEASE OF SAND PINE PINUS-CLAUSA IN FLORIDA USA.**

BARNARD E L, ENGLISH J T, ANDERSON R L and BLAKESLEE G M.
PhytopathologyAUG. 8-12, 1982
FLA DEP AGRIC CONSUM SERV, GAINESVILLE 32602, USA

Abstr. 74TH ANNUAL MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY 1982, 72 (7)

ENGLISH

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142. **LEPTOGRAPHIUM - PROCERUM GROWTH IN PINE SEEDLING STEMS FOLLOWING OZONE EXPOSURE.**

CARLSON J A and ALEXANDER S A.
PhytopathologyMARCH 18-20, 1992.
DEP PLANT PATHOL PHYSIOL WEED SCIENCE, VPI SU, BLACKSBURG, VA 24061-0330, USA

Abstr. MEETING OF THE POTOMAC DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY 1982, 82 (6)

ENGLISH

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143. **OCCURRENCE AND PATHOGENICITY OF VERTICICLADIELLA - PROCERA IN CHRISTMAS TREE PLANTATIONS IN VIRGINIA USA.**

A. L. LACKNER and S. A. ALEXANDER.

DEP PLANT PATHOL PHYSIOL, VA POLYTECHNIC INST, STATE UNIV, BLACKSBURG 24061, USA

Plant Dis. 1982, 66 (3): 211-212

ENGLISH

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145. **VERTICICLADIELLA - PROCERA NEW-RECORD ON PINES IN MINNESOTA USA.**

W. H. LIVINGSTON and M. J. WINGFIELD.

DEP PLANT PATHOL, UNIV MINN, ST PAUL, 55108

Plant Dis. 1982, 66 (3): 260-261

ENGLISH

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146. **FIRST REPORT OF VERTICICLADIELLA PROCERA ON PINES IN MINNESOTA.**

W. H. LIVINGSTON and M. J. WINGFIELD.

Plant Dis. 1982, 66 (3): 260-261

English

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147. **Incidence of resin-soaked roots in subsoiled loblolly pine seed orchards on sandy soils.**

R. S. Webb and S. A. Alexander.

Dep. Pl. Pathol. & Physiol., Virginia Polytech. Inst. & State Univ., Blacksburg, VA, USA.

Southern Journal of Applied Forestry 1982, 6 (2): 104-107

English

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148. **VERTICICLADIELLA - PROCERA ASSOCIATED WITH ROOT WEEVIL DAMAGE.**

WINGFIELD M J.
PhytopathologyJUNE 16-18, 1982
DEP PLANT PATHOL, UNIV MINN, ST PAUL, MN 55108, USA

Abstr. ANNUAL MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY, NORTH CENTRAL DIVISION 1982, (72): 8

ENGLISH

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149. **Verticicladiella procera Kendrick, the pathogen of wilt of Eastern white pine in conifers. Verticicladiella procera Kendrick uzrocnik venuca americkog borovca u kulturama cetinjaca.**

M. Halambek.

For. Inst., Jastrebarsko, Yugoslavia.

Zastita Bilja 1981, 32 (3): 313-323

English

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150. **VERTICICLADIELLA - PROCERA PATHOGENIC ON PINUS-STROBUS AND PINUS-TAEDA.**

LACKNER A L and ALEXANDER S A.
PhytopathologyAUG. 24-28, 1980
DEP PLANT PATHOL PHYSIOL, VPI AND SU, BLACKSBURG, VA 24061, USA

Abstr, 72ND ANNUAL MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY AND THE CANADIAN PHYTOPATHOLOGICAL SOCIETY 1981, 71 (2)

ENGLISH

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151. **ASSOCIATION OF ROOT DISEASE AND INSECT INFESTATIONS WITH EASTERN WHITE PINES EXPRESSING SENSITIVITY TO AIR POLLUTION.**

A. L. LACKNER and S. A. ALEXANDER.

Phytopathology 1981, 71 (7): 769

English

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152. **SELECTIVE MEDIUM FOR RECOVERING VERTICICLADIELLA - PROCERA FROM SOILS AND SYMPTOMATIC WHITE PINES PINUS-STROBUS.**

I. S. SWAI and D. F. HINDAL.

DIV PLANT SOIL SCI, COLL AGRIC FORESTRY, W VA UNIV, MORGANTOWN 26506, USA

Plant Dis. 1981, 65 (12): 963-965

ENGLISH

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153. **METHOD FOR RECOVERING VERTICICLADIELLA - PROCERA FROM BASAL CANKERS ON EASTERN WHITE PINE.**

R. L. ANDERSON.

FOREST INSECT AND DISEASE MANAGEMENT, SOUTHEASTERN AREA, STATE AND PRIVATE FORESTRY, USDA FOREST SERVICE, ASHEVILLE, NC 28803, USA

Plant Dis. 1980, 64 (7): 680

ENGLISH

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154. **Abstracts of papers presented at the 1980 meeting of the Potomac Division of the American Phytopathological Society.**

Anon.

USA, American Phytopathological Society

Phytopathology 1980, 70 (7): 687-694

English

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155. **ALTERATION OF BEECH WOOD FAGUS-SYLVATICA BY FUSARIUM-SOLANI AND VERTICICLADIELLA - PROCERA .**

G. KILBERTUS, F. MANGENOT and D. RADTKE.

LAB BOT MICROBIOL, UNIV NANCY I CENT DE 2E CYCLE, CASE OFFICIELLE NO 140, 54037 NANCY CEDEX, FR

Cryptogamie Mycologie 1980, 1 (2): 97-104

FRENCH

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156. **SELECTIVE MEDIUM FOR VERTICICLADIELLA - PROCERA .**

K. A. MCCALL and W. MERRILL.

DEP PLANT PATHOL, PA STATE UNIV, UNIVERSITY PARK, PA 16802, USA

Plant Dis. 1980, 64 (3): 277-278

ENGLISH

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157. **A selective medium for Verticicladiella procera.**

K. A. McCall and W. Merrill.

Dep. Pl. Path., Pennsylvania State Univ., University Park, PA 16802, USA.

Phytopathology 1980, 70 (5): 465

English

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159. **Tree and shrub pathogens new or noteworthy in New York state.**

W. A. Sinclair and G. W. Hudler.

Cornell Univ., Ithaca, NY, USA.

Plant Disease 1980, 64 (6): 590-592

English

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160. **A defined agar medium for isolating Verticicladiella procera [white pine root decline] from naturally infested soils.**

I. S. Swai and D. F. Hindal.

Brooks Hall, West Virginia Univ., Morgantown, WV 26506, USA.

Phytopathology 1980, 70 (7): 693

English

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161. **VERTICICLADIELLA-ALACRIS NEW-SPECIES ASSOCIATED WITH A ROOT DISEASE OF PINES IN SOUTH AFRICA.**

M. J. WINGFIELD and W. F. O. MARASAS.

PLANT PROT RES INST, PRIV BAG X5017, STELLENBOSCH, 7600 S AFR

Transactions of the British Mycological Society 1980, 75 (1): 21-28

ENGLISH

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162. **TWO DIFFERENT FUNGAL GROUPS IN 55-YEAR-OLD SCOTCH PINES ON A GROUNDWATER-INFLUENCED SANDY SITE.**

H. COURTOIS.

ANGEW.BOTAN 1979, 53 (5-6): 255-260

English

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163. **BIOLOGICAL CONTROL OF HETEROBASIDION ANNOSUM /FR./ BREF. /FOMES ANNOSUS/ IN FINLAND.**

T. KALLIO and A. M. HALLAKSELA.

EUR.J.FOREST PATHOL. 1979, 9 (5): 298-308

English

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164. **BIOLOGICAL CONTROL OF HETEROBASIDION-ANNOSUM FOMES-ANNOSUS IN FINLAND.**

T. KALLIO and A. -M HALLAKSELA.

FINN FOR RES INST, UNIONINKATU 40A, SF-00170 HELSINKI 17, FINL

Eur.J.For.Pathol. 1979, 9 (5): 298-308

ENGLISH

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165. **Incidence of root disease in bark beetle-infested eastern Oregon and Washington true firs.**

B. B. Lane and D. J. Goheen.

Coll. For. Resour., Univ. Washington, Seattle, Wash. 98105, USA.

Plant Disease Reporter 1979, 63 (4): 262-266

English

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166. **Decomposition du bois de pin et de hetre par des champignons lignicoles.Recherche d'une methode standard d'etude**

F. MANGENOT, D. RADTKE, G. KILBERTUS and P. JODIN.

UNIVERSITE DE NANCY 1, LABORATOIRE DE BOTANIQUE ET MICROBIOLOGIE, NANCY, France; DELEGATION GENERALE A LA RECHERCHE SCIENTIFIQUE ET TECHNIQUE, PARIS, France (patr.)

Action concertee: Materiau-bois 1979, 6 (2)

French

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167. **LEPTOGRAPHIUM-RECONDITUM NEW-SPECIES AND OBSERVATIONS IN VERTICICLADIELLA.**

W. J. JOOSTE.

Transactions of the British Mycological Society 1978, 70 (1): 152-155

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168. **THE OCCURRENCE OF VERTICICLADIELLA - PROCERA IN PENNSYLVANIA 1976.**

B. TOWERS.

Plant Disease Reporter 1977, 61 (6): 477

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Appendix 3. Search strategy on potentially adverse effects of *Leptographium* or related genera.

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* * * * * * * * *   Welcome to STN International   * * * * * * * * * *
=> index safety, toxicology

41 FILES IN THE FILE LIST IN STNINDEX

=> s leptographium or verticicladiella or hantzchia or scopularia or
phialocephala or grosmannia

  24 FILES HAVE ONE OR MORE ANSWERS,   41 FILES SEARCHED IN STNINDEX

L1   QUE LEPTOGRAPHIUM OR VERTICICLADIELLA OR HANTZCHIA OR SCOPULARIA OR
PHIALO
         CEPHALA OR GROSMANNIA

=> s l1 and (safe? or health or tox? or carcino? or teratogen? or adverse)


  17 FILES HAVE ONE OR MORE ANSWERS,   41 FILES SEARCHED IN STNINDEX

L2   QUE L1 AND (SAFE? OR HEALTH OR TOX? OR CARCINO? OR TERATOGEN? OR
ADVERSE)

=> file hits

=> s l2
L3         217 L2

=> s l3 not patent/dt
L4         214 L3 NOT PATENT/DT

=> duplicate
L5           82 DUPLICATE REMOVE L4 (132 DUPLICATES REMOVED)
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Additional manual deduplication because of inaccurate technological deduplication. 60 unique papers remained.

Appendix 4. **Potentially adverse effects of Leptographium or related genera.**

1. **Bacteria Associated with a Tree-Killing Insect Reduce Concentrations of Plant Defense Compounds.**

Celia K. Boone, Ken Keefover-Ring, Abigail C. Mapes, Aaron S. Adams, Joerg Bohlmann and Kenneth F. Raffa.

Univ Wisconsin, Dept Entomol, 1630 Linden Dr, Madison, WI 53706 USA raffa@entomology.wisc.edu

J.Chem.Ecol. 2013, 39 (7, Sp Iss SI): 1003-1006

English

ABSTRACT Bark beetles encounter a diverse array of constitutive and rapidly induced terpenes when attempting to colonize living conifers. Concentrations of these compounds at entry sites can rapidly reach levels ***toxic*** to beetles, their brood, and fungal symbionts. Large numbers of beetles can overwhelm tree defenses via pheromone-mediated mass attacks, but the mechanisms are poorly understood. We show that bacteria associated with mountain pine beetles can metabolize monoterpenes and diterpene acids. The abilities of different symbionts to reduce concentrations of different terpenes appear complementary. Serratia reduced concentrations of all monoterpenes applied to media by 55-75 %, except for alpha-pinene. Beetle-associated Rahnella reduced (-)- and (+)-alpha-pinene by 40 % and 45 %, respectively. Serratia and Brevundimonas reduced diterpene abietic acid levels by 100 % at low concentrations. However, high concentrations exhausted this ability, suggesting that opposing rates of bacterial metabolism and plant induction of terpenes are critical. The two major fungal symbionts of mountain pine beetle, ***Grosmannia*** clavigera and Ophiostoma montium were highly susceptible to abietic acid. ***Grosmannia*** clavigera did not reduce total monoterpene concentrations in lodgepole pine turpentine. We propose the ability of bark beetles to exert landscape-scale impacts may arise partly from micro-scale processes driven by bacterial symbionts.

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2. **Ophiostomatoid fungi associated with three spruce-infesting bark beetles in Slovenia.**

Andreja Repe, Thomas Kirisits, Barbara Piskur, Maarten de Groot, Bojka Kump and Maja Jurc.

Univ Ljubljana, Dept Forestry and Renewable Forest Resources, Biotech Fac, Vecna Pot 83, Ljubljana 1000, Slovenia andreja.repe@bf.uni-lj.si

Ann.For.Sci. 2013, 70 (7): 717-727

English

ABSTRACT Ophiostomatoid fungi can severely affect the ***health*** and economic value of Norway spruce trees (Picea abies). Although the diversity of ophiostomatoid species and their associations with insects have been well-investigated in central and northern Europe, little is known about the conditions in south-eastern Europe.This study aims to study the assemblages of ophiostomatoid fungi associated with three bark beetle species (Ips typographus, Ips amitinus, and

Pityogenes chalcographus) that infect Norway spruce in Slovenia.Bark beetles were sampled in four phytogeographic regions in Slovenia. The fungi found on the bark beetles were identified based on morphology, DNA sequence comparisons of ITS regions and phylogenetic analysis. The species compositions of the fungal associates of the three insect species were compared and the pairwise associations of the occurrence of the fungal species were analysed.Thirteen different species were found. The most commonly encountered fungal associates of the beetles were Ophiostoma bicolor, Ophiostoma brunneo-ciliatum, ***Grosmannia*** piceiperda, Ophiostoma ainoae, Ceratocystiopsis minuta, and ***Grosmannia*** penicillata. The composition of the fungal associates differed among the bark beetle species, but not among the phytogeographic regions.This study confirms that ophiostomatoid species are common associates of the investigated bark beetle species. Many ophiostomatoid species have strong host associations. I. typographus and P. chalcographus can act as effective vectors for O. bicolor, O. ainoae, G. piceiperda and O. brunneo-ciliatum, whereas I. amitinus often carries G. piceiperda and C. minuta in Slovenian forests.

