



Item 2

Reviewer: Esther Seto , Date Dec. 5, 2001

Peer Review: Zigfridas Vaituzis, PhD
Microbial Pesticides Branch
Biopesticides and Pollution Prevention Division
U.S. Environmental Protection Agency

STUDY TYPE: Freshwater Fish Testing, Tier I
PMRA DATA CODE M9.4.1 / USEPA OPPTS 885.4200

TEST MATERIAL (PURITY): *Pseudozyma flocculosa* - DAOM 196992 (National Collection of Fungus Cultures) (TGAI)
Sporodex L (EP)

SYNONYMS: *Stephanoascus flocculosa*
Sporothrix flocculosa

CITATION: Traquair, J, Hale, J. and Brown, W. (2000). Sporodex WP Waiver in Support of Not Submitting Freshwater Fish Data. January 24, 2000.

SPONSOR: Plant Products Co. Ltd.

EXECUTIVE SUMMARY: Plant Products Co. Ltd. submitted a justification for a data waiver from freshwater fish studies (DACO M9.4.1 and OPPTS 885.4200). The waiver request was based on the rationale that *Pseudozyma flocculosa* is a naturally-occurring microorganism whose level in the environment will not significantly increase with the use of Sporodex L and that an extensive literature search yielded no reports of adverse effects in freshwater fish.

Use of Sporodex L in commercial greenhouses is not expected to result in increased exposure or adverse effects in freshwater fish. Consequently, testing is considered unnecessary to assess the risks of Sporodex L to freshwater fish. The request for a waiver of freshwater fish testing is accepted.

STUDY AUTHOR'S SUMMARY AND DISCUSSION:

The applicant did not conduct studies on the effects of *P. flocculosa* on freshwater fish. Instead, a waiver request, based on the following rationales, was submitted:

- 1. Increased exposure to *P. flocculosa*, due to use of the end-use product, Sporodex L, will be minimal:** *Pseudozyma* species are ubiquitous in nature and have been isolated from a wide variety of plant surfaces including leaf litter (Bandoni, 1985), clover, maize (Boekhout

2. *et al.*, 1995) and cucumber (Urquhart *et al.*, 1997). No adverse effects to freshwater fish, due to natural populations of *Pseudozyma flocculosa*, have been noted in the published literature.

Use of Sporodex L will be limited to foliar, greenhouse applications on cucumbers and roses, thus minimizing direct exposure and risk to freshwater fish. Roses are usually grown in containers which allow for the collection and re-use of water and run-off, while most greenhouse cucumbers are grown in plastic-covered rockwool slabs which are placed on plastic ground covers. Both of these methods of production limit run-off. Any *P. flocculosa* that reaches aquatic systems, in the form of run-off, is expected to behave as it would in the wild.

3. **No evidence of adverse effects:** A literature search of the AGRICOLA, AGRIS, CAB ABSTRACTS, BIOLOGICAL ABSTRACTS, CHEMTOX (Hazardous and Regulated Chemicals Database), RTECS (Registry of the Toxic Effects of Chemical Substances) databases for the period 1980-1999 was conducted. For the purposes of this literature search, *Pseudozyma flocculosa* and other species in the genus *Pseudozyma*, as well as synonyms of *P. flocculosa* (eg. *Sporothrix flocculosa*) were used as the main search words. Searches based on the principle fungitoxic agents of *P. flocculosa* including the unsaturated fatty acids heptadecenoic acid, methyl-heptadecanoic acid, heptadecadienoic acid and methyl-heptadecadienoic acid, were also conducted. Other unrelated basidiomycetous yeasts known to be common epiphytes on plant surfaces such as *Sporobolomyces*, *Rhondotorula*, *Ustilago* anamorphs and *Tilletiopsis* ssp. were also searched.

The references were manually scanned for information related to genotoxicity, carcinogenicity, allergenicity, mutagenicity, toxicity, antibiotics, mycotoxins, mycocins, pathogenicity, environmental fate and interactions with freshwater fish. No cases or evidence of adverse effects, due to *P. flocculosa*, were reported in the literature.

REVIEWER'S COMMENTS AND CONCLUSION:

Pseudozyma flocculosa is a saprophytic fungal epiphyte and a hyperparasite of powdery mildew and other leaf-surface moulds. *Pseudozyma flocculosa* is widely distributed in North America (Canada and USA) and in Europe on aerial plant surfaces in field or greenhouse agricultural ecosystems. Heavy rainfall likely carries *P. flocculosa* into neighbouring aquatic environments, where growth and survival of terrestrial fungi such as *P. flocculosa* is limited. Neither *P. flocculosa* nor its associated fungitoxins have been associated with adverse effects in freshwater fish species.

Sporodex L will be used in commercial greenhouses only. Freshwater fish will not be directly exposed to the product at the time of application. Greenhouse practices designed to limit exposure to the outside environment will limit post-application exposure to freshwater fish.

Use of Sporodex L in commercial greenhouses is not expected to result in increased exposure or adverse effects in freshwater fish. The waiver rationale is acceptable and testing to assess the risks of Sporodex L to freshwater fish is not required.

References Cited

- Bandoni, R.J. (1985). On an undescribed, pleomorphic hyphomycete from litter. *Botanical Journal of the Linnean Society* **91**:37-43.
- Boekhout, T., Fell, J.W. and O'Donnell, K. (1995). Molecular systematics of some yeast-like anamorphs belonging to the Ustilginales and Tilletiales. *Studies in Mycology* **38**:175-183.
- Urquhart, E.J., Sun, L.J., and Punha, Z.K. (1997). Identification of species of *Tilletiopsis* using random amplified polymorphic DNA analysis. *Canadian Journal of Plant Pathology* **19**:380-389.



Reviewer: Esther Seto , Date Nov. 21, 2001

Peer Review: Zigfridas Vaituzis, PhD
Microbial Pesticides Branch
Biopesticides and Pollution Prevention Division
U.S. Environmental Protection Agency

STUDY TYPE: Avian Oral Toxicity Testing, Tier I,
PMRA DATA CODE M9.2.1 / USEPA OPPTS 885.4050
Avian Pulmonary/Inhalation/Injection, Tier I,
PMRA DATA CODE M9.2.2 / USEPA OPPTS 885.4100

TEST MATERIAL (PURITY): *Pseudozyma flocculosa* - DAOM 196992 (National Collection of Fungus Cultures) (TGAI)
Sporodex L (EP)

SYNONYMS: *Stephanoascus flocculosa*
Sporothrix flocculosa

CITATION: Traquair, J. (2000). Sporodex WP Waiver in Support of Not Submitting Avian Oral Toxicity Study. January 24, 2000.

SPONSOR: Plant Products Co. Ltd.

EXECUTIVE SUMMARY: Plant Products Co. Ltd. submitted a justification for a data waiver from avian oral toxicity studies (DACO M9.2.1 and OPPTS 885.4050) and avian pulmonary/inhalation/injection studies (DACO M9.2.2 and OPPTS 885.4100). The waiver request was based on the rationale that *Pseudozyma flocculosa* is a naturally-occurring soil microorganism whose level in the environment will not significantly increase with the use of Sporodex L and that an extensive literature search yielded no reports of adverse effects in birds.

Use of Sporodex L in commercial greenhouses is not expected to result in increased exposure or adverse effects in birds. Consequently, testing is considered unnecessary to assess the risks of Sporodex L to avian wildlife. The request for a waiver of avian oral and avian pulmonary/inhalation/injection testing is accepted.

STUDY AUTHOR'S SUMMARY AND DISCUSSION:

The applicant did not conduct studies on the effects of *P. flocculosa* on birds. Instead, a waiver request, based on the following rationales, was submitted:

- 4. Increased exposure to *P. flocculosa*, due to use of the end-use product, Sporodex L, will be minimal:** *Pseudozyma* species are ubiquitous in nature and have been isolated from a wide variety of plant surfaces including leaf litter (Bandoni, 1985), clover, maize (Boekhout *et al.*, 1995) and cucumber (Urquhart *et al.*, 1997). No adverse effects to birds, due to natural populations of *Pseudozyma flocculosa*, have been noted in the published literature. Use of Sporodex L will be limited to foliar, greenhouse applications on cucumbers and roses, thus minimizing direct exposure to birds. Roses are usually grown in containers which allow for the collection and re-use of water and run-off, while most greenhouse cucumbers are grown in plastic-covered rockwool slabs which are placed on plastic ground covers. Both of these methods of production limit run-off. Any *P. flocculosa* that reaches the soil, in the form of either run-off or overspray, is expected to behave as it would in the wild.
- 5. No evidence of adverse effects:** A literature search of the AGRICOLA, AGRIS, CAB ABSTRACTS, BIOLOGICAL ABSTRACTS, CHEMTOX (Hazardous and Regulated Chemicals Database), RTECS (Registry of the Toxic Effects of Chemical Substances) databases for the period 1980-1999 was conducted. For the purposes of this literature search, *Pseudozyma flocculosa* and other species in the genus *Pseudozyma*, as well as synonyms of *P. flocculosa* (eg. *Sporothrix flocculosa*) were used as the main search words. Searches based on the principle fungitoxic agents of *P. flocculosa* including the unsaturated fatty acids heptadecenoic acid, methyl-heptadecanoic acid, heptadecadienoic acid and methyl-heptadecadienoic acid, were also conducted. Other unrelated basidiomycetous yeasts known to be common epiphytes on plant surfaces such as *Sporobolomyces*, *Rhondotorula*, *Ustilago* anamorphs and *Tilletiopsis* ssp. were also searched. The references were manually scanned for information related to genotoxicity, carcinogenicity, allergenicity, mutagenicity, toxicity, antibiotics, mycotoxins, mycocins, pathogenicity, environmental fate and interactions with birds. No cases or evidence of adverse effects, due to *P. flocculosa*, were reported in the literature. *Pseudozyma* species have been shown to grow at 30°C but not at 37°C (Middelhoven, 1997). The body temperature of duck and quail species is approximately 40°C.

REVIEWER'S COMMENTS AND CONCLUSION:

Pseudozyma flocculosa is a saprophytic fungal epiphyte and a hyperparasite of powdery mildew and other leaf-surface moulds. *P. flocculosa* is widely distributed in North America (Canada and USA) and in Europe on aerial plant surfaces in field or greenhouse agricultural ecosystems. Rapid death and collapse of susceptible host cells is brought about by the secretion of three fungitoxic unsaturated fatty acids and an acyclic norterpene. Neither *P. flocculosa* or its associated fungitoxins have been associated with adverse effects in avian species.

Sporodex L will be used in commercial greenhouses only. Birds will not be directly exposed to the product at the time of application. Greenhouse practices designed to limit exposure to the outside environment will limit post-application exposure to birds.

Use of Sporodex L in commercial greenhouses is not expected to result in increased exposure or adverse effects in birds. The waiver rationale is acceptable and testing to assess the risks of Sporodex L to birds is not required.

References Cited

Bandoni, R.J. (1985). On an undescribed, pleomorphic hyphomycete from litter. *Botanical Journal of the Linnean Society* **91**:37-43.

Boekhout, T., Fell, J.W. and O'Donnell, K. (1995). Molecular systematics of some yeast-like anamorphs belonging to the Ustilginales and Tilletiales. *Studies in Mycology* **38**:175-183.