*****

4. *****Toxins*** from a symbiotic fungus, ***Leptographium*** qinlingensis associated with Dendroctonus armandi and their in vitro ***toxicities*** to Pinus armandi seedlings.**

Xiao-Jun Li, Jin-Ming Gao, Hui Chen, An-Ling Zhang and Ming Tang.

NW A and F Univ, Coll Sci, Chem Biol Res Inst, Yangling 712100, Shaanxi, Peoples R China jinminggao@nwsuaf.edu.cn

Eur.J.Plant Pathol. 2012, 134 (2): 239-247

English

ABSTRACT ***Leptographium*** qinlingensis is an ophiostomatoid fungal pathogen associated with a forest pest Dendroctonus armandi, which causes enormous deaths of its host trees Pinus armandi. The objective of this research was to isolate and characterise some ***toxic*** metabolites from cultures of the pathogen. Three ***toxins*** , 6-methoxymethyleugenin (compound 1), maculosin (cyclo(L-Pro-L-Tyr), compound 5) and cerevisterol (compound 12), together with 11 other compounds, have been isolated from the ethyl acetate extracts of the cultures of L. qinlingensis by several chromatographic methods. Their structures were characterized on the basis of spectroscopic analyses including ESIMS, H-1-NMR, and C-13-NMR, in comparison with those reported in the literature. Phytotoxic test showed that these three compounds were active in vitro against both aseptic Pinus armandi seedlings and field P. armandi seedlings. The present investigation demonstrated that three ***toxic*** metabolites including 6-methoxymethyleugenin, maculosin and cerevisterol, which were biosynthesized by L. qinlingensis isolated from the insect Dendroctonus armandi on Pinus armandi tree, could be phytotoxic to P. armandi seedlings.

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5. **Mycorrhiza reduces ***adverse*** effects of dark septate endophytes (DSE) on growth of conifers.**

V. Reininger and T. N. Sieber.

Department of Environmental Systems Science, ETH Zurich, Zurich, Switzerland. EMAIL:

Vanessa.Reininger@env.ethz.ch

PLoS ONE 2012, 7 (8): e42865

English

ABSTRACT Mycorrhizal roots are frequently colonized by fungi of the ***Phialocephala*** fortinii s.l. - Acephala applanata species complex (PAC). These ascomycetes are common and widespread colonizers of tree roots. Some PAC strains reduce growth increments of their hosts but are beneficial in protecting roots against pathogens. Nothing is known about the effects of PAC on mycorrhizal fungi and the PAC-mycorrhiza association on plant growth, even though these two fungal groups occur closely together in natural habitats. We expect reduced colonization rates and reduced negative effects of PAC on host plants if roots are co-colonized by an ectomycorrhizal fungus (ECM). Depending on the temperature regime interactions among the partners in this tripartite ECM-PAC-plant system might also change. To test our hypotheses, effects of four PAC genotypes (two pathogenic and two non-pathogenic on the Norway spruce), mycorrhization by Laccaria bicolor (strain S238N) and two temperature regimes (19.degree.C and 25.degree.C) on the biomass of the Douglas-fir (Pseudotsuga menziesii) and Norway spruce (Picea abies) seedlings were studied. Mycorrhization compensated the ***adverse*** effects of PAC on the growth of the Norway spruce at both temperatures. The growth of the Douglas-fir was not influenced either by PAC or mycorrhization at 19.degree.C, but at 25.degree.C mycorrhization had a similar protective effect as in the Norway spruce. The compensatory effects probably rely on the reduction of the PAC-colonization density by mycorrhizae. Temperature and the PAC strain only had a differential effect on the biomass of the Norway spruce but not on the Douglas-fir. Higher temperature reduced mycorrhization of both hosts. We conclude that ectomycorrhizae form physical and/or physiological barriers against PAC leading to reduced PAC-colonization of the roots. Additionally, our results indicate that global warming could cause a general decrease of mycorrhization making primary roots more accessible to other symbionts and pathogens.

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6. **Host species and strain combination determine growth reduction of spruce and birch seedlings colonized by root-associated dark septate endophytes.**

Vanessa Reininger, Christoph R. Gruenig and Thomas N. Sieber.

ETH, Inst Integrat Biol Forest Pathol and Dendrol, Univ Str 16, CH-8092 Zurich, Switzerland
vanessa.reininger@env.ethz.ch

Environ.Microbiol. 2012, 14 (4): 1064-1076

English

ABSTRACT Interactions of Betula pendula and Picea abies with dark septate endophytes of the ***Phialocephala*** fortinii - Acephala applanata species complex (PAC) were studied. PAC are ubiquitous fungal root symbionts of many woody plant species but their ecological role is largely unknown. Sterile birch and spruce seedlings in monoculture and mixed culture were exposed to four PAC strains, added either singularly or paired in all possible combinations at 18 degrees C and 23 degrees C. Plant and fungal biomass was determined after 4 months. The most significant factors were strain and host combination. One of the strains significantly reduced biomass gain of spruce but

not of birch. Plant biomass was negatively correlated with total endophytic fungal biomass in half of the strain - plant combinations. Endophytic PAC biomass was four times higher in spruce (approximate to 40 mg g(-1) drw) than in birch (approximate to 10 mg g-1 drw). Competition between strains was strain-dependent with some strains significantly reducing colonization density of other strains, and, thus, attenuating ***adverse*** effects of 'pathogenic' strains on plant growth in some strain - plant combinations. Biomass gain of spruce but not of birch was significantly reduced at higher temperature. In conclusion, host, fungal genotype, colonization density and presence of a competing PAC strain were the main determining factors for plant growth.

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7. **Do colonization by dark septate endophytes and elevated temperature affect pathogenicity of oomycetes?.**

Christoph Tellenbach and Thomas N. Sieber.

Eawag, Aquat Ecol, Uberlandstr 133, CH-8600 Dubendorf, Switzerland tellench@eawag.ch

FEMS Microbiol.Ecol. 2012, 82 (1): 157-168

English

ABSTRACT ***Phialocephala*** subalpina is one of the most frequent dark septate root endophytes in tree roots but its function in forest ecosystems is largely unknown. A full-factorial infection experiment was performed, using six P. subalpina isolates, two pathogenic oomycetes (Phytophthora plurivora [syn. Phytophthora citricola s.l.] and Elongisporangium undulatum [syn. Pythium undulatum]) and two temperature regimes (17.9 and 21.6 degrees C) to examine the ability of P. subalpina to protect Norway spruce seedlings against root pathogens. Seedling survival, disease intensity and seedling growth were affected by P. subalpina genotype, temperature and pathogen species. Some P. subalpina isolates effectively reduced mortality and disease intensity caused by the two pathogens. Elevated temperature adversely affected seedling growth but did not aggravate the effect of the pathogens. Elongisporangium undulatum but not P. plurivora significantly reduced plant growth. Colonization density of P. subalpina measured by quantitative PCR was not affected by temperature or the presence of the pathogens. In conclusion, P. subalpina confers an indirect benefit to its host and might therefore be tolerated in natural ecosystems, despite negative effects on plant ***health*** and plant growth.

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9. **Influence of water deficit on the induced and constitutive responses of pines to infection by mountain pine beetle fungal associates.**

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26 Jun 2011-02 Jul 2011Bahia, Brazil
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BMC Proceedings 2011, 5 (Suppl. 7): 029

English

ABSTRACT Background: The ongoing outbreak of mountain pine beetle (MPB; Dendroctonus ponderasae Hopkins) and its associated pathogenic fungi (e.g. ***Grosmannia*** clavigera [Robinson-Jeffrey and Davidson] Zipfel, de Beer and Wingfield) in western North America has resulted in the loss of more than 13 million hectares of pines since 1999 in British Columbia alone [1]. MPB has principally attacked lodgepole pine (Pinus contorta Dougl. ex Loud. var. latifolia) in British Columbia. However, since 2006 MPB has spread into northern Alberta, where lodgepole pine hybridizes with jack pine (Pinus banksiana Lamb.) [2]. Few studies have compared lodgepole and jack pine defense responses, but given that lodgepole pine and MPB share a co-evolutionary history [1] whereas jack pine is a new host for MPB [2], it is reasonable to expect that differences might exist. Some regions affected by the current outbreak have experienced drought conditions during the last decade. Water deficit can limit carbon assimilation, potentially increasing tree susceptibility to MPB and their symbiotic fungi [3]. We are testing the hypotheses that lodgepole and jack pine defenses against MPB and G. clavigera differ, and that water deficit affects these responses. Materials and methods: The relationship between water availability and tree defense was evaluated in (1) two year old lodgepole and jack pine seedlings in growth rooms, and (2) ca. sixty year old lodgepole x jack pine hybrids in naturally regenerated, thinned stands. Soil relative water content was monitored using time-domain reflectometry. Seedlings were subjected to watering or water deficit for one week prior to wounding or wounding plus G. clavigera inoculation. Mature trees were either watered or water limited via tarps for six weeks before wounding plus G. clavigera inoculation. In both experiments, water deficit conditions were maintained throughout the time course. Tree physiological status was evaluated by measuring gas exchange and stomatal conductance using a LiCor 6400, stem hydraulic conductivity using a low-pressure flow meter and safranin dye xylem perfusion, and HPLC. Defense responses were assessed by lesion measurements histochemistry, and qRT-PCR. Results and discussion: Stomatal conductance and photosynthesis significantly decreased under water deficit for both lodgepole and jack pine seedlings, but seedling hydraulic conductivity was not affected. The mild water deficit applied to the mature trees reduced stomatal conductance and photosynthesis, but not significantly. Stem lesions are a means of killing and compartmentalizing invading organisms [4]. G. clavigera-induced lesions developed more slowly in jack pine than lodgepole pine seedlings. Stem hydraulic conductivity decreased in inoculated lodgepole but not jack pine seedlings, likely because of greater tracheid occlusion caused by increased fungal growth and/or resin production in lodgepole pine [5]. Water deficit reduced lesion development rates at early time points in inoculated lodgepole and jack pine seedlings, as well as in mature trees at 5 weeks post-inoculation. Lesion length has been considered an indicator of tree defense capacity [6], with longer lesions reported to reflect increased release of ***toxic*** and/or inhibitory substances [7]. Accordingly, we interpret slower lesion development to indicate a slower defense response. Our results suggest (1) more rapid defense responses to G. clavigera in the co-evolved lodgepole pine host than in the new jack pine host, and (2) defense responses are slowed by water limitation. We then examined the effect of water deficit on transcript abundance corresponding to genes classically associated with drought and defense responses. We conducted qRT-PCR transcript abundance profiling of secondary phloem from mature lodgepole x jack pine hybrids. We first profiled four aquaporin and five DREB genes, families associated with water stress responses. Although the mild water deficit did not significantly alter expression of these genes, expression of one aquaporin and one DREB decreased in response to G. clavigera inoculation. We then profiled five chitinase and four terpene synthase defense-associated genes. Expression of two chitinases was significantly induced by water deficit but not G. clavigera. Expression of other chitinases significantly increased in response to fungalinoculation, but the response was attenuated by water deficit. Expression of one terpene synthase significantly increased with fungal inoculation, but this response was also attenuated under water deficit. In contrast, water deficit increased constitutive expression of another

terpene synthase. Higher constitutive expression of the monoterpene synthase under mild water stress suggests a pre-emptive defense via higher biosynthesis of volatile monoterpenes. Microarray and qRT-PCR analyses of the lodgepole and jack pine seedling experiment are underway. Conclusion: Our analyses suggest that defense responses of jack pine differ from those of lodgepole pine. Molecular analyses are underway to further characterize these differences. Both constitutive and induced defense responses are modulated in pines by water deficit, and this response appears to be gene-specific. This study shows evidence of cross talk between the water stress and defense responses of pine trees.

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10. **The Effects of Copper-Carbon Core-Shell Nanoparticles On Three Blue-Stain Fungi**

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Masters Abstracts International 2011, 50 (3): 1586

English

ABSTRACT Over the past few years, nanomaterials have opened a tremendous interest because they offer an opportunity to deliver unprecedented material performance. Effects of Copper-Carbon Core-Shell Nanoparticles (CCCNPs) on the growth of different blue-stain fungi infecting forest trees will be examined. A series of CCCNPs treatments with pure copper concentrations ranging from 0 parts per million (ppm) to 5,000ppm were incorporated into malt extract agar (MEA) media (3.5% MEA to Water). For the growth assay studies, three blue-stain fungi Ophiostoma minus, ***Leptographium*** huntii and ***Grosmannia*** huntii were inoculated onto the media in Petri-dishes with four replicates per concentration per species. The mycelia growth was measured daily for at least 30 days depending on species. The fungal colony was photographed every other day. Mycelia growth measurements were compared and analyzed statistically. The in-vivo tests show that CCCNPs treatments with pure copper concentrations at 1,223ppm and above strongly inhibited Ophiostoma minus growth, while CCCNPs treatments with pure copper concentrations at 1,000ppm and above strongly inhibited the growth of both ***Leptographium*** huntii and ***Grosmannia*** huntii. This research indicates that the proper formulation of CCCNPs has a potential to control blue-stain fungi, thus, helping to improve tree and forest ***health*** .

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11. **Mycobiota in Needles and Shoots of Pinus nigra following Infection by Dothistroma septosporum.**

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Phyton (Horn) 2011, 51 (2): 277-287

English

ABSTRACT KOWALSKI T. & DROZYNSKA K. 2011. Mycobiota in needles and shoots of Pinus nigra following infection by Dothistroma septosporum. - Phyton (Horn, Austria) 51 (2): 277-287, with 1 figure.A 25-year-old Pinus nigra ARN. seed plantation in Miechow Forest District, in the south of Poland, was studied. The P nigra had shown symptoms of red band needle blight caused by Dothistroma septosporum (DOROG.) MORELET (Mycosphaerellaceae) since 1997. This disease leads to premature needle death and defoliation followed by dying of twigs and branches. The aims of the study were: 1. to recognize the extent to which needle infection by D. septosporum affects the structure of the needle endophyte community and colonization by other fungi which may contribute to needle loss, and 2. to determine whether stress caused by D. septosporum infection over many years facilitates latent infection of shoots by other potential pathogens, including contributors to bark and cambium necroses. Mycological analyses were done on three groups of P nigra needles, i.e. A type needles (living, symptomless), B type (living, with necrotic bands typical of D. septosporum infection) and C type (prematurely dead as a result of primary infection by D. septosporum), and on living, 1-4-year-old shoots. Needles and shoots were colonized by 47 and 38 fungal species, respectively. Anthostomella formosa, Cenangium ferruginosum, Cyclaneusma minus, Lophodermium pinastri, Sclerophoma pythiophila and Verticicladium trifidum colonized more than 10% of needles. Fungi occurred at significantly different frequencies in needles with different ***health*** status. Cystodendron sp., Fusicoccum sp., Geniculosporium serpens, Mollisia cinerea, Pezicula eucrita, ***Phialocephala*** cf. dimorphospora, Phomopsis occulta, Sirodothis sp.1 and Therrya pini colonized more than 10% of shoots. Among the well-known causal agents of diseases of shoots, C. ferruginosum was recorded only sporadically, Gremmeniella abietina and Sphaeropsis sapinea were not isolated at all. The significance and importance of individual fungi in induction of diseases of needles and of shoot dieback are discussed.

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12. **Antifungal activity of a Pinus monticola antimicrobial peptide 1 (Pm-AMP1) and its accumulation in western white pine infected with Cronartium ribicola**

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Can.J.Microbiol. 2011, 57 (8): 667-679

English

ABSTRACT Pinus monticola antimicrobial peptide 1 (Pm-AMP1) was expressed and purified from bacterial cell lysate and its identity and purity confirmed by Western blot anal. using the Pm-AMP1 antibody. Application of Pm-AMP1 resulted in visible hyphal growth inhibition of Cronartium ribicola, Phellinus sulphurascens, Ophiostoma montium, and Ophiostoma clavigerum 3-12 days post-treatment. Pm-AMP1 also inhibited spore germination of several other phytopathogenic fungi by 32%-84% 5 days post-treatment. Microscopic examn. of C. ribicola hyphae in contact with Pm-AMP1 showed distinct morphol. changes. Seven western white pine (Pinus monticola Douglas ex D. Don) families (Nos. 1, 2, 5, 6, 7, 8, 10) showing partial resistance to C. ribicola in the form of bark reaction (BR) were assessed by Western immunoblot for assocns. between Pm-AMP1 accumulation and family, phenotype, canker no., and virulence of C. ribicola. There was a significant difference ($p < 0.001$) in mean Pm-AMP1 protein accumulation between families, with higher levels detected in the full-sib BR families (Nos. 1, 2, 5) than the half-sib BR families (Nos. 6, 7). Family 8, previously described as a Mechanism 'X' BR family, had the highest no. of BR seedlings and displayed high Pm-

AMP1 levels, whereas the susceptible family (No. 10) showed the lowest levels ($p < 0.05$). Family 1 showed a significant assocn. between Pm-AMP1 accumulation and overall seedling ***health*** ($p < 0.01$, $R = 0.533$), with higher protein levels obsd. in healthy vs. severely infected seedlings. In general, low Pm-AMP1 levels were obsd. with an increase in the no. of cankers per seedling ($p < 0.05$), and seedlings inoculated with the avirulent source of C. ribicola showed significantly higher Pm-AMP1 levels ($p < 0.05$) in the majority of BR families. Cis-acting regulatory elements, such as CCAAT binding factors, and an AG-motif binding protein were identified in the Pm-AMP1 promoter region. Multiple polymorphic sites were identified within the 5' untranslated region and promoter regions. Our results suggest that Pm-AMP1 is involved in the western white pine defense response to fungal infection, as obsd. by its antifungal activity on C. ribicola and a range of phytopathogens as well as through its assocn. with different indicators of resistance to C. ribicola.

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13. **State of Pine Decline in the Southeastern United States.**

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South.J.Appl.For. 2010, 34 (3): 138-141

English

ABSTRACT Pine decline is an emerging forest ***health*** issue in the southeastern United States. Observations suggest pine decline is caused by environmental stress arising from competition, weather, insects and fungi, anthropogenic disturbances, and previous management. The problem is most severe for loblolly pine on sites that historically supported longleaf pine, are highly eroded, or are not managed. The purposes of this technical note are (1) to describe the symptomology and extent of pine decline in the southeastern United States; (2) to describe its connection with root disease, resource stress, and silviculture; and (3) to summarize the consensus opinion of scientists and land managers during a workshop sponsored by the US Army Strategic Environmental Research and Development Program regarding the scope of this syndrome and the best research avenues to counter its potential effect on the sustainability of southern pine forests.

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14. **Effect of hot air treatment on postharvest mould decay in Chinese bayberry fruit and the possible mechanisms**

Kaituo Wang, Shifeng Cao, Peng Jin, Huaijing Rui and Yonghua Zheng.

Wang, Kaituo; Cao, Shifeng; Jin, Peng; Rui, Huaijing; Zheng, Yonghua (College of Food Science and Technology, Nanjing Agricultural University, Nanjing 210095, Jiangsu (CN)) EMAIL: zhengyh@njau.edu.cn

International Journal of Food Microbiology 2010, 141 (1-2): 11-16

English

ABSTRACT The effect of hot air treatment (HAT) on reducing natural fungal decay and green mould decay caused by ***Leptographium*** abietinum on postharvest Chinese bayberry fruit and the possible mechanisms were investigated. Freshly harvested Chinese bayberry fruit were firstly pretreated with hot air at 36-60 .degree.C for 1-3. h, and then stored at 20 .degree.C for 3. d or at 1 .degree.C for 12. d to investigate the optimum condition of hot air treatment (HAT) for inhibiting decay development. Results demonstrated that HAT at 48 .degree.C for 3. h was the most effective in reducing natural decay without impairing quality. This treatment also enhanced the resistance of Chinese bayberry fruit against green mould rot caused by L. abietinum and reduced the severity of the disease. The activities of defense-related enzymes including chitinase, .BETA.-1, 3-glucanase, peroxidase and polyphenol oxidase were significantly enhanced by HAT. In addition, the in vitro experiment showed that HAT significantly inhibited spore germination, germ tube elongation and mycelial growth of L. abietinum. These results indicate that HAT can effectively reduce fruit decay possibly by directly inhibiting pathogen growth and indirectly inducing disease resistance. .COPYRGT. 2010 Elsevier B.V.

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15. **De novo genome sequence assembly of a filamentous fungus using Sanger, 454 and Illumina sequence data.**

S. DiGuistini, N. Y. Liao, D. Platt, G. Robertson, M. Seidel, S. K. Chan, T. R. Docking, I. Birol, R. A. Holt, M. Hirst, E. Mardis, M. A. Marra, R. C. Hamelin, J. Bohlmann, C. Breuil and S. J. M. Jones.

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Genome biology 2009, 10 (9, R94): 24

English

ABSTRACT Sequencing-by-synthesis technologies can reduce the cost of generating de novo genome assemblies. We report a method for assembling draft genome sequences of eukaryotic organisms that integrates sequence information from different sources, and demonstrate its effectiveness by assembling an approximately 32.5 Mb draft genome sequence for the forest pathogen ***Grosmannia*** clavigera, an ascomycete fungus. We also developed a method for assessing draft assemblies using Illumina paired end read data and demonstrate how we are using it to guide future sequence finishing. Our results demonstrate that eukaryotic genome sequences can be accurately assembled by combining Illumina, 454 and Sanger sequence data.

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17. **Biological issues in Kitora Tumulus during relocation works of the mural paintings (2008)**

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Hozon Kagaku 2009, 48 167-174

Japanese

ABSTRACT In Jan. 2004, excavation of the Kitora Tumulus took place and the relocation of the paintings started. However, after people began to go into the tumulus, we started to see fungal growth. In the beginning, fungi such as Trichoderma sp., Penicillium sp. and Fusarium sp. were seen inside the Tumulus. Ethanol was mainly used to kill and remove such fungal colonies, as it was thought to be one of the mildest of fungicides with respect to the pigments used in the murals. In early 2005, small colonies of viscous gel appeared on some parts of the walls. In the summer of 2005, the viscous gels (biofilms) suddenly developed to form biofilms on the plaster walls. In the fall of 2005, small holes with black substances inside became obvious on the plaster walls; the holes seemed to have developed from the back side of the plaster. Such holes might have been caused by the activity of microbes, esp. by an acetic acid bacterium, Gluconacetobacter sp. The acetic acid bacterium had been isolated from one of the black substance from a hole of the ceiling. In 2008, almost all of the paintings on the side walls and the star charts on the ceiling were relocated, except for those which might have been hidden by a thin layer of mud on the walls. By a survey in June 2008, species of the fungal genera Penicillium, Clonostachys, Cladosporium, Phialophora, Ophiostoma, Cylindrocarpon, ***Phialocephala*** and Trichoderma were isolated from the eight gel samples from the interior of the stone chamber. But the basidiomycetous fungus Burgoa sp. and the anamorphic fungus Acremonium (sect. Gliomastix) sp. were not isolated this time. An acetic acid bacterium, Gluconacetobacter sp., was isolated from three samples out of eight, which suggested that this bacterium might spread to various parts of the stone chamber's interior. As this bacterium was shown to decomp. CaCO3, there is strong concern on its effect on the plaster. Chems. to treat microbes in Kitora Tumulus has been selected from consideration of their efficacy on microbes, possible ***adverse*** effects on paintings and ***toxicity*** to human. Mainly, ethanol, isopropanol, formalin and isothiazolones (Kathon CG) have been used depending on conditions of the plaster or the stones and purposes. Selection and the way of using the chems. must be carefully designed in line with the timing of further relocation of the plaster. Some chems. might be a carbon source to encourage growth of some microbes or secretion of acids by them. Special caution to such side effects is necessary.

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18. **Application of temporal temperature gradient gel electrophoresis for characterisation of fungal endophyte communities of Salix caprea L. in a heavy metal polluted soil.**

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Sci.Total Environ. 2009, 407 (24): 6179-6187

English

ABSTRACT Fungal endophytes can affect the heavy metal uptake of their host plants and increase the tolerance of their host plants to heavy metal stress. Therefore, in the present study, a wide-range screening of the fungal endophyte communities was conducted to determine the fungal distribution and diversity on S. caprea roots on a metal polluted site. Fungal communities were screened using amplification with the 5.8S-ITS2-28S part of the rDNA operon, with the resulting

amplicons analysed by temporal temperature gradient gel electrophoresis (TTGE) and sequencing. This technique is reproducible and shows good coverage of ascomycete and basidiomycete taxa, as 68% and 32% of all of the sequences, respectively. No clear shift in fungal ITS-TTGE profiles from S. caprea roots was seen along the secondary succession stages. Ascomycetes dominated the more polluted plots, while there was a greater diversity of basidiomycetes in the less polluted and control plots, suggesting greater tolerance of ascomycetes in comparison with basidiomycete fungi. The high diversity of DSEs was confirmed at the highly metal-enriched locations, with species belonging to the genera Phialophora, ***Phialocephala*** and Leptodontidium. Furthermore, the DSE colonisation of S. caprea roots and the frequency of the sequences showing affinity towards DSE genus Phialophora. showed good correspondence with soil Pb, Cd and plant-available P concentrations. possibly indicating that DSEs improve metal tolerance of willows to high heavy metal contamination. (C) 2009 Elsevier B.V. All rights reserved.

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19. **Horizontal transmission of the Picea glauca foliar endophyte ***Phialocephala*** scopiformis CBS 120377.**

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Fungal Ecology 2009, 2 (Part 2): 98-101

English

ABSTRACT We have studied the presence of the foliar endophtye of Picea glauca (white spruce) ***Phialocephala*** scopiformis CBS 120377 and its affect on the growth of Choristoneura fumiferana (spruce budworm). Here we examine the transmission of this fungus from 50 trees planted in a test field site to 250 P. glauca seedlings planted under the emerging canopies. After 3 y, the endophyte spread to 40 % of these trees (now 20-30 cm) with an average rugulosin (an anti-insect ***toxin***) concentration of 1 mu g g(-1). All woody plants within 2 m of the test trees were collected. These were all shown to be negative for P. scopiformis except for some spruce seedlings that arose from seeds (natural generation). This is positive evidence for the horizontal transmission of P. scopiformis and its apparent specificity to P. glauca under field conditions. (C) 2009 Elsevier Ltd and The British Mycological Society. All rights reserved.

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20. **Anti-insect secondary metabolites from fungal endophytes of conifer trees.**

M. W. Sumarah and J. D. Miller.

Natural Product Communications 2009, 4 (11): 1497-1504

English

ABSTRACT Choristoneura fumiferana is the most economically-important insect pest in eastern North America. Historically, strategies to control epidemics have relied on chemical pesticides that are no longer approved for use. The presence of fungal endophytes in cool area grass species and

their role in reducing the impact of herbivorous insects is well understood. Recent work has demonstrated that foliar endophytes of conifers also produce anti-insect ***toxins*** . Field and nursery studies testing trees infected with the rugulosin producing endophyte ***Phialocephala*** scopiformis reduced the growth and development of C. fumiferana. The study of foliar endophytes from a variety of conifers including: Picea mariana, P. rubens and P. glauca as well as Abies balsamea and Larix laricina for the discovery of other anti-insect ***toxins*** are discussed. These endophytes are horizontally transmitted thus they are not present in nursery seedlings. Inoculating seedlings with ***toxigenic*** endophyte strains has been demonstrated to be effective in providing the tree with tolerance to herbivorous insects.

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21. **Topographic features associated with loblolly pine decline in central Alabama.**

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For.Ecol.Manage. 2008, 255 (5-6): 1735-1739

English

ABSTRACT Loblolly pine (Pinus taeda L.) decline has been present in upland sites of central Alabama since the 1960s. Symptoms of loblolly pine decline (fine root deterioration, short chlorotic needles, sparse crowns, reduced radial growth) begin in the 30-40 year age class, resulting in premature death at ages 35-50. Loblolly pine decline occurs on sandy, well-drained soils and is associated with ***Leptographium*** spp., as well as with root-feeding bark beetles and weevils. The present article discusses the results of a comparison of biological factors associated with pine decline and topographical features, using the analysis of tree ***health*** at the Talladega National Forest and Westervelt (formerly Gulf State Paper) Company in central Alabama. Results of this study suggest that an ecological pattern of tree decline and mortality exists. Loblolly stands were more prone to develop symptoms at sites with increased slope and south/southwest orientation. This report indicates that the dominant determinants, or predictors, of loblolly decline are identifiable topographical features. (C) 2007 Elsevier B.V. All rights reserved.