Middelhoven, W.J. (1997). Identity and biodegradative abilities of yeasts isolated from plants growing in an arid climate. *Antonie van Leeuwenhoek* **72**:81-89.

Urquhart, E.J., Sun, L.J., and Punha, Z.K. (1997). Identification of species of *Tilletiopsis* using random amplified polymorphic DNA analysis. *Canadian Journal of Plant Pathology* **19**:380-389.



Reviewer: Esther Seto , Date Dec. 5, 2001

Peer Review: Zigfridas Vaituzis, PhD
Microbial Pesticides Branch
Biopesticides and Pollution Prevention Division
U.S. Environmental Protection Agency

STUDY TYPE: Aquatic Arthropods, Tier I
PMRA DATA CODE M9.5.2 / USEPA OPPTS 885.4240

TEST MATERIAL (PURITY): *Pseudozyma flocculosa* - DAOM 196992 (National Collection of Fungus Cultures) (TGAI)
Sporodex L (EP)

SYNONYMS: *Stephanoascus flocculosa*
Sporothrix flocculosa

CITATION: Traquair, J., Hale, J. and Brown, W. (2000). Sporodex WP Waiver in Support of Not Submitting Aquatic Arthropod Data. January 24, 2000.

SPONSOR: Plant Products Co. Ltd.

EXECUTIVE SUMMARY: Plant Products Co. Ltd. submitted a justification for a data waiver from aquatic arthropod studies (DACO M9.5.2 and OPPTS 885.4240). The waiver request was based on the rationale that *Pseudozyma flocculosa* is a naturally-occurring microorganism whose level in the environment will not significantly increase with the use of Sporodex L and that an extensive literature search yielded no reports of adverse effects in aquatic arthropods.

Use of Sporodex L in commercial greenhouses is not expected to result in increased exposure or adverse effects in aquatic arthropods. Consequently, testing is considered unnecessary to assess the risks of Sporodex L to aquatic arthropods. The request for a waiver for aquatic arthropod testing is accepted.

STUDY AUTHOR'S SUMMARY AND DISCUSSION:

The applicant did not conduct studies on the effects of *P. flocculosa* on aquatic arthropods. Instead, a waiver request, based on the following rationales, was submitted:

- 6. Increased exposure to *P. flocculosa*, due to use of the end-use product, Sporodex L, will be minimal:** *Pseudozyma* species are ubiquitous in nature and have been isolated from a wide variety of plant surfaces including leaf litter (Bandoni, 1985), clover, maize (Boekhout *et al.*, 1995) and cucumber (Urquhart *et al.*, 1997). No adverse effects to aquatic arthropods,

due to natural populations of *Pseudozyma flocculosa*, have been noted in the published literature.

Use of Sporodex L will be limited to foliar, greenhouse applications on cucumbers and roses, thus minimizing direct exposure to aquatic arthropods. Roses are usually grown in containers which allow for the collection and re-use of water and run-off, while most greenhouse cucumbers are grown in plastic-covered rockwool slabs which are placed on plastic ground covers. Both of these methods of production limit run-off. Any *P. flocculosa* that reaches aquatic systems, in the form of either run-off or overspray, is expected to behave as it would in the wild.

- 7. No evidence of adverse effects:** A literature search of the AGRICOLA, AGRIS, CAB ABSTRACTS, BIOLOGICAL ABSTRACTS, CHEMTOX (Hazardous and Regulated Chemicals Database), RTECS (Registry of the Toxic Effects of Chemical Substances) databases for the period 1980-1999 was conducted. For the purposes of this literature search, *Pseudozyma flocculosa* and other species in the genus *Pseudozyma*, as well as synonyms of *P. flocculosa* (eg. *Sporothrix flocculosa*) were used as the main search words. Searches based on the principle fungitoxic agents of *P. flocculosa* including the unsaturated fatty acids heptadecenoic acid, methyl-heptadecanoic acid, heptadecadienoic acid and methyl-heptadecadienoic acid, were also conducted. Other unrelated basidiomycetous yeasts known to be common epiphytes on plant surfaces such as *Sporobolomyces*, *Rhondotorula*, *Ustilago* anamorphs and *Tilletiopsis* ssp. were also searched.

The references were manually scanned for information related to genotoxicity, carcinogenicity, allergenicity, mutagenicity, toxicity, antibiotics, mycotoxins, mycocins, pathogenicity, environmental fate and interactions with aquatic arthropods. No cases or evidence of adverse effects, due to *P. flocculosa*, were reported in the literature.

REVIEWER'S COMMENTS AND CONCLUSION:

Pseudozyma flocculosa is a saprophytic fungal epiphyte and a hyperparasite of powdery mildew and other leaf-surface moulds. *Pseudozyma flocculosa* is widely distributed in North America (Canada and USA) and in Europe on aerial plant surfaces in field or greenhouse agricultural ecosystems. Heavy rainfall likely carries *P. flocculosa* into neighbouring aquatic environments, where growth and survival of terrestrial fungi such as *P. flocculosa* is limited. Neither *P. flocculosa* nor its associated fungitoxins have been associated with adverse effects in aquatic arthropods.

Sporodex L will be used in commercial greenhouses only. Aquatic arthropods will not be directly exposed to the product at the time of application. Greenhouse practices designed to limit exposure to the outside environment will limit post-application exposure to aquatic arthropods.

Use of Sporodex L in commercial greenhouses is not expected to result in increased exposure or adverse effects in aquatic arthropods. The waiver rationale is acceptable and testing to assess the risks of Sporodex L to aquatic arthropods is not required.