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22. **Biological issues in Kitora Tumulus during relocation work of the mural paintings (2007)**

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Hozon Kagaku 2008, 47 129-134

Japanese

ABSTRACT Excavation of Kitora Tumulus started at the end of Jan. 2004. Some parts of the beautiful and very fragile mural paintings on a thin plaster layer had become detached. Thus it was

decided to relocate the mural paintings and to restore them in a ***safe*** environment. This report describes the biol. issues encountered during restoration work in 2007. As we had reported earlier, in the summer of 2005 and 2006, a viscous gel (biofilm) developed on the plaster walls. From such biofilms, many kinds of fungi were isolated in a survey of March 2007: Penicillium sp., Paecilomyces sp., Gliocladium sp., Acremonium, (sect.Gliomastix) sp., ***Phialocephala*** sp., Phialophora sp., Phoma sp., Ophiostoma sp. and Cladosporium sp. It seemed that diversity of fungi and bacteria in the tumulus was increasing. A biocide based on two isothiazolones (for example, Kathon CG) was applied to the biofilm to prevent its further growth, where it was necessary. But its use had to be carefully planned in consideration of the timing of relocation of the plaster parts. Black fungi such as basidiomycetous fungus Burgoa sp. and ascomycetous fungus Acremonium (sect. Gliomastix) sp. were also seen on the walls in 2006 and 2007. Penicillium sp. was also often seen on the floor stones. On the plasters of the ceiling and walls, Trichoderma sp. and Verticillium sp. etc. were seen like white strings or clumps. Special care must be taken until all the plaster paintings are relocated, and preventive measures must be carefully considered together with the conditions of the plasters and timing of the relocation work.

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23. **Interactions among scolytid bark beetles and the associated fungi during attacking the living conifers.**

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Chinese Bulletin of Entomology 2008, 45 (4): 518-527

Chinese

ABSTRACT Scolytid bark beetles that colonize living conifers are frequently associated with specific fungi that are carried in specialized structures or on the body Surface. These fungi are introduced into the tree during the attack process. The association suggests that there is mutual benefit to the fitness of both beetles and fungi. The fungal species may benefit from the association with the beetles by being transported to new host trees while beetle species may benefit from the association with fungi by feeding on the fungi, or by the fungi contributing to the death of the host trees through mycelial penetration of host tissue or ***toxin*** release. Extensive researches have been directed towards characterizing the interactions of beetle-fungal complexes with live host conifers and determining the ecological advantages for maintaining the associations. However, differences among systems and how species interact under different population and environmental conditions make it difficult to generalize about the importance of the separate biological components in successful host colonization.

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24. **Seasonal water stress and the resistance of Pinus yunnanensis to a bark-beetle-associated fungus**

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Tree Physiol. 2008, 28 (5): 679-687

English

ABSTRACT We examined the influence of seasonal water stress on the resistance of Pinus yunnanensis (Franch.) to inoculation with ***Leptographium*** yunnanense, a pathogenic fungus associated with the aggressive bark beetle, Tomicus n. sp. Experiments took place between October 1997 and November 1999 in two plots located at the top and at the foot of a hill in Shaogiu, China, a region characterized by dry winters and wet summers. Following isolated and mass fungal inoculations, we observed the reaction zone length, fungal growth in the phloem, and the occlusion, blue-staining and specific hydraulic conductivity of the sapwood. Measurements of soil and needle water contents and predawn needle water potentials confirmed that trees were subject to mild water stress during winter, especially at the drier hilltop site. Measures of tree resistance to fungal infection of phloem and sapwood were congruent and indicated that trees were most susceptible to inoculation during the wet summer, especially at the lower-elevation plot. Specific hydraulic conductivity decreased after inoculation in summer. The results indicate that mild seasonal water stress is not likely responsible for the recent extensive damage to young P. yunnanensis stands by Tomicus n. sp. in the vicinity of our study plots. Rather, the results suggest that mild water stress enhances tree resistance to fungal pathogens associated with Tomicus n. sp.

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25. **Diversity and structure of ectomycorrhizal and co-associated fungal communities in a serpentine soil**

Alexander Urban, Alexander Urban, Alexander Urban, Joseph Strauss, Markus Gorfer, Alexander Urban, Joseph Strauss, Markus Gorfer and Markus Puschenreiter.

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MYCORRHIZA 2008, 18 (6-7): 339-354

English

ABSTRACT The community of ectomycorrhizal (ECM) and co-associated fungi from a serpentine site forested with Pinus sylvestris and Quercus petraea was explored, to improve the understanding of ECM diversity in naturally metalliferous soils. ECM fungi were identified by a combination of morphotyping and direct sequencing of the nuclear ribosomal internal transcribed spacer region 2 and of a part of the large-subunit region. Co-associated fungi from selected ECM were identified by restriction fragment length polymorphism and sequencing of representative clones from libraries. Polymerase chain reaction with species-specific primers was applied to assess patterns of association of ECM and co-associated fungi. Twenty ECM species were differentiated. Aphyllophoralean fungi

representing several basidiomycete orders and Russulaceae were dominant. ***Phialocephala*** fortinii was the most frequently encountered taxon from the diverse assemblage of ECM co-associated fungi. A ribotype representing a deeply branching ascomycete lineage known from ribosomal deoxyribonucleic acid sequences only was detected in some ECM samples. A broad taxonomic range of fungi have the potential to successfully colonise tree roots under the extreme edaphic conditions of serpentine soils. Distribution patterns of ECM-co-associated fungi hint at the importance of specific inter-fungal interactions, which are hypothesised to be a relevant factor for the maintenance of ECM diversity.

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26. **Two new bisorbicillinoids isolated from a deep-sea fungus, ***Phialocephala*** sp. FL30r.**

D. H. Li, F. P. Wang, S. X. Cai, X. Zeng, X. Xiao, Q. Q. Gu and W. M. Zhu.

Univ.Ocean-China

J.Antibiot. 2007, 60 (5): 317-320

English

ABSTRACT This study reported the isolation, structure elucidation, and cytotoxic activities of 2 new bisorbicillinoids, named oxosorbiquinol (1) and dihydrooxosorbiquinol (2). They were isolated from a deep-sea fungus, ***Phialocephala*** sp., and they were characterized using MS. The absolute configurations of (1 and 2) were determined by their biosynthesis route and analysis of the CD spectrum. Except for the A549 cell line, (1 and 2) showed weak cytotoxic activities against the other cell lines (P388, HL60, BEL7402 and K562). (No EX).

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27. **Trisorbicillinone A, a novel sorbicillin trimer, from a deep sea fungus, ***Phialocephala*** sp. FL30r.**

D. H. Li, F. P. Wang, X. Xiao, Y. C. Fang, T. J. Zhu, Q. Q. Gu and W. M. Zhu.

UNIV.CHINA-OCEAN; INST.OCEANOGR.THIRD-CHINA

Tetrahedron Lett. 2007, 48 (30): 5235-5238

English

ABSTRACT Trisorbicillinone A (1) is the 1st example with the novel trimeric sorbicillin skeleton. Trisorbicillinone A (1) was isolated from ***Phialocephala*** sp. FL30r and was tested for its in-vitro cytotoxic activity against P388, A549, HL60, BEL7402 and K562 cell lines by the MTT method. Compound was characterized using IR, MS, PMR, CMR, and 2D-NMR. Trisorbicillinone A (1) showed cytotoxicity against P388 and HL60 cells, with HL60 cells being the most sensitive. IC50 values of trisorbicillinone A (1) against P388, A549, HL60, BEL7402 and K562 cell lines were 9.10, higher than 100, 3.14, 60.28 and 30.21 uM, respectively. (No EX).

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28. **Mountain pine beetle associated blue-stain fungi cause lesions on jack pine, lodgepole pine, and lodgepole x jack pine hybrids in Alberta.**

Adrianne V. Rice, Markus N. Thormann and David W. Langor.

Nat Resources Canada, Canadian Forest Serv, No Forestry Ctr, 5320-122 St, Edmonton, AB T6H 3S5, Canada arice@nrcan.gc.ca

Canadian Journal of Botany 2007, 85 (3): 307-315

English

ABSTRACT Mountain pine beetles (Dendroctonus ponderosae Hopkins; (MPB)) have spread into lodgepole x jack pine hybrid (Pinus contorta Douglas x Pinus banksiana Lambert) forests in Alberta and are predicted to spread into jack pine forests. Their success in these forests is uncertain but will be influenced by multiple factors, including the ability of their associated blue-stain fungi to colonize the trees and the ***health*** of the encountered trees. Healthy and dwarf mistletoe infected pines at three sites across Alberta (one site per pine species) were inoculated with three isolates each of ***Grosmannia*** clavigera (Robinson-Jeffrey and Davidson) Zipfel, de Beer and Wingfield and Ophiostoma montium (Rumbold) von Arx. Both fungi grew and caused lesions on all hosts, suggesting that MPB will not be limited by a lack of fungal growth. Both fungi caused longer lesions in jack and hybrid pines than in lodgepole pines, indicating that susceptibility varies among hosts and is greater in the novel systems than in the co-evolved one. G. clavigera caused longer lesions than O. montium in hybrids and lodgepole pines, while the two species caused similar-sized lesions on jack pine. Intraspecific variation was high in G. clavigera, with one isolate producing much shorter lesions than the other two. Dwarf mistletoe infestation had little effect on infection lesion length.

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29. **Microbial Root Endophytes.**

[Editor] Schulz BJE, [Editor] Boyle CJC and [Editor] Sieber TN.

Tech Univ Carolo Wilhelmina Braunschweig, Inst Microbiol, Spielmannstr 7, D-38106 Braunschweig, Germany
2006,
English

ABSTRACT This 367-page book is volume 9 in the series ''Soil Biology'', and this volume focuses on the interactions of non-mycorrhizal microbial endophytes with plant roots. This volume contains 19 individually-authored chapters. The first chapter provides an introduction to microbial endophytes. The remaining 18 chapters are divided among 3 major parts. Part I of the book focuses on endophytic bacteria, and contains chapters 2-6, inclusive. Topics covered in these 5 chapters include, respectively: spectrum and population dynamics of bacterial root endophytes; bacterial endophytes as elicitors of induced systemic resistance; control of plant pathogenic fungi with bacterial endophytes; the role of proteins secreted by rhizobia in symbiotic interactions with leguminous roots; and research on endophytic bacteria, in particular recent advances in forest trees. Endophytic fungi are the focus of part II, which contains chapters 7-16, inclusive. Specific chapter topics in this second part include: biodiversity of fungal root-endophyte communities and populations, in particular of the dark septate endophyte ***Phialocephala*** fortinii; endophytic

root colonization by Fusarium species, in terms of histology, plant interactions, and ***toxicity*** ; microbial endophytes of orchid roots; fungal endophytes in submerged roots; nematophagous fungi as root endophytes; molecular diversity and ecological roles of mycorrhiza-associated sterile fungal endophytes in Mediterranean ecosystems; Oidiodendron maius as a saprobe in sphagnum peat, and a mutualist in ericaeous roots; mycorrhizal and endophytic fungi of epacrids; mutualistic interactions with fungal root endophytes; and understanding the roles of multifunctional mycorrhizal and endophytic fungi. The third part of the book focuses on methods, and contains chapters 17-19, inclusive. Individual chapter topics in this third part include: isolation procedures for endophytic microorganisms; microbial interactions with plants; and application of molecular fingerprinting techniques to explore the diversity of bacterial endophytic communities. Each chapter is internally structured into more specific sections within the chapter scope, and each chapter is independently referenced. The text is written in English, and the book is indexed by subject. The book is illustrated with 29 figures, 4 of which are in color. The book includes a list of the contributors and their respective institutions. This book will appeal to students and researchers in the fields of microbiology, botany, ecology, agriculture, forestry, and soil science.

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30. **Effects of four monoterpenes on the growth in vitro of some Heterobasidion spp. and two ***Leptographium*** species**

L. Zamponi, M. Michelozzi and P. Capretti.

Univ Florence, Dept Agr Biotechnol, Piazzale Cascine 28, I-50144 Florence, Italy (Reprint) E-mail: letizia.zamponi@unifi.it; Univ Florence, Dept Agr Biotechnol, I-50144 Florence, Italy; CNR, Inst Plant Genet, I-50019 Florence, Italy

J. PLANT DIS, PROT, 2006, 113 (4): 164-167

English

ABSTRACT The inhibitory effects of the monoterpenes (-)-alpha-pinene, (-)-beta-pinene, delta-3-carene and myrcene, in both the contact and the volatile phase, on Heterobasidion spp, ***Leptographium*** wingfleldii and ***Leptographium*** serpens were studied in vitro. Inhibition was usually greater when the monoterpenes were in contact with the fungi than when the fungi were exposed to the monoterpene vapours. Myrcene was the most strongly inhibiting compound for H. annosum s.l., followed by delta-3-carene; the Leptographiurn spp. were inhibited most strongly by delta-3-carene. Exposure to the monoterpenes reduced conidiogenesis in H. abietinum. After exposure of the fungi to the monoterpenes was discontinued, there were in general no residual effects on fungal growth, apart from ***Leptographium*** spp. that had been exposed to delta-3-carene.

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31. **Monitoring loblolly pine decline in the western zone of the Southern Region**

N. Hess, L. Eckhardt, R. Menard and J. Jones.

USDA Forest Service, Pineville, LA, USA

Phytopathology 2005, 95 (6 Suppl):

ABSTRACT More than one million acres of southern yellow pine stands across the south are estimated to be declining. Loblolly pine stands in 3 southern states (with a predominance of sites in central and southern Alabama) are reported to be declining based on data from Forest ***Health*** Monitoring plots established in the 1990's. Current studies of affected stands show a strong correlation with (upland) site, deterioration of fine roots, presence of a fairly constant set of insect associates, and infection by ***Leptographium*** spp. affecting the primary roots. The influence of fire, previous agriculture, and landform are other factors that may have a role in the loblolly decline complex. The symptoms of the decline are sparse crowns, short chlorotic needles, and reduced radial growth primarily above 40 years of age and first apparent in trees in the 40-50 year age class. Mortality can occur beginning as little as two to three years after first symptom expression. Although the symptoms of the decline complex are similar to those of littleleaf disease, the site conditions on stands are found expressing this decline are different. On upland sites on federal lands, loblolly pine is the major forest type since it was used for watershed restoration and is easily regenerated. Many of these sites are now managed for red-cockaded woodpecker (RCW) habitat and decline of the pine stands is impacting the long-term habitat needs of this endangered species. One of mitigations recommended for decline sites is to convert them to longleaf pine where site conditions are suitable to fulfill the habitat requirements for RCW. Additional monitoring plots are being installed on National Forest upland sites in Texas, Louisiana, Arkansas, and Mississippi. This monitoring process will help define the range and impact of loblolly pine decline and will further assess ***Leptographium*** spp. component of the pine decline complex.