References Cited

- Bandoni, R.J. (1985). On an undescribed, pleomorphic hyphomycete from litter. *Botanical Journal of the Linnean Society* **91**:37-43.
- Boekhout, T., Fell, J.W. and O'Donnell, K. (1995). Molecular systematics of some yeast-like anamorphs belonging to the Ustilginales and Tilletiales. *Studies in Mycology* **38**:175-183.
- Urquhart, E.J., Sun, L.J., and Punha, Z.K. (1997). Identification of species of *Tilletiopsis* using random amplified polymorphic DNA analysis. *Canadian Journal of Plant Pathology* **19**:380-389.



Reviewer: Esther Seto , Date Dec. 11, 2001

Peer Review: Zigfridas Vaituzis, PhD
Microbial Pesticides Branch
Biopesticides and Pollution Prevention Division
U.S. Environmental Protection Agency

STUDY TYPE: Aquatic Plants, Tier I
PMRA DATA CODE M9.8.2

TEST MATERIAL (PURITY): *Pseudozyma flocculosa* - DAOM 196992 (National Collection of Fungus Cultures) (TGAI)
Sporodex L (EP)

SYNONYMS: *Stephanoascus flocculosa*
Sporothrix flocculosa

CITATION: Traquair, J., Hale, J. and Brown, W. (2000). Sporodex WP Waiver in Support of Not Submitting Aquatic Plant Data. January 24, 2000.

SPONSOR: Plant Products Co. Ltd.

EXECUTIVE SUMMARY: Plant Products Co. Ltd. submitted a justification for a data waiver from aquatic plant testing (DACO M9.8.2). The waiver request was based on observations of no adverse effects in greenhouse trials on non-target terrestrial plants, the natural occurrence of *Pseudozyma flocculosa*, the proposed use pattern and an extensive literature search which yielded no reports of adverse effects in aquatic plants.

Use of Sporodex L in commercial greenhouses is not expected to result in increased exposure or adverse effects in aquatic plants. Consequently, testing is considered unnecessary to assess the risks of Sporodex L to aquatic plants. The request for a waiver for aquatic plant testing is accepted.

STUDY AUTHOR'S SUMMARY AND DISCUSSION:

The applicant did not conduct studies on the effects of *P. flocculosa* on aquatic plants. Instead, a waiver request, based on the following rationales, was submitted:

8.Increased exposure to *P. flocculosa*, due to use of the end-product, Sporodex L, will be minimal: *Pseudozyma* species are ubiquitous in nature and have been isolated from a wide variety of plant surfaces including leaf litter (Bandoni, 1985), clover, maize (Boekhout *et al.*, Page 10/29

1995) and cucumber (Urquhart *et al.*, 1997). No adverse effects to terrestrial plants, due to natural populations of *Pseudozyma flocculosa*, have been noted.

Use of Sporodex L will be limited to foliar, greenhouse applications, thus minimizing direct exposure and risk to non-target aquatic plants. Roses are usually grown in containers which allow for the collection and re-use of water and run-off, while most greenhouse cucumbers are grown in plastic-covered rockwool slabs which are placed on plastic ground covers. Both of these methods of production limit run-off. Any *P. flocculosa* that reaches aquatic environments, in the form of either run-off or overspray, is expected to behave as it would in the wild.

9. No evidence of adverse effects: A literature search of the AGRICOLA, AGRIS, CAB ABSTRACTS, BIOLOGICAL ABSTRACTS, CHEMTOX (Hazardous and Regulated Chemicals Database), RTECS (Registry of the Toxic Effects of Chemical Substances) databases for the period 1980-1999 was conducted. For the purposes of this literature search, *Pseudozyma flocculosa* and other species in the genus *Pseudozyma*, as well as synonyms of *P. flocculosa* (eg. *Sporothrix flocculosa*) were used as the main search words. Searches based on the principle fungitoxic agents of *P. flocculosa* including the unsaturated fatty acids heptadecenoic acid, methyl-heptadecanoic acid, heptadecadienoic acid and methyl-heptadecadienoic acid, were also conducted. Other unrelated basidiomycetous yeasts known to be common epiphytes on plant surfaces such as *Sporobolomyces*, *Rhondotorula*, *Ustilago* anamorphs and *Tilletiopsis* ssp. were also searched.

The references were manually scanned for information related to genotoxicity, carcinogenicity, allergenicity, mutagenicity, toxicity, antibiotics, mycotoxins, mycocins, pathogenicity, environmental fate and interactions with aquatic plants. No cases or evidence of adverse effects, due to *P. flocculosa*, were reported in the literature.

REVIEWER'S COMMENTS AND CONCLUSION:

Pseudozyma flocculosa is a saprophytic fungal epiphyte and a hyperparasite of powdery mildew and other leaf-surface moulds. *Pseudozyma flocculosa* is widely distributed in North America (Canada and USA) and in Europe on aerial plant surfaces in field or greenhouse agricultural ecosystems. Heavy rainfall likely carries *P. flocculosa* into neighbouring aquatic environments, where growth and survival of terrestrial fungi such as *P. flocculosa* is limited. Neither *P. flocculosa* nor its associated fungitoxins have been associated with adverse effects in aquatic plants.

Sporodex L will be used in commercial greenhouses only. Aquatic plants will not be directly exposed to the product at the time of application. Greenhouse practices designed to limit exposure to the outside environment will limit post-application exposure to aquatic plants.

Use of Sporodex L in commercial greenhouses is not expected to result in increased exposure or adverse effects in aquatic plants. The waiver rationale is acceptable and testing to assess the risks of Sporodex L to aquatic plants is not required.

References Cited

- Bandoni, R.J. (1985). On an undescribed, pleomorphic hyphomycete from litter. *Botanical Journal of the Linnean Society* **91**:37-43.
- Boekhout, T., Fell, J.W. and O'Donnell, K. (1995). Molecular systematics of some yeast-like anamorphs belonging to the Ustilginales and Tilletiales. *Studies in Mycology* **38**:175-183.
- Urquhart, E.J., Sun, L.J., and Punha, Z.K. (1997). Identification of species of *Tilletiopsis* using random amplified polymorphic DNA analysis. *Canadian Journal of Plant Pathology* **19**:380-389.



Reviewer: Esther Seto, Date February 12, 2002

Peer Review: Zigfridas Vaituzis, PhD
Microbial Pesticides Branch
Biopesticides and Pollution Prevention Division
U.S. Environmental Protection Agency

STUDY TYPE: Wild Mammal Testing, Tier I
PMRA DATA Code M9.3 / USEPA OPPTS 885.4150

TEST MATERIAL (PURITY): *Pseudozyma flocculosa* - DAOM 196992 (National Collection of Fungus Cultures) (TGAI)
Sporodex L (EP)

SYNONYMS: *Stephanoascus flocculosa*
Sporothrix flocculosa

CITATION: Traquair, J. and Mudryj, G.J. (2000). Sporodex WP Waiver in Support of Not Submitting Wild Mammal Data. January 24, 2000.

SPONSOR: Plant Products Co. Ltd.

EXECUTIVE SUMMARY: Plant Products Co. Ltd. submitted a justification for a data waiver from wild mammal studies (DACO M9.2.1 and OPPTS 885.4050). The waiver request was based on the rationale that *Pseudozyma flocculosa* is a naturally-occurring soil microorganism whose level in the environment will not significantly increase with the use of Sporodex L and that an extensive literature search and the studies submitted to address human health issues yielded no reports or evidence of significant adverse effects in wild mammals.

Use of Sporodex L in commercial greenhouses are not expected to result in increased exposure or adverse effects in wild mammals. Consequently, testing is considered unnecessary to assess the risks of Sporodex L to wild mammals. The request for a waiver of wild mammal testing is accepted.

STUDY AUTHOR'S SUMMARY AND DISCUSSION:

The applicant did not conduct studies on the effects of *P. flocculosa* on wild mammals. Instead, a waiver request, based on the following rationales, was submitted:

10. Increased exposure to *P. flocculosa*, due to use of the end-use product, Sporodex L, will be minimal: *Pseudozyma* species are ubiquitous in nature and have been isolated from a wide variety of plant surfaces including leaf litter (Bandoni, 1985), clover, maize (Boekhout

et al., 1995) and cucumber (Urquhart *et al.*, 1997). No adverse effects to wild mammals, due to natural populations of *Pseudozyma flocculosa*, have been noted in the published literature.

Use of Sporodex L will be limited to foliar, greenhouse applications on cucumbers and roses, thus minimizing direct exposure to wild mammals. Roses are usually grown in containers which allow for the collection and re-use of water and run-off, while most greenhouse cucumbers are grown in plastic-covered rockwool slabs which are placed on plastic ground

covers. Both of these methods of production limit run-off. Any *P. flocculosa* that reaches the soil, in the form of either run-off or overspray, is expected to behave as it would in the wild.

11. No evidence of adverse effects: A literature search of the AGRICOLA, AGRIS, CAB ABSTRACTS, BIOLOGICAL ABSTRACTS, CHEMTOX (Hazardous and Regulated Chemicals Database), RTECS (Registry of the Toxic Effects of Chemical Substances) databases for the period 1980-1999 was conducted. For the purposes of this literature search, *Pseudozyma flocculosa* and other species in the genus *Pseudozyma*, as well as synonyms of *P. flocculosa* (eg. *Sporothrix flocculosa*) were used as the main search words. Searches based on the principle fungitoxic agents of *P. flocculosa* including the unsaturated fatty acids heptadecenoic acid, methyl-heptadecanoic acid, heptadecadienoic acid and methyl-heptadecadienoic acid, were also conducted. Other unrelated basidiomycetous yeasts known to be common epiphytes on plant surfaces such as *Sporobolomyces*, *Rhondotorula*, *Ustilago* anamorphs and *Tilletiopsis* ssp. were also searched.

The references were manually scanned for information related to genotoxicity, carcinogenicity, allergenicity, mutagenicity, toxicity, antibiotics, mycotoxins, mycocins, pathogenicity, environmental fate and interactions with wild mammals. No cases or evidence of adverse effects, due to *P. flocculosa*, were reported in the literature.

3. Mammalian toxicity/pathogenicity studies: According to the toxicological information submitted under Part M4 Human Health and Safety Testing, *Sporothrix flocculosa* (now termed *Pseudozyma flocculosa*) was not toxic or pathogenic to Fisher 344 rats following oral gavage of Sporodex WP (an alternative end-use formulation) at a dose of 5.8×10^8 CFU/animal. Exposure via intraperitoneal injection of 3.5×10^7 CFU/animal indicated that *S. flocculosa* was of slight toxicity (due to decreased body weight gain coupled with increased food consumption of treated male rats) but was not pathogenic. No signs of dermal irritation or dermal toxicity were noted in rabbits after dermal exposure to Sporodex WP. Slight ocular irritation was observed up to 24 hours after exposure to Sporodex WP, but all signs of irritation subsided by the 48-hour scoring interval. Sporodex L is expected to be less irritating, than Sporodex WP, to the skin and eyes due to the reduction of an irritating component of the final formulation.