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32. **Seeking the elusive function of the root-colonising dark septate endophytic fungi**

K. Mandyam and A. Jumpponen.

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STUDIES IN MYCOLOGY 2005, 53 173-189

English

ABSTRACT A comparison of published estimates of mycorrhizal and dark septate endophyte (DSE) colonisation from various ecosystems suggests that DSE may be as abundant as mycorrhizal fungi as judged by the proportion of host plants colonised in mixed plant communities, or by the extent of colonisation in sampled root systems. While many strides have been made in understanding the ecological significance of the mycorrhizal fungi, our knowledge about the role of DSE fungi is in its infancy. In order to provide a framework of testable hypotheses, we review and discuss the most likely functions of this poorly understood group of root-associated fungi. We propose that, like mycorrhizal symbioses, DSE-plant symbioses should be considered multifunctional and not limited to nutrient acquisition and resultant positive host growth responses. Admittedly, many mycorrhizal and endophyte functions, (e.g. stress tolerance, pathogen or herbivore deterrence) are likely to be mediated by improved nutritional status and increased fitness of the host. Accordingly, it is pivotal to establish whether or not the DSE fungi are involved in host nutrient acquisition, either from inorganic and readily soluble sources, or from organic and recalcitrant sources. Facilitation by DSE of the use of organic nitrogen, phosphorus and sulphur sources by plants is a topic that warrants further attention and research. Even in the absence of a clear nutrient uptake function, the extensive DSE colonisation that occurs is likely to pre-emptively or competitively deter

pathogens by minimising the carbon available in host rhizosphere environment. The DSEs' high melanin levels and their potential production of secondary metabolites ***toxic*** or inhibitory to herbivores are also likely to be factors influencing host performance. Finally, the broad host ranges speculated for most DSE fungi thus far suggest that they are candidates for controlling plant community dynamics via differential host responses to colonisation. We emphasise the need for simple experiments that allow unravelling of the basic biological functions of DSE fungi when they colonise their hosts.

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34. **Southern Interior Forest Region: forest ***health*** stand establishment decision aids.**

A. Stock, M. Duthie-Holt, S. Walsh, J. Turner and K. Swift.

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Journal of Ecosystems and Management 2005, 6 (1): 55-73

English

ABSTRACT This paper presents the Stand Establishment Decision Aid (SEDA) for the Southern Interior Forest Region, British Columbia, Canada, for different forest ***health*** problems such as the black army cutworm [Actebia fennica [Dissimactebia fennica]], black stain root disease [***Leptographium*** wageneri [Ophiostoma wageneri]], comandra blister rust [Cronartium comandrae], rhizina root disease [Rhizina undulata], spruce/white pine weevil [Pissodes strobi], western gall rust [Endocronartium harknessii], western hemlock looper [Lambdina fiscellaria lugubrosa], western spruce budworm [Choristoneura occidentalis], and white pine blister rust [Cronartium ribicola]. Each SEDA provides a hazard rating system that identifies the specific biogeoclimatic zone and subzone where the forest ***health*** problem potentially occurs, a detailed description of the characteristics of susceptible stands, and some general information on the insect's or pathogen's biology. In addition, harvest and silviculture strategies to consider when managing susceptible stands are presented, as well as the potential productivity implications of infestations. Each SEDA concludes with a resource section outlining where more information can be located.

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35. **Bioassay of the pathogenic ***toxin*** of ***Leptographium*** yunnanense, a fungus associated with Tomicus piniperda.**

Zhou-yu Liao and Hui Ye.

Sch Life Sci, Yunnan Univ, Kunming, Yunnan, 650091, China

For.Res. 2004, 17 (1): 102-105

Chinese

ABSTRACT Pathogenic ***toxin*** of ***Leptographium*** yunnanense associated with Tomicus piniperda could pathogeny not only to Pinus yunnanensis, but also to P. armandii and P.

kesiya var. langbianensis. The symptoms were very complicated and not easy to be observed and determined when bioassaying the ***toxin*** with seedlings cutted roots, however, when bioassaying the ***toxin*** with sampling trees and its wood blocks, the symptoms would be observed clearly, and as the same as they were induced by inoculating the fungus to the host trees. The bioassay results would be observed sooner (about 5 days) when bioassaying the ***toxin*** with wood block, so it was better to inoculate the pathogenic ***toxin*** to the wood blocks for bioassay.

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37. **Determination and basic properties about the pathogenic ***toxin*** of ***Leptographium*** yunnanense, a blue-stain fungus associated with Tomicus piniperda.**

Liao ZhouYu, Ye Hui, Z. Y. Liao and H. Ye.

Institute of Ecology and Geobotany, Yunnan University, Kunming, 650091, China. EMAIL: liaozhyu@sohu.com; yehui@ynu.edu.cn

Journal of Beijing Forestry University 2003, 25 (5): 38-41

English

ABSTRACT A study was conducted to determine the properties of the pathogenic ***toxin*** of ***Leptographium*** yunnanense, a blue-stain fungus associated with Tomicus piniperda attacking Pinus yunnanensis. The fungus could produce pathogenic ***toxin*** when cultured in PD liquid medium for 30 days. The protein part of the original ***toxin*** liquid possesses the pathogenic activity. The ***toxin*** could keep its activity for 20 to 40 days under cold storage or room temperature. Increase in temperature decreased the ***toxin*** activity gradually. At 60.degree.C, the pathogenic ***toxin*** could still keep some activities, but lose fully when the temperature exceeded 80.degree.C. The pH value and osmotic potential of the ***toxin*** extraction liquid was 5.1 and -120 kPa, respectively, but the ***toxicity*** of the extract liquid was not induced by pH value and osmotic potential.

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38. **Decline in diversity and abundance of endophytic fungi in twigs of Fagus sylvatica L. after experimental long-term exposure to sodium dodecylbenzene sulphonate (SDBS) aerosol**

Roberto Danti, Giovanni Sanguineti, Paolo Raddi, Vincenzo Di Lonardo and Thomas N. Sieber.

Danti, Roberto; Sanguineti, Giovanni; Raddi, Paolo; Di Lonardo, Vincenzo (C. N. R., Ist. Patol. Alberi Forestali, Piazzale delle Cascine 28, I-50144 Firenze (IT)); Sieber, Thomas N. (Swiss Fed. Institute of Technology, Department of Forest Sciences, ETH-Zentrum, CH-8092 Zurich (CH)) EMAIL: thomas.sieber@fowi.ethz.ch

Environmental microbiology 2002, 4 (11): 696-702

English

ABSTRACT Sodium dodecylbenzene sulphonate (SDBS) is an anionic synthetic detergent found in

polluted sea aerosol and is known for its harmful effects on leaf surface ultrastructure on conifers and broadleaved trees. Four-year-old saplings of European beech were sprayed weekly for three consecutive growing seasons with either a 50 mg l -1 solution of SDBS in deionized water or with pure deionized water (control). Two- to three- year-old twigs were collected from SDBS-treated and control plants during the growing season one year after the last treatment to isolate endophytic fungi. The frequency of colonization by endophytic fungi was significantly lower on SDBS-treated plants (63.8%) than on control plants (85.4%). Multiple colonization of twigs occurred more frequently and diversity of endophyte species was higher in control plants than in SDBS-treated plants. Thirty-six fungal species were isolated from 360 twigs. Cladosporium cladosporioides, Coryneum compactum, ***Phialocephala*** dimorphospora, and a species each of Mycosphaerella and Phomopsis were the most abundant endophytes with frequencies of colonization of more than 5%. The abundance of the Phomopsis species proved to be significantly reduced by the SDBS treatment. Within the limits of the indoor experimental conditions, the obtained results suggest that long-term exposure of aerial parts of beech to SDBS can affect the amount and composition of endophytic fungal communities of lignified twigs. Degradation of the leaf epicuticular wax layer and changes of the assimilation capacity and leaf water content (transpiration) of the crowns are presumed to be responsible for the reduction of endophytic fungi detected in twigs of SDBS-treated plants.

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39. **Association of wildfire with tree ***health*** and numbers of pine bark beetles, reproduction weevils and their associates in Florida.**

James L. Hanula, James R. Meeker, Daniel R. Miller and Edward L. Barnard.

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For.Ecol.Manage. 2002, 170 (1-3): 233-247

English

ABSTRACT Wildfires burned over 200,000 ha of forest lands in Florida from April to July 1998. This unique disturbance event provided a valuable opportunity to study the interactions of summer wildfires with the activity of pine feeding insects and their associates in the southeastern United States. We compared tree mortality with abundance of bark and ambrosia beetles, reproduction weevils and wood borers relative to fire severity. Over 27% of residual live trees in stands that experienced high fire severity died between October 1998 and May 1999. An additional 2-3% of trees that initially survived the fire died during the second year compared to <1% mortality in unburned stands. One year after the fire, more than 75% of the trees surviving in high fire severity stands had roots infected with one or more species of ***Leptographium*** and/or Graphium spp. and nearly 60% of the sampled roots were infected. No such fungi were recovered from roots of trees in unburned stands. Significantly, more root weevils, Hylobius pales and Pachylobius picovorus, were captured in unbaited pitfalls in the moderate and high fire severity stands than in the controls. Mean trap catches of Ips grandicollis, Dendroctonus terebrans and Hylastes salebrosus, three common bark beetles that feed on phloem tissue of pines, were lower in Lindgren traps in the fire-damaged areas than in the control stands. In contrast, catches of the ambrosia beetles. Xyleborus spp. and Monarthrum mali, were higher in burned stands than in control stands. The generalist predator, Temnochila virescens (Coleoptera: Trogositidae), showed a strong positive relationship

between abundance and fire severity, while the flat bark beetle, Silvanus sp. (Coleoptera: Sylvanidae), exhibited the reverse trend. Our results show that most tree mortality occurred within 1 year of the fire. Ips or Dendroctonus bark beetle populations did not build up in dead and weakened trees and attack healthy trees in nearby areas. The prevalence of ***Leptographium*** spp. in roots may be a symptom of, or result in, weakened trees that may affect the trees' susceptibility to bark beetles in the future.

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40. **Assessment of loblolly pine decline in Central Alabama.**

N. J. Hess, W. J. Otrosina, E. A. Carter, J. R. Steinman, J. P. Jones, L. G. Eckhardt, A. M. Weber, Walkinshaw, C. H. Editor: Outcalt,K.W., P. A. Outcalt and R. B. Tucker.

USDA Forest Service, Pineville, Louisiana, USA.

General Technical Report - Southern Research Station, USDA Forest Service 2002, (SRS-48): 558-564

English

ABSTRACT Loblolly pine (Pinus taeda) decline has been prevalent on upland sites of central Alabama (USA) since the 1960s. The purpose of this study was to compare Forest ***Health*** Monitoring (FHM) standards and protocols with root ***health*** evaluations relative to crown, stem, and site measurements. Thirty-nine 1/6 acre plots were established on loblolly decline sites in nine central Alabama counties. Sites were selected on federal, state, and private industrial lands to measure variables of decline symptoms, age classes and management procedures. A two-root sampling procedure, selective media, and soil baiting assay methods were used to isolate pathogenic root infecting fungi. Pitfall traps collected root-feeding insects from which ***Leptographium*** species were recovered. FHM indicators of tree crown conditions were recorded on all pines in the plots. Preliminary results showed a significant correlation between live crown ratio and incidence of ***Leptographium*** spp. We recovered ***Leptographium*** from damaged roots in eighty-four percent of plots. The pine basal area was significantly reduced with increased incidence of Phytophthora cinnamomi, with P. cinnamomi being recovered from the soil root zone in 50% of the plots. Histological examination of root damage indicated a significant correlation between reduced growth and root wounding.

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41. **Association, population dynamics, and underlying behaviors of bark beetle predators in the ***health*** of red pine plantations (Pinus resinosa)**

Nadir [Ph D.]. Erbilgin and Kenneth F. [adviser] Raffa.

The University of Wisconsin - Madison (0262)

Dissertation Abstracts International 2001, 62 (7B): 3050

English

ABSTRACT Stem and root colonizing beetles, and their associated staining fungi and predators,

were associated with declining red pine, Pinus resinosa, stands in Wisconsin. Monitoring of seventeen declining and healthy plantations over 3 years showed that populations of predators of Ips, particularly Thanasimus dubius, Platysoma cylindrica, and P. parallelum , were higher in healthy stands. Declining stands had more lower stem infesting Dendroctonus valens and Pissodes spp. Furthermore, declining stands had higher Ips and lower predator populations early in the season, whereas healthy stands had higher numbers of predator populations early in the season. Populations of root weevils were higher in healthy stands early in the season, but were higher in declining stands later in the season. The staining fungi ***Leptographium*** procerum and ***Leptographium*** terebrantis were more frequently isolated from roots in declining than healthy stands and more frequently associated with trees at the pocket margin than in the asymptomatic portions of declining stands. Thanasimus dubius and P. cylindrica were attracted to cues associated with feeding on bark-phloem disks by two scolytids that produce adult pheromones, I. pini and I. grandicollis, but not to other phloeophagous species that colonize the same trees. We conducted additional experiments to evaluate the responses of bark beetles and their predators to various blends of Ips spp. pheromones and conifer monoterpenes. Ips pini were not attracted to monoterpenes alone. However, with the exception of 3-carene, the effect of monoterpenes on I. pini's response to its pheromone, ipsdienol plus lanierone, was inhibitory. In contrast, (-)-.alpha.-pinene both attracted I. grandicollis, and enhanced the attraction of I. grandicollis to its pheromone, ipsenol. No monoterpene inhibited the response of I. grandicollis to its pheromone. Monoterpenes alone were not attractive to predators. However, some monoterpenes, such as myrcene and to a lesser extent 3-carene, reduced, whereas others, such as .alpha.-pinene, consistently enhanced, predator attraction to the pheromones of their Ips prey. Furthermore, (+)-.alpha.-Pinene synergized predator responses to the pheromone of I. pini, whereas (-)-.alpha.-pinene synergized responses to the pheromone of I. grandicollis. This pattern occurred for all major predator species.