The acute pulmonary toxicity/infectivity study was not acceptable due to a large number of deaths in the treatment and control groups, likely due to improper dosing technique. A subsequent range-finding study indicated that *S. flocculosa* was not toxic at doses of up to 4.2×10^7 CFU/animal. This study, however, was considered supplemental because the highest

dose administered was below the minimum dose required of 10^8 CFU/animal and because infectivity/pathogenicity was not addressed.

REVIEWER'S COMMENTS AND CONCLUSION:

Pseudozyma flocculosa is a saprophytic fungal epiphyte and a hyperparasite of powdery mildew and other leaf-surface moulds. *P. flocculosa* is widely distributed in North America (Canada and USA) and in Europe on aerial plant surfaces in field or greenhouse agricultural ecosystems. Rapid death and collapse of susceptible host cells is brought about by the secretion of three fungitoxic unsaturated fatty acids and an acyclic norterpene. Neither *P. flocculosa* nor its associated fungitoxins have been associated with adverse effects in wild mammals.

Studies submitted to address human health concerns indicate that *P. flocculosa* is not toxic or pathogenic when administered by the oral route. Slight toxicity was observed following administration by the intraperitoneal route, however, this route of exposure is unlikely in wild mammals. No signs of dermal toxicity or dermal irritation were observed. Slight ocular irritation was noted with an alternative end-use formulation which, when compared to the present Sporodex L formulation, contained a higher level of a potentially irritating formulation.

Although data addressing exposure via the pulmonary route was found to be lacking, Sporodex L will be used in commercial greenhouses only. Wild mammals will not be directly exposed to the product by this route at the time of application. Greenhouse practices designed to limit exposure to the outside environment will limit post-application exposure to wild mammals.

Greenhouse use of Sporodex L is not expected to result in increased exposure or adverse effects in wild mammals. The waiver rationale is acceptable and testing to assess the risks of Sporodex L to wild mammals is not required.

References Cited

Bandoni, R.J. (1985). On an undescribed, pleomorphic hyphomycete from litter. *Botanical Journal of the Linnean Society* **91**:37-43.

Boekhout, T., Fell, J.W. and O'Donnell, K. (1995). Molecular systematics of some yeast-like anamorphs belonging to the Ustilginales and Tilletiales. *Studies in Mycology* **38**:175-183.

Urquhart, E.J., Sun, L.J., and Punha, Z.K. (1997). Identification of species of *Tilletiopsis* using random amplified polymorphic DNA analysis. *Canadian Journal of Plant Pathology* **19**:380-389.



Reviewer: Esther Seto , Date Dec. 11, 2001

Peer Review: Zigfridas Vaituzis, PhD
Microbial Pesticides Branch
Biopesticides and Pollution Prevention Division
U.S. Environmental Protection Agency

STUDY TYPE: Terrestrial Plants, Tier I
PMRA DATA CODE M9.8.1 / USEPA OPPTS 885.4300

TEST MATERIAL (PURITY): *Pseudozyma flocculosa* - DAOM 196992 (National Collection of Fungus Cultures) (TGAI)
Sporodex L (EP)

SYNONYMS: *Stephanoascus flocculosa*
Sporothrix flocculosa

CITATION: Traquair, J., Hale, J. and Brown, W. (2000). Sporodex WP Waiver in Support of Not Submitting Terrestrial Plant Data. January 24, 2000.

SPONSOR: Plant Products Co. Ltd.

EXECUTIVE SUMMARY: Plant Products Co. Ltd. submitted a justification for a data waiver from terrestrial plant studies (DACO M9.8.1 and OPPTS 885.4300). The waiver request was based on observations of no adverse effects in greenhouse trials, the natural occurrence of *Pseudozyma flocculosa*, the proposed use pattern and an extensive literature search which yielded no reports of adverse effects in terrestrial plants.

Use of Sporodex L in commercial greenhouses is not expected to result in increased exposure or adverse effects in terrestrial plants. Consequently, testing is considered unnecessary to assess the risks of Sporodex L to terrestrial plants. The request for a waiver for terrestrial plant testing is accepted.

STUDY AUTHOR'S SUMMARY AND DISCUSSION:

The applicant did not conduct studies on the effects of *P. flocculosa* on terrestrial plants. Instead, a waiver request, based on the following rationales, was submitted:

1. **No adverse effects observed in greenhouse trials:** Nine research trials on cucumbers (cv. ventura, jessica and flamingo) and a single trial on tomatoes (cv. aromata) were conducted to determine the efficacy of Sporodex WP (a wettable powder formulation of *P. flocculosa*). 16

Visual evaluations for phytotoxicity and crop status were included in each trial. No adverse effects on the target plants were observed.

In a commercial rose trial comparing the efficacy of *P. flocculosa* against chemical fungicides (prochloraz and dodemorph-acetate), the quality of the roses treated with the biological fungicide were comparable to those treated with chemical products (Bureau, unpublished).

2. Increased exposure to *P. flocculosa*, due to use of Sporodex L, will be minimal:

Pseudozyma species are ubiquitous in nature and have been isolated from a wide variety of plant surfaces including leaf litter (Bandoni, 1985), clover, maize (Boekhout *et al.*, 1995) and cucumber (Urquhart *et al.*, 1997). No adverse effects to terrestrial plants, due to natural populations of *Pseudozyma flocculosa*, have been noted.

Use of Sporodex L will be limited to foliar, greenhouse applications, thus minimizing direct exposure and risk to non-target terrestrial plants. Roses are usually grown in containers which allow for the collection and re-use of water and run-off, while most greenhouse cucumbers are grown in plastic-covered rockwool slabs which are placed on plastic ground covers. Both of these methods of production limit run-off. Any *P. flocculosa* that reaches the soil, in the form of either run-off or overspray, is expected to behave as it would in the wild.