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42. **Impact of biocontrol Pseudomonas fluorescens CHA0 and a genetically modified derivative on the diversity of culturable fungi in the cucumber rhizosphere.**

M. Girlanda, S. Perotto, Y. Moenne-Loccoz, R. Bergero, A. Lazzari, G. Defago, P. Bonfante and A. M. Luppi.

Appl.Environ.Microbiol. 2001, (Volume 67, Number 4): pp 1851

English

ABSTRACT Summary: Little is known about the effects of Pseudomonas biocontrol inoculants on nontarget rhizosphere fungi. This issue was addressed using the biocontrol agent Pseudomonas fluorescens CHA0-Rif, which produces the anti-microbial polyketides 2,4-diacetylphloroglucinol (Phl) and pyoluteorin (Plt) and protects cucumber from several fungal pathogens, including Pythium spp., as well as the genetically modified derivative CHA0-Rif-(pME3424). Strain CHA0-Rif(pME3424) overproduces Phl and Plt and displays improved biocontrol efficacy compared with CHA0-Rif. Cucumber was grown repeatedly in the same soil, which was left uninoculated, was inoculated with CHA0-Rif or CHA0-Rif(pME3424), or was treated with the fungicide metalaxyl (Ridomil). Treatments were applied to soil at the start of each 32-day-long cucumber growth cycle, and their effects on the diversity of the rhizosphere populations of culturable fungi were assessed at the end of the first and fifth cycles. Over 11,000 colonies were studied and assigned to 105 fungal species (plus several sterile morphotypes). The most frequently isolated fungal species (mainly belonging to the genera Paecilomyces, ***Phialocephala*** , Fusarium, Gliocladium, Penicillium, Mortierella, Verticillium,

Trichoderma, Staphylotrichum, Coniothyrium, Cylindrocarpon, Myrothecium, and Monocillium) were common in the four treatments, and no fungal species was totally suppressed or found exclusively following one particular treatment. However, in each of the two growth cycles studied, significant differences were found between treatments (e.g., between the control and the other treatments and/or between the two inoculation treatments) using discriminant analysis. Despite these differences in the composition and/or relative abundance of species in the fungal community, treatments had no effect on species diversity indices, and species abundance distributions fit the truncated lognormal function in most cases. In addition, the impact of treatments at the 32-day mark of either growth cycle was smaller than the effect of growing cucumber repeatedly in the same soil.

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43. **Oak ***health*** in Europe and its perspectives.**

T. Editor: Radoglou Oszako K.
27 August-1 September 2001
Forest Research Institute in Sekocin Las, 05-090 Raszyn, Poland. EMAIL: T.Oszako@ibles.waw.pl

Proceedings International Conference Forest Research: a challenge for an integrated European approach, 2001, 1 (245): 252

English

ABSTRACT Cases of abnormal oak mortality have already occurred repeatedly in Europe. From the point of view of symptomatology and aetiology it is a part of the broader phenomenon called oak decline which has its own specificity, dynamics and local dissimilarity. Major symptoms of oak decline such as progressive crown transparency, dieback and bark necroses show regional and temporal differences. From the upper part of declining crowns or dead trunks or roots of oaks it has been possible to isolate many fungi species. Inoculation tests carried out showed that they could not be responsible for the mass decline of oak. Using criteria concerning ecological functions these fungi could be divided into: * fungi which are pathogens: e.g. Hypoxylon mediteraneum, Pthytophthora spp., Fusicoccum quercus, Pezicula cinnamomea, Phomopsis quercella, Phellinus robustus, Armillaria spp.; Collybia fusipes * fungi colonising weakened and declining tissues: e.g. Amphiporthe leiphaemia, Colpoma quercinum, Cytospora intermedia, Arternaria alternata, Aureobasidium pullulans, Botrytis cinerea; * fungi causing discoloration of wood: e.g. Aposphaeria spp., Phialophora melini, ***Phialocephala*** dimorphospora, Ceratocystis/Ophiostoma spp.; * fungi existing with insects: e.g. Ambriosiella sp.; * fungi typical of other environments which colonised the investigated tissues only by chance: e.g. Mucor sp., Mortiella sp. Very often the colonisation of oak tissues by fungi was a secondary one which could only enhance the process of decline. Assuming that natural and anthropogenic stresses and biotic factors have predisposed trees, every actual stress factor will reduce the vitality of oaks and be followed by killing by secondary organisms. On the basis of the assumption that the present oak decline is not an epidemic disease but mainly geared by extreme weather conditions, further development will depend on weather circumstances.

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44. **Root diseases in Eastern Oregon and Washington. Special issue: Forest ***health*** and productivity in eastern Oregon and Washington**

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Northwest Science 2001, 75 (Special Issue): 38-45

English

ABSTRACT Root diseases are important natural disturbance agents affecting all tree species and all forest ecosystems of the Pacific Northwest. They influence stand structure, density, composition, function, and yield. The root diseases of greatest concern in eastern Washington and Oregon, USA are annosus root disease (caused by Heterobasidion annosum), Armillaria root disease (caused by Armallaria ostoyae), laminated root rot (caused by Phellinus weirii), and black stain root disease (caused by ***Leptographium*** wageneri). For these diseases, some information is available on root disease ecology, population dynamics, biology of the pathogens, and the natural processes that regulate them. Less is known about disease effects at the watershed or landscape level or how diseases interact with other disturbances and how they respond to management strategies. Decision support, such as the Western Root Disease Model, is being developed. In response to a shift in forest species and structure following a century of fire suppression and partial cutting, root diseases are believed to have spread causing more inoculum on more sites than existed in historical times. Stand management to restore historical vegetation conditions may intensify disease; however, in cases where diseased stands are converted to seral species, incidence and severity of root diseases will likely decline. Treatments that retain high stocking of host species, or allow host regeneration, will likely result in increased levels of root diseases because inoculum remains on the site. Spore infections may increase with partial cutting where stumps are not chemically treated. Disturbance increases activity of some insects, which may act as vectors of root disease pathogens.

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45. **Dark septate hyphomycetes in swiss conifer forest soils surveyed using Norway-spruce seedlings as bait.**

Karin Ahlich, Daniel Rigling, Ottmar Holdenrieder and Thomas N. Sieber.

Dep. Wood For. Sci., Sect. For. Pathol. Dendrol., Swiss Federal Inst. Technol., ETH-Zentrum, CH-8092 Zurich, Switzerland

Soil Biol.Biochem. 1998, 30 (8-9): 1069-1075

English

ABSTRACT Soil samples were collected under Norway-spruce trees (Picea abies (L.) Karst.) on 72 sites throughout Switzerland, passed through a disinfected sieve and put into autoclaved clay pots. Surface-sterilized Norway-spruce seeds were sown into these pots and the seedlings were reared in a growth cabinet for 4 months. The root systems of nine seedlings per site were surface-sterilized and one 23 mm long segment of each of two root-diameter classes (class 1: dia < 1 mm, class 2: dia 1-2 mm) was aseptically excised from each root system and plated out on 2% (w/v) malt-extract agar to isolate fungi. Dark septate hyphomycetes (DSH) could be isolated from the soil samples of all except two sites. The frequency of seedling roots colonized by DSH ranged from 11-100%, the average being 76%. Colonization of root diameter class 2 was significantly higher than the one of class 1. No

site-dependent pattern of colonization could be recognized except for the soil pH value. The frequency of DSH was correlated with the pH value of the collection sites and reached the maximum between pH values 3.5 and 4.5. The ***health*** status of the seedlings as measured by needle color, presence or absence of needle tip chlorosis, the number of living seedlings per pot and the dry weight was not correlated with the colonization of the roots by DSH. About 49% of the DSH sporulating after 1 y of incubation at 4degreeC in the dark were identified as ***Phialocephala*** fortinii. Advantages and disadvantages of the baiting technique are discussed.

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46. **Sapwood and crown symptoms in ponderosa pine infected with black-stain and annosum root disease.**

Rick G. Kelsey, Gladwin Joseph and Walter G. Thies.

USDA For. Serv., Pac. Northwest Res. Stn., Corvallis, OR 97331, USA

For.Ecol.Manage. 1998, 111 (2-3): 181-191

English

ABSTRACT Crown growth parameters, extent of disease in roots, and volatile compounds from sapwood were measured for diseased and healthy ponderosa pine (Pinus ponderosa Dougl. ex Laws.). The pathogens were black-stain root disease, caused by ***Leptographium*** wageneri var. ponderosum (Harrington's and Cobb), annosum root disease, caused by Heterobasidion annosum (Fr.) Bref., or both. Samples of sapwood collected near the root collar were heated in sealed vials and volatile compounds analyzed by gas chromatography. Acetaldehyde, acetone, ethanol, methanol, and 2-propanol were quantified. Acetaldehyde was selected by logistic regression to be the best predictor for distinguishing healthy from diseased trees. Acetaldehyde concentrations were significantly lower in healthy trees. Concentrations of methanol and acetaldehyde from sapwood were positively correlated to the percentage of non-functional root area caused by disease, while length of the terminal leader and water content of sapwood from near the root collar were negatively related. Crown growth parameters did not change significantly until more than one-third of the root system was diseased. The mean ethanol concentration from diseased trees was significantly higher than that from healthy trees. About half of the diseased pine contained high ethanol concentrations, with substantial variation in concentrations among cardinal positions sampled around the root collar. While high ethanol concentrations were associated with diseased trees, low ethanol concentrations were not a good indicator of ***health*** . All volatiles, except ethanol, were partially or entirely generated by thermal decomposition of sapwood constituents when heated in sealed vials during analysis. Nevertheless, some may be useful chemical markers for identifying trees with root disease before the disease reaches an advanced stage. Also, they might be useful in estimating what proportion of a root system is diseased and non-functional.

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47. **Isolation of root associated fungi from Erica herbacea and their tolerance to lead.**

Dominik Vodnik, Mirjam Mihelcic and Nada Gogala.

Dep. Biol., Biotech. Fac., Univ. Ljubljana, Vecna pot 111, SI-1000 Ljubljana, Slovenia

Acta Biologica Slovenica 1997, 41 (4): 35-42

English

ABSTRACT Root associated fungi were isolated from Erica herbacea growing in two different localities. Two fungi were isolated from the plants growing in lead contaminated soil: Cladosporium herbarum and Cylindrocarpon destructans. The endophyte ***Phialocephala*** fortinii and the fungus Rhizoctonia sp. were isolated from plants growing in non-polluted soil. The lack of true cricoid endosymbionts was probably due to the method of isolation used. The lead tolerance of isolated fungi and two ericoid mycorrhizal fungi, Oidiodendron griseum and O. rhodogenum were tested in liquid culture.

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49. **Development of bluestain in commercially harvested logs in Britain.**

A. Uzunovic, J. F. Webber and D. J. Dickinson.

Forestry Commission Research Station, Alice Holt Lodge, Farnham, Surrey GU10 4LH, UK.

Document - International Research Group on Wood Preservation 1996, (IRG/WP/96-10150): 11

English

ABSTRACT In Britain, mechanized harvesting of conifer forestry crops is now the preferred method of felling where terrain and access allows. However, use of mechanized harvesters can lead to excessive debarking, loosening of the bark and wood splintering, with on average about a third of the bark removed from the more severely damaged logs. More bark has also been observed to be lost from logs harvested early in the summer (June) than later in the season (August). In an experiment in the UK which ran from June-October in 1993, mechanically harvested logs with only relatively small amounts of bark damage (mean 12%) were found to be much more susceptible to attack by blue stain fungi than those processed manually, and the damaged areas were associated with extensive blue stain development around the log circumference. The most dramatic staining was produced by Ceratocystis coerulescens and ***Leptographium*** wingfieldii. Bark beetles which act as vectors of some blue stain fungi were excluded from the experimental logs, but other insect genera were found to act as casual vectors of the stain fungi. Harvester design and improved skills of harvester operators cannot significantly reduce the potential amount of blue stain degrade as significant reduction of stain only comes with the very low amounts of bark damage (0-10% of circumference) and this is practically unachievable with mechanized methods. Thus rapid delivery of logs for further processing remains the ***safest*** way of minimizing the opportunity for fungal attack.

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50. **Interaction of pre-attack and induced monoterpene concentrations in host conifer defense against bark beetle-fungal complexes.**

K. F. Raffa and E. B. Smalley.

Department of Entomology, University of Wisconsin, Madison, WI 53706, USA.

Oecologia 1995, 102 (3): 285-295

English

ABSTRACT In studies in Wisconsin, 21- or 25-year-old trees of 2 pine species (Pinus resinosa, P. banksiana) responded to inoculation with fungi (Ophiostoma ips, O. nigrocarpa and ***Leptographium*** terebrantis) carried by bark beetles (Ips pini) by rapidly increasing monoterpene concentrations at the entry site. Changes in total monoterpenes were more pronounced than changes in proportionate compositions. The extent and rate of host response was affected by fungal species, the viability of the inoculum, and host tree species. In general, host responses were highest to fungi that are phytopathogenic and consistently associated with the major bark beetles in the study region. Simple mechanical wounding cannot account for the observed allelochemical changes, as aseptic inoculations elicited only minor reactions. Similarly, inoculation with autoclaved inviable fungi generally elicited intermediate responses, suggesting that both structural and metabolic fungal properties are important. Responses by jack pine, P. banksiana, were generally more rapid and variable than those of red pine, P. resinosa. Dose ***toxicity*** experiments with synthetic compounds demonstrated that monoterpene concentrations present in vivo only a few days after simulated attack are lethal to most beetles. Constitutive (pre-attack) monoterpene levels can also exert some ***toxicity*** . Because bark beetles engage in pheromone-mediated mass attacks that can deplete host defences, constitutive monoterpene levels, while a necessary early phase of successful plant defence, appear insufficient by themselves. Such interactions between constitutive and induced defence chemistry may be important considerations when evaluating general theories of plant defence.

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51. **Response of Lutz, Sitka and white spruce to attack by Dendroctonus rufipennis (Coleoptera: Scolytidae) and blue stain fungi.**

Richard A. Werner and Barbara L. Illman.

Inst. Northern Forestry, Pacific Northwest Res. Stn., Forest Serv., USDA, Fairbanks, AK 99775-5500, USA

Environ.Entomol. 1994, 23 (2): 472-478

English

ABSTRACT Mechanical wounding and wounding plus inoculation with a blue-stain fungus, ***Leptographium*** abietinum (Peck), associated with the spruce beetle, Dendroctonus rufipennis (Kirby), caused an induced reaction zone or lesion around the wound sites in Lutz spruce, Picea lutzii Little, Sitka spruce, P. sitchensis (Bong.) Carr., and white spruce, P. glauca (Moench) Voss, in south-central Alaska. The effects of tree species on lesion length were nonsignificant; however, the effects of wounding versus wounding plus blue-stain inoculate were highly significant. Lesion length was significantly longer in high-flow Lutz spruce compared with low-flow trees that were wounded. There was a significant change in monoterpene composition in the induced reaction zones of wounded phloem compared with unwounded phloem. The total percentage of potential ***toxic*** monoterpenes such as limonene, myrcene, 3-carene, and beta phellandrene increased in all three host species. Egg gallery length and the area of phloem consumed by larvae outside of lesions was

significantly less for trees with wounds caused by fungal inoculation compared with mechanical wounds only. Trees with fungal inoculations appeared to deter larval feeding. Hydroxystilbenes were not found in the three species of spruce; however, differences were found in the chemical content of the reaction and the nonreaction zones. Two unknown chemicals present in nonreaction zones were not found in the induced reaction zone. One chemical appears to be a dimer with a carbohydrate moeity. A low molecular weight chemical found in the induced reaction zone could not be identified by mass spectroscopy.

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53. **Fungi associated with decay in treated southern pine utility poles in the eastern United States**

Robert A. Zabel, Frances F. Lombard, C. J. K. Wang and Fred Terracina.

Coll. Environ. Sci. For., SUNY, Syracuse, NY, 13210, USA

Wood Fiber Sci. 1985, 17 (1): 75-91

English

ABSTRACT The fungi exhibited a wide range in tolerance to creosote or pentachlorophenol [87-86-5]. The soft rot fungi were generally more tolerant to the preservatives than the Basidiomycete decayers. Cladosporium resinae, The most tolerant fungus to creosote, was isolated primarily from creosote-treated poles at all service ages. Rhinocladiella atrovirens Was isolated primarily from pentachlorophenol-treated poles, which may be related to its high tolerance to this ***toxicant*** . Also, Phlebia subserialis, which was most tolerant to pentachlorophenol, was isolated only from pentachlorophenol-treated poles. Isolation, identification and grouping of fungi assocd. with wood decay are described. OSC.G 6 THERE ARE 6 CAPLUS RECORDS THAT CITE THIS RECORD (6 CITINGS)

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54. **Biologically active phenolic metabolites of a ***Verticicladiella*** species.**

W. A. Ayer, L. M. Browne and S. H. Lovell.

Phytochemistry 1983, (Volume 22, Number 10, pp 2267-2271): ill

English

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55. **ROOT DISEASE AND INSECT INFESTATIONS ON AIR POLLUTION SENSITIVE PINUS-STROBUS AND STUDIES OF PATHOGENICITY OF ***VERTICICLADIELLA*** -PROCERA.**

A. L. LACKNER and S. A. ALEXANDER.

DEP PLANT PATHOL, NC STATE UNIV, RALEIGH, NC 27650, USA

Plant Dis. 1983, 67 (6): 679-681

ENGLISH

ABSTRACT Two lateral roots were excavated from each of 25 air-pollution-sensitive and 18 air-pollution-tolerant eastern white pines [P. strobus] in the Blue Ridge Mountains in Virginia [USA]. V. procera, Heterobasidion annosum, V. serpens and a Graphium sp. were isolated from 24, 8, 8 and 4% of the sensitive trees, respectively. Two species of weevils, Pissodes approximatus and a Hylobius sp., were isolated from 20% of the sensitive trees. No pathogenic fungi or insects were isolated from the tolerant trees. The pathogenicity of 3 V. procera isolates was tested on eastern white pine and loblolly pine seedlings. Seedlings were inoculated using a root-dip method. Fifty-nine of 60 eastern white pines and 57 of 60 loblolly pines were dead 7 wk after inoculation. The total number of eastern white pines and loblolly pines killed was not significantly different (P = 0.05). One isolate killed significantly fewer loblolly pines than eastern white pines (P = 0.05).

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56. **L'alteration des bois d'oeuvre de hetre (Fagus sylvatica L.) par Fusarium solani (Mart.) Sacc. et ***Verticicladiella*** procera Kendrick. Alteration of beech wood (Fagus sylvatica L.) by Fusarium solani (Mart.) Sacc. and ***Verticicladiella*** procera Kendrick.**

G. Kilbertus, F. Mangenot and D. Radtke.

Cryptogamie; mycologie 1980, 1 (2): 97-104

English

57. **Un caso di "moria" di Pinus pinea associata alla presenza di ***Verticicladiella*** sp. (nota preliminare) Decline of Pinus pinea associated with the presence of ***Verticicladiella*** sp. (preliminary note)**

G. Lorenzini and P. Gambogi.

Informatore fitopatologico 1976, 5 5-8

Italian

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58. **Prospects for reducing forest damages from the black stain root disease caused by ***Verticicladiella*** wageneri.**

R. S. Smith Jr.

California University, Berkeley Agricultural Extension Service

Calif Plant Pathol Univ Calif Agric Ext 1974, 1-2

English

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59. **ANTAGONISM OF SCYTALIDIUM ISOLATES AGAINST DECAY FUNGI.**

A. E. KLINGSTROM and S. M. JOHANSSON.

Phytopathology 1973, 63 (4): 473-479

Unavailable

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60. **Wood protection chemistry. XI. The antiblueing action of various compounds and its dependence on chemical constitution**

Wilhelm Sandermann, Reinhard Casten and Henning Thode.

Holzforschung 1963, 17 (4): 97-105

ABSTRACT cf. Holz RohWerkstoff 19(1), 20-1(1961); CA 53, 20792c. Present-day fungicides have not proved entirely satisfactory in preventing the "blueing" of wood. It is therefore proposed to test about 500 different org. compds. for their possible antiblueing action. The initial screening methods involve a previously discussed technique to show their activity against ***Scopularia*** phycomyces (and in certain cases against the more resistant Pullularia pullulans), and the present paper reports tentatively on the work with about 300 compds. When the preliminary screening procedures are complete, the compds. that show promise will be tested by other methods and the soly., volatility, ***toxicity*** , availability, and cost will also be considered. Besides their potential antiblueing action, fungicides will be tested for the preservation of chem. and mech. pulps, papers, and boards. Compds. tested include cyclic hydrocarbons and their Cl and NO2 derivs., a variety of phenols (with various ring substituents), aromatic carboxylic acids and their derivs., cyclic oxides and pyrones, quinones, stilbenes and stilbazoles, N compds., and a series of compds., most of them cyclic, all of which contained S, and in some cases N, O, and (or) Cl. 45 references.

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Annex 7.2.A

Pages 000171-000181 have been removed in accordance with copyright laws. The removed references are:

Steensma, A., Van Dijck, P.W. and Hempenius, R.A. (2004) Safety evaluation of phosphodiesterase derived from Leptographium procerum, Food Chem. Toxicol. 42 (6) 935-944

Steensma, A., Van Dijck, P.W. and Hempenius, R.A. (2005) Corrigendum to "Safety evaluation of phosphodiesterase derived from Leptographium procerum" [Food and Chemical Toxicology 42 (2004)935–944], Food Chem. Toxicol. 43 1567.

Annex 7.2.B

Table C.3 Name list of food grade enzyme solutions and their source

表 C.3 食品用酶制剂及其来源名单

Number 序号	酶 Enzyme	来　源[a] Source	供　体[b] Donor
1	α-半乳糖苷酶 Alpha-galactosidase	黑曲霉 *Aspergillus niger*	
2	α-淀粉酶 Alpha-amylase	地衣芽孢杆菌 *Bacillus licheniformis*	
			地衣芽孢杆菌 *Bacillus licheniformis*
			嗜热脂肪芽孢杆菌 *Bacillus stearothermophilus*
		黑曲霉 *Aspergillus niger*	
		解淀粉芽孢杆菌 *Bacillus amyloliquefaciens*	
		枯草芽孢杆菌 *Bacillus subtilis*	
			嗜热脂肪芽孢杆菌 *Bacillus stearothermophilus*
		米根霉 *Rhizopus oryzae*	
		米曲霉 *Aspergillus oryzae*	
		嗜热脂肪芽孢杆菌 *Bacillus stearothermophilus*	
		猪或牛的胰腺 hog or bovine pancreas	
3	α-乙酰乳酸脱羧酶 Alpha-acetolactate decarboxylase	枯草芽孢杆菌 *Bacillus subtilis*	短小芽孢杆菌 *Bacillus brevis*
4	β-淀粉酶 beta- amylase	大麦、山芋、大豆、小麦和麦芽 barley, taro, soya, wheat and malted barley	
		枯草芽孢杆菌 *Bacillus subtilis*	
5	β-葡聚糖酶 beta-glucanase	地衣芽孢杆菌 *Bacillus licheniformis*	
		孤独腐质霉 *Humicola insolens*	
		哈次木霉 *Trichoderma harzianum*	
		黑曲霉[c] *Aspergillus niger*	
		枯草芽孢杆菌 *Bacillus subtilis*	
		李氏木霉 *Trichoderma reesei*	

表 C.3(续) Table C.3 (continued)

序号	酶	来　源[a]	供　体[b]
5	β-葡聚糖酶 beta-glucanase	解淀粉芽孢杆菌 *Bacillus amyloliquefaciens*	解淀粉芽孢杆菌 *Bacillus amyloliquefaciens*
		Disporotrichum dimorphosporum	
		埃默森篮状菌 *Talaromyces emersonii*	
		绿色木霉 *Trichoderma viride*	
6	阿拉伯呋喃糖苷酶 Arabinofuranosidease	黑曲霉 *Aspergillus niger*	
7	氨基肽酶 Aminopeptidase	米曲霉 *Aspergillus oryzae*	
8	半纤维素酶 Hemicellulase	黑曲霉 *Aspergillus niger*	
9	菠萝蛋白酶 Bromelain	菠萝 *Ananas* spp.	
10	蛋白酶（包括乳凝块酶） Protease (including milk clotting enzymes)	寄生内座壳（栗疫菌）*Cryphonectria parasitica*（*Endothia parasitica*）	寄生内座壳（栗疫菌）*Cryphonectria parasitica*（*Endothia parasitica*）
		地衣芽孢杆菌 *Bacillus licheniformis*	
		黑曲霉 *Aspergillus niger*	
		黑曲霉 *Aspergillus niger*	黑曲霉 *Aspergillus niger*
		解淀粉芽孢杆菌 *Bacillus amyloliquefaciens*	
		解淀粉芽孢杆菌 *Bacillus amyloliquefaciens*	解淀粉芽孢杆菌 *Bacillus amyloliquefaciens*
		枯草芽孢杆菌 *Bacillus subtilis*	
		寄生内座壳（栗疫菌）*Cryphonectria parasitica*（*Endothia parasitica*）	
		米黑根毛霉 *Rhizomucor miehei*	
		米曲霉 *Aspergillus oryzae*	
		乳克鲁维酵母 *Kluyveromyces lactis*	小牛胃 *Calf stomach*
		微小毛霉 *Mucor pusillus*	
		蜂蜜曲霉 *Aspergillus melleus*	
11	单宁酶 Tannase	米曲霉 *Aspergillus oryzae*	
12	多聚半乳糖醛酸酶 Polygalacturonase	黑曲霉[c] *Aspergillus niger*	
		米根霉 *Rhizopus oryzae*	

表 C.3(续)

序号	酶	来　源[a]	供　体[b]
13	谷氨酰胺酶 Glutaminase	解淀粉芽孢杆菌 *Bacillus amyloliquefaciens*	
14	谷氨酰胺转氨酶 Glutamine Transaminase	茂原链轮丝菌 *Streptoverticillium mobaraense*	
15	果胶裂解酶 Pectinlyase	黑曲霉 *Aspergillus niger*	
		黑曲霉 *Aspergillus niger*	黑曲霉 *Aspergillus niger*
16	果胶酶 Pectinase	黑曲霉 *Aspergillus niger*	
		米根霉 *Rhizopus oryzae*	
17	果胶酯酶（果胶甲基酯酶）Pectinesterase (Pectin methylesterase)	黑曲霉 *Aspergillus niger*	
		黑曲霉 *Aspergillus niger*	黑曲霉 *Aspergillus niger*
		米曲霉 *Aspergillus oryzae*	针尾曲霉 *Aspergillus aculeatus*
18	过氧化氢酶 Catalase	黑曲霉 *Aspergillus niger*	
		牛、猪或马的肝脏 bovine, pig or horse liver	
		溶壁微球菌 *Micrococcus lysodeicticus*	
19	核酸酶 Nuclease	橘青霉 *penicillium citrinum*	
20	环糊精葡萄糖苷转移酶 Cyclomaltodextin glucanotransferase	地衣芽孢杆菌 B*acillus licheniformis*	高温厌氧杆菌 *Thermoanaerobacter* sp.
21	己糖氧化酶 Hexose oxidase	（多形）汉逊酵母 *Hansenula polymorpha*	皱波角叉菜 *Chondrus crispus*
22	菊糖酶 Inulinase	黑曲霉 *Aspergillus niger*	
23	磷脂酶 Phospholipase	胰腺 pancreas	
24	磷脂酶 A2 Phospholipase A2	猪胰腺组织 porcine pancreas	
		黑曲霉 *Aspergillus niger*	猪胰腺组织 porcine pancreas
25	磷脂酶 C Phospholipase C	巴斯德毕赤酵母 *Pichia pastoris*	从土壤中分离的编码磷脂酶 C 基因的微生物
26	麦芽碳水化合物水解酶（α-、β-麦芽碳水化合物水解酶）Malt carbohydrases (alpha- and beta-amylase)	麦芽和大麦 malted barley & barley	
27	麦芽糖淀粉酶 Maltogenic amylase	枯草芽孢杆菌 *Bacillus subtilis*	嗜热脂肪芽孢杆菌 *Bacillus stearothermophilis*
28	木瓜蛋白酶 Papain	木瓜 *Carica papaya*	

表 C.3(续)