3. No evidence of adverse effects: A literature search of the AGRICOLA, AGRIS, CAB ABSTRACTS, BIOLOGICAL ABSTRACTS, CHEMTOX (Hazardous and Regulated Chemicals Database), RTECS (Registry of the Toxic Effects of Chemical Substances) databases for the period 1980-1999 was conducted. For the purposes of this literature search, *Pseudozyma flocculosa* and other species in the genus *Pseudozyma*, as well as synonyms of *P. flocculosa* (eg. *Sporothrix flocculosa*) were used as the main search words. Searches based on the principle fungitoxic agents of *P. flocculosa* including the unsaturated fatty acids heptadecenoic acid, methyl-heptadecanoic acid, heptadecadienoic acid and methyl-heptadecadienoic acid, were also conducted. Other unrelated basidiomycetous yeasts known to be common epiphytes on plant surfaces such as *Sporobolomyces*, *Rhondotorula*, *Ustilago* anamorphs and *Tilletiopsis* ssp. were also searched.

The references were manually scanned for information related to genotoxicity, carcinogenicity, allergenicity, mutagenicity, toxicity, antibiotics, mycotoxins, mycocins, pathogenicity, environmental fate and interactions with terrestrial plants. No cases or evidence of adverse effects, due to *P. flocculosa*, were reported in the literature.

REVIEWER'S COMMENTS AND CONCLUSION:

Pseudozyma flocculosa is a saprophytic fungal epiphyte and a hyperparasite of powdery mildew and other leaf-surface moulds. *Pseudozyma flocculosa* is widely distributed in North America (Canada and USA) and in Europe on aerial plant surfaces in field or greenhouse agricultural ecosystems. Despite the common occurrence of *P. flocculosa*, an extensive search of the published literature failed to produce references indicating adverse effects on terrestrial plants. The strain of *P. flocculosa* used in Sporodex L was, in fact, isolated from the leaves of red clover

grown in Harrow, Ontario. Furthermore, visual inspections of cucumber and tomato plants, treated with *P. flocculosa* for numerous efficacy trials, yielded no signs of phytotoxicity.

Sporodex L will be used in commercial greenhouses only. Non-target terrestrial plants will not be directly exposed to the product at the time of application. Greenhouse practices designed to limit exposure to the outside environment will limit post-application exposure to non-target terrestrial plants.

Use of Sporodex L in commercial greenhouses is not expected to result in increased exposure or adverse effects in terrestrial plants. The waiver rationale is acceptable and testing to assess the risks of Sporodex L to non-target terrestrial plants is not required.

References Cited

- Bandoni, R.J. (1985). On an undescribed, pleomorphic hyphomycete from litter. *Botanical Journal of the Linnean Society* **91**:37-43.
- Boekhout, T., Fell, J.W. and O'Donnell, K. (1995). Molecular systematics of some yeast-like anamorphs belonging to the Ustilginales and Tilletiales. *Studies in Mycology* **38**:175-183.
- Bureau, A.M. (2000). Commercial Scale Control of Rose Powdery Mildew with the Biofungicide Sporodex in Typical Columbian Greenhouses. Draft thesis.
- Urquhart, E.J., Sun, L.J., and Punha, Z.K. (1997). Identification of species of *Tilletiopsis* using random amplified polymorphic DNA analysis. *Canadian Journal of Plant Pathology* **19**:380-389.



Reviewer: Esther Seto , Date Dec. 11, 2001

Peer Review: Zigfridas Vaituzis, PhD
Microbial Pesticides Branch
Biopesticides and Pollution Prevention Division
U.S. Environmental Protection Agency

STUDY TYPE: Terrestrial Arthropods, Tier I
PMRA DATA CODE M9.5.1 / USEPA OPPTS 885.4340
Honey Bee, Tier I
USEPA OPPTS 885.4380

TEST MATERIAL (PURITY): *Pseudozyma flocculosa* - DAOM 196992 (National Collection of Fungus Cultures) (TGAI)
Sporodex L (EP)

SYNONYMS: *Stephanoascus flocculosa*
Sporothrix flocculosa

CITATION: Traquair, J., Hale, J. and Brown, W. (2000). Sporodex WP Waiver in Support of Not Submitting Terrestrial Arthropod and Honey Bee Testing Data. January 24, 2000.

SPONSOR: Plant Products Co. Ltd.

EXECUTIVE SUMMARY: Plant Products Co. Ltd. submitted a justification for a data waiver from terrestrial arthropod and honey bee testing (DACO M9.5.1 and USEPA OPPTS 885.4340 and OPPTS 885.4380). The waiver request was based on the rationale that *Pseudozyma flocculosa* is a naturally-occurring microorganism whose level in the environment will not significantly increase with the use of Sporodex L and that an extensive literature search yielded no reports of adverse effects in terrestrial arthropods.

Use of Sporodex L in commercial greenhouses is not expected to result in increased exposure or adverse effects in terrestrial arthropods and honeybees. Consequently, testing is considered unnecessary to assess the risks of Sporodex L to terrestrial arthropods and honey bees. The request for a waiver of terrestrial arthropod and honey bee testing is accepted.