序号	酶	来　源[a]	供　体[b]
29	木聚糖酶 Xylanase	*Fusarium venenatum*	棉状嗜热丝孢菌 *Thermomyces lanuginosus*
	木聚糖酶 Xylanase	毕赤氏酵母 *Pichia paseoris*	
		孤独腐质霉 *Humicola insolens*	
		黑曲霉 *Aspergillus niger*	
		黑曲霉 *Aspergillus niger*	黑曲霉 *Aspergillus niger*
		李氏木霉 *Trichoderma reesei*	
		绿色木霉 *Trichoderma viride*	
		枯草芽孢杆菌 *Bacillus subtilis*	枯草芽孢杆菌 *Bacillus subtilis*
		米曲霉 *Aspergillus oryzae*	棉状嗜热丝孢菌 *Thermomyces lanuginosus*
		米曲霉 *Aspergillus oryzae*	黑曲霉[c] *Aspergillus niger*
30	凝乳酶 A Chymosin A	大肠杆菌 K-12 *Eschorichia Coli* K-12	小牛前凝乳酶 A 基因 calf prochymosin A gene
31	凝乳酶 B Chymosin B	黑曲霉泡盛变种 *Aspergillus niger* var. *awamori*	小牛前凝乳酶 B 基因 calf prochymosin B gene
		乳克鲁维酵母 *Kluyveromyces lactis*	小牛前凝乳酶 B 基因 calf prochymosin B gene
32	凝乳酶或粗制凝乳酶 Chymosin or Rennet	小牛、山羊或羔羊的皱胃 calf, kid, or lamb abomasum	
33	葡糖淀粉酶（淀粉葡糖苷酶） Glucoamylase（amyloglucosidase）	戴尔根霉 *Rhizopus delemar*	
		黑曲霉 *Aspergillus niger*	
			黑曲霉 *Aspergillus niger*
			埃默森篮状菌 *Talaromyces emersonii*
		米根霉 *Rhizopus oryzae*	
		米曲霉 *Aspergillus oryzae*	
		雪白根霉 *Rhizopus niveus*	
34	葡糖氧化酶 Glucose oxidase	黑曲霉 *Aspergillus niger*	
		米曲霉 *Aspergillus oryzae*	黑曲霉 *Aspergillus niger*
35	葡糖异构酶（木糖异构酶） Glucose isomerase (xylose isomerase)	橄榄产色链霉菌 *Streptomyces olivochromogenes*	
		橄榄色链霉菌 *Streptomyces olivaceus*	
		密苏里游动放线菌 *Actinoplanes missouriensis*	
		凝结芽孢杆菌 *Bacillus*	

表 C.3(续)

序号	酶	来　源[a]	供　体[b]
		coagulans	
		锈棕色链霉菌 *Streptomyces rubiginosus*	
		紫黑吸水链霉菌 *Streptomyces violaceoniger*	
		鼠灰链霉菌 *Streptomyces murinus*	
36	普鲁兰酶 Pullulanase	产气克雷伯氏菌 *Klebsiella aerogenes*	
		枯草芽孢杆菌 *Bacillus subtilis*	
		枯草芽孢杆菌 *Bacillus subtilis*	嗜酸普鲁兰芽孢杆菌 *Bacillus acidopullulyticus*
		嗜酸普鲁兰芽孢杆菌 *Bacillus acidopullulyticus*	
		枯草芽孢杆菌 *Bacillus subtilis*	*Bacillus deramificans*
		地衣芽孢杆菌 *Bacillus licheniformis*	*Bacillus deramificans*
37	漆酶 Laccase	米曲霉 *Aspergillus oryzae*	嗜热毁丝霉 *Myceliophthora thermophila*
38	溶血磷脂酶（磷脂酶 B）Lysophospholipase (lecithinase B)	黑曲霉 *Aspergillus niger*	
		黑曲霉 *Aspergillus niger*	黑曲霉 *Aspergillus niger*
39	乳糖酶 （β-半乳糖苷酶）Lactase (beta-galactosidase)	脆壁克鲁维酵母 *Kluyveromyces fragilis*	
		黑曲霉 *Aspergillus niger*	
		米曲霉 *Aspergillus oryzae*	
		乳克鲁维酵母 *Kluyveromyces lactis*	
		乳克鲁维酵母 *Kluyveromyces lactis*	乳克鲁维酵母 *Kluyveromyces lactis*
40	天门冬酰胺酶 Asparaginase	黑曲霉 *Aspergillus niger*	黑曲霉 *Aspergillus niger*
		米曲霉 *Aspergillus oryzae*	米曲霉 *Aspergillus oryzae*
41	脱氨酶 Deaminase	蜂蜜曲霉 *Aspergillus melleus*	
42	胃蛋白酶 Pepsin	猪、小牛、小羊、禽类的胃组织 hog, calf, goat(kid) or poultry stomach	
43	无花果蛋白酶 Ficin	无花果 *Ficus* spp.	
44	纤维二糖酶 Cellobiase	黑曲霉 *Aspergillus niger*	

表 C.3(续)

序号	酶	来　源[a]	供　体[b]
45	纤维素酶 Cellulase	黑曲霉 *Aspergillus niger*	
		李氏木霉 *Trichoderma reesei*	
		绿色木霉 *Trichoderma viride*	
46	胰蛋白酶 Typsin	猪或牛的胰腺 porcine or bovine pancreas	
47	胰凝乳蛋白酶（糜蛋白酶）Chymotrypsin	猪或牛的胰腺 porcine or bovine pancreas	
48	脂肪酶 Lipase	黑曲霉 *Aspergillus niger*	
		黑曲霉 *Aspergillus niger*	南极假丝酵母 *Candida antarctica*
		米根霉 *Rhizopus oryzae*	
		米黑根毛霉 *Rhizomucor miehei*	
		米曲霉 *Aspergillus oryzae*	
		米曲霉 *Aspergillus oryzae*	尖孢镰刀菌 *Fusarium oxysporum*
			棉状嗜热丝孢菌 *Thermomyces lanuginosus*
		小牛或小羊的唾液腺或前胃组织 salivary glands or forestomach of calf, kid, or lamb	
		雪白根霉 *Rhizopus niveus*	
		羊咽喉 goat gullets	
		猪或牛的胰腺 hog or bovine pancreas	
		米曲霉 *Aspergillus oryzae*	米黑根霉 *Rhizomucor miehei*
49	酯酶 Esterase	黑曲霉 *Aspergillus niger*	
		李氏木霉 *Trichoderma reesei*	
		米黑根毛霉 *Rhizomucor miehei*	
50	植酸酶 phytase	黑曲霉 *Aspergillus niger*	
51	转化酶（蔗糖酶）Invertase (saccharase)	酿酒酵母 *Saccharomyces cerevisiae*	
52	转葡糖苷酶 Transglucosidase	黑曲霉 *Aspergillus niger*	

[a] 指用于提取酶制剂的动物、植物或微生物。a. Referring to the animals, plants and microorganisms used to extract the enzyme solution

[b] 指为酶制剂的生物技术来源提供基因片段的动物、植物或微生物。b. Referring to the animals, plants and microorganisms providing the gene fragment for enzyme solution biotechnology

表 C.3(续)

序号	酶	来　源[a]	供　体[b]
[c]包括针尾曲霉 *Aspergillus aculeatus* 和泡盛曲霉 *A. awamori*。			

Annex 7.3.1.A

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POSITION PAPER

ETA Position On Food Allergen Labeling of Microbially Derived Enzymes Under FALCPA as it Applies to Fermentation Media Raw Materials

It is the position of the Enzyme Technical Association (ETA) that microbially derived enzymes do not fall within the scope of the Food Allergy Labeling and Consumer Protection Act (FALCPA) and that labeling for food allergens is not triggered by the use of a microbially derived enzyme preparation. There may be other reasons why a manufacturer labels a food product with regard to allergen content, but the use of a microbially derived enzyme preparation is not a reason for such labeling.

Enzymes are not one of the eight major allergenic foods, often referred to as the big 8, so they do not fit within the first requirement of FALCPA. In addition, microbial enzymes are not byproducts of nor are they derived from the major food allergens. Although enzymes are not major food allergens,[1] many enzymes are produced with microorganisms and the nutrient media used to feed these microorganisms may contain protein from one or more of the major food allergens. The enzymes are not derived from raw materials containing major food allergens, but rather are obtained from the microorganisms which are used to produce the enzyme proteins. In other words, enzymes obtained from fermentation are directly derived from microorganisms fed on media that may include protein obtained from one or more of the major food allergens. Proteins and other nitrogenous material are consumed by the microorganisms for cell growth, cell maintenance, and production of enzyme protein. It is the intent of the enzyme manufacturer to supply enzymes, therefore it is critical that the ratio of nutrient to enzyme yield is carefully controlled. It is also the intent of the manufacturer that these raw materials are added to the fermentation as food to be consumed by the microorganism and are not added as formulation ingredients.

In arriving at its position ETA also considered that:

- The regulatory agencies in the EU and Japan have determined that enzyme preparations are not required to have allergen labeling for the raw materials used in the fermentation process. Indeed, the European Commission's Health & Consumer Protection Directorate General has clearly stated that enzymes

[1] To the extent the enzyme producer uses an allergenic material, such as wheat flour diluent in the final product formulation, labeling may be required.

are outside the scope of the Directive 2003/89/EC which amended the EU Food Labelling Regulations.

- Enzyme broths are normally processed to separate biomass and fermentation materials from the enzyme, to concentrate the enzymatic activity, and formulated to achieve a uniform and stable enzyme product.

- The unique role of enzymes in food processing is as a catalyst. Due to the specific nature of enzymes, only small amounts are required to make desired modifications to the property of a food.

- Many enzymes do not become a component of the food ingredient or final food. Some enzymes are used in an immobilized form or are denatured during processing. Further, processing of the food ingredient after the enzyme catalyst has performed the expected function often reduces or eliminates the enzyme from the product.

- ETA has made an extensive review of the published scientific literature and has found no reports that even suggest there has been an allergenic reaction to a component of the fermentation media which was used to feed the microorganism that produced the enzyme.

The above position paper and accompanying report were provided to FDA on September 12, 2005 and to date ETA has received no comment.

Annex 7.3.1.B

UNIVERSITY OF Nebraska Lincoln

INSTITUTE OF AGRICULTURE AND NATURAL RESOURCES
FOOD ALLERGY RESEARCH AND RESOURCE PROGRAM

EXPERT OPINION STATEMENT
FOOD ALLERGY RESEARCH & RESOURCE PROGRAM
UNIVERSITY OF NEBRASKA

Testing of Microbially Derived Enzymes for Potential Allergens from Fermentation Media Raw Materials

August 13, 2013

Prepared by: Steve L. Taylor, Ph.D., Co-Director
and
Joe L. Baumert, Ph.D., Co-Director

with assistance from Enzyme Technical Association

Microbially derived enzymes are used by food processors as additives and processing aids in a wide variety of foods. Enzymes obtained from microbial fermentation are directly derived from microorganisms fed on sterilized media[1] that may include protein sources obtained from one or more of the recognized commonly allergenic foods (e.g., milk, soybean) or from a cereal source of gluten (e.g., wheat, barley). This paper addresses the relevance of testing microbial enzymes for allergenic material from the fermentation growth media.[2]

It has been the long-standing position of the Food Allergy Research & Resource Program (FARRP) at the University of Nebraska that testing of the products of fermentation (with limited exceptions), including microbially derived enzymes is unreliable using enzyme-linked immunosorbent assays (ELISAs).

While various fermentation media may contain one or more of the major food allergens, the biochemical reactions that occur during fermentation result in the breakdown of the fermentation media proteins. The extent of proteolysis is dependent upon the fermentation culture and the resultant enzyme (e.g., some enzymes are proteases). As proteins are digested, the resulting amino acids, along with other

[1] Aunstrup, K., O. Andresen, E.A. Falch, and T.K. Nielsen (1979) *Microbial Technology*. (Perlman and Peppler, eds.) Academic Press, pp. 281-309.

[2] For this paper, FARRP's analysis is limited to microbially derived enzymes that are intended for additive and processing aid applications in food.

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nitrogenous material, are consumed by the microorganisms for cell growth, cell maintenance, and production of enzyme protein.

Upon completion of fermentation, remaining fermentation media that are not consumed by the microorganism are typically separated and/or purified from the enzyme in the recovery process. Enzymes are recovered from the fermentation broth by standard chemical engineering operations, such as filtration and centrifugation, broadly used in enzyme production.[3,4] (See Appendices for further information.) The recovery steps result in separation of microbial biomass and other fermentation solids from the enzyme, concentration of the enzyme, and removal of impurities prior to final formulation with food-grade ingredients.

Any potential residual fragments from the food allergen would be difficult to measure as there is no reliable assay. Commercial ELISAs are able to detect only intact proteins in most cases. Any peptides, even larger ones, would not likely be detected, although this possibility has not been well investigated. Results would typically be reported as below the limit of quantitation for the enzyme preparation. Further, if any residual but undetected fragments of the food allergen remain, the relevance of any such residual material to food allergenicity is unproven. Accordingly, testing of fermented product does not result in reliable or useful data.

In addition, due to the specific catalytic nature of enzymes, only very small amounts of enzymes are generally required and used by food processors to make the desired modifications to the property of a food, and therefore any *de minimis* amount of fermentation media protein that may survive the fermentation process will not pose a significant public health risk to the consumer.[5]

FARRP also notes that regulatory agencies in the European Union and Japan do not require allergen labeling of enzyme preparations for the raw materials used in the fermentation process.

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[3] Atkinson, B. and F. Mavituna (1991) *Biochemical Engineering and Biotechnology Handbook.* (Atkinson, B. and Mavituna, F., eds.) Stockton Press, Hampshire, pp. 1146-1158.

[4] Kroschwitz, J.I. (1994) *Enzyme Applications in Encyclopedia of Chemical Technology.* 4th edition, Volume 9. (Kroschwitz, J.I., ed.), pp. 567-620.

[5] To the extent the enzyme producer uses an allergen as diluent to formulate the final product, labeling for such allergen is appropriate and required under Food Allergen Labeling and Consumer Protection Act.

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March 5, 2014

VIA FEDERAL EXPRESS

Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835

Re: Clarification to GRAS Notification for Phosphodiesterase I Produced with *Leptographium procerum*

Dear Sir or Madam:

On behalf of DSM Food Specialties ("DSM"), we are submitting three paper copies and one eCopy of this letter detailing DSM's response to a question posed about its generally recognized as safe ("GRAS") notification for phosphodiesterase I produced with *Leptographium procerum* ("*L. procerum*"). The eCopy, which contains a copy of the original submission, is provided on a disc which is virus free.

DSM has determined through scientific procedures that the phosphodiesterase I enzyme preparation from *L. procerum* is GRAS for use in the production of yeast extracts and yeast autolysates, that can be applied as an ingredient in a wide range of savory foods such as soups and sauces, snacks, processed cheese, dressings and spreads, flavours and seasonings. The Food and Drug Administration ("FDA") has requested clarification regarding this proposed use of the phosphodiesterase I. Specifically, FDA wished to know if the enzyme would be used in soups and products containing meat products, which would require additional review by the US Department of Agriculture. It is not the intention of DSM to use the phosphodiesterase I product in soups or any food products that are subject to USDA meat inspection.

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OFFICE OF
FOOD ADDITIVE SAFETY

March 5, 2014

VIA FEDERAL EXPRESS

Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835

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If you have any questions regarding this letter or the previously submitted notification, please do not hesitate to contact me via email at gyingling@morganlewis.com or by telephone, (202)739-5610.

Sincerely,

Gary L. Yingling

cc: DSM Food Specialties

SUBMISSION END