STUDY AUTHOR'S SUMMARY AND DISCUSSION:

The applicant did not conduct studies on the effects of *P. flocculosa* on terrestrial arthropods. Instead, a waiver request, based on the following rationales, was submitted:

4. Increased exposure to *P. flocculosa*, due to use of the end-use product, Sporodex L, will be minimal: *Pseudozyma* species are ubiquitous in nature and have been isolated from a wide variety of plant surfaces including leaf litter (Bandoni, 1985), clover, maize (Boekhout *et al.*, 1995) and cucumber (Urquhart *et al.*, 1997). No adverse effects to terrestrial arthropods or honey bees, due to natural populations of *Pseudozyma flocculosa*, have been noted. Use of Sporodex L will be limited to foliar, greenhouse applications, thus minimizing direct exposure and risk to terrestrial arthropods and honey bees in the outside environment. Roses are usually grown in containers which allow for the collection and re-use of water and run-off, while most greenhouse cucumbers are grown in plastic-covered rockwool slabs which are placed on plastic ground covers. Both of these methods of production limit run-off. Any *P. flocculosa* that reaches the soil is expected to behave as it would in the wild. Plant material will be disposed of by composting. The applicant reasoned that *P. flocculosa* will not persist on composted vegetation because it is a parasite of powdery mildew, which in turn is an obligate parasite of plant material. No evidence or data was provided to support this claim. The inability of *P. flocculosa* to survive at high temperatures, (i.e. >37°C; Middelhoven, 1997), however, indicates that it will not persist on composted vegetation.

5. No evidence of adverse effects: A literature search of the AGRICOLA, AGRIS, CAB ABSTRACTS, BIOLOGICAL ABSTRACTS, CHEMTOX (Hazardous and Regulated Chemicals Database), RTECS (Registry of the Toxic Effects of Chemical Substances) databases for the period 1980-1999 was conducted. For the purposes of this literature search, *Pseudozyma flocculosa* and other species in the genus *Pseudozyma*, as well as synonyms of *P. flocculosa* (eg. *Sporothrix flocculosa*) were used as the main search words. Searches based on the principle fungitoxic agents of *P. flocculosa* including the unsaturated fatty acids heptadecenoic acid, methyl-heptadecanoic acid, heptadecadienoic acid and methyl-heptadecadienoic acid, were also conducted. Other unrelated basidiomycetous yeasts known to be common epiphytes on plant surfaces such as *Sporobolomyces*, *Rhondotorula*, *Ustilago* anamorphs and *Tilletiopsis* ssp. were also searched. The references were manually scanned for information related to genotoxicity, carcinogenicity, allergenicity, mutagenicity, toxicity, antibiotics, mycotoxins, mycocins, pathogenicity, environmental fate and interactions with terrestrial arthropods. Although *Stephanoascus flocculosus* (anamorph *Sporothrix flocculosa*) was isolated from mosquito eggs (*Culex pipines quinquefoscatus*) and dead spider mites (*Tetranychus urticae*) (Traquair *et al.*, 1988), no cases or evidence of adverse effects, due to *P. flocculosa*, were reported in the literature.

REVIEWER'S CONCLUSIONS:

Pseudozyma flocculosa is a saprophytic fungal epiphyte and a hyperparasite of powdery mildew and other leaf-surface moulds. *Pseudozyma flocculosa* is widely distributed in North America (Canada and USA) and in Europe on aerial plant surfaces in field or greenhouse agricultural ecosystems. Despite the common occurrence of *P. flocculosa*, an extensive search of the

published literature failed to produce references indicating adverse effects on terrestrial arthropods.

Sporodex L will be used in commercial greenhouses only. Terrestrial arthropods, outside of greenhouse facilities using Sporodex L, will not be directly exposed to the product at the time of application. Greenhouse practices designed to limit exposure to the outside environment will limit post-application exposure to terrestrial arthropods.

Use of Sporodex L in commercial greenhouses is not expected to result in increased exposure or adverse effects in terrestrial arthropods. The waiver rationale is acceptable and testing to assess the risks of Sporodex L to terrestrial arthropods is not required.

References Cited

- Bandoni, R.J. (1985). On an undescribed, pleomorphic hyphomycete from litter. *Botanical Journal of the Linnean Society* **91**:37-43.
- Boekhout, T., Fell, J.W. and O'Donnell, K. (1995). Molecular systematics of some yeast-like anamorphs belonging to the Ustilginales and Tilletiales. *Studies in Mycology* **38**:175-183.
- Middelhoven, W.J. (1997). Identity and biodegradative abilities of yeasts isolated from plants growing in an arid climate. *Antonie van Leeuwenhoek* **72**:81-89.
- Traquair, J.A., Shaw, L.A., and Jarvis, W.R. (1988). New species of *Stephanoascus* with *Sporothrix* anamorphs. *Canadian Journal of Botany* **66**:926-933.



Reviewer: Esther Seto , Date Dec. 5, 2001

Peer Review: Zigfridas Vaituzis, PhD
Microbial Pesticides Branch
Biopesticides and Pollution Prevention Division
U.S. Environmental Protection Agency

STUDY TYPE: Non-Arthropod Invertebrates, Tier I
PMRA DATA CODE M9.6

TEST MATERIAL (PURITY): *Pseudozyma flocculosa* - DAOM 196992 (National Collection of Fungus Cultures) (TGAI)
Sporodex L (EP)

SYNONYMS: *Stephanoascus flocculosa*
Sporothrix flocculosa

CITATION: Traquair, J., Hale, J. and Brown, W. (2000). Sporodex WP Waiver in Support of Not Submitting Non-Arthropod Invertebrate Data. January 24, 2000.

SPONSOR: Plant Products Co. Ltd.

EXECUTIVE SUMMARY: Plant Products Co. Ltd. submitted a justification for a data waiver from non-arthropod invertebrate studies (DACO M9.6). The waiver request was based on the rationale that *Pseudozyma flocculosa* is a naturally-occurring microorganism whose level in the environment will not significantly increase with the use of Sporodex L and that an extensive literature search yielded no reports of adverse effects in aquatic arthropods.

Use of Sporodex L in commercial greenhouses is not expected to result in increased exposure or adverse effects in non-arthropod invertebrates. Consequently, testing is considered unnecessary to assess the risks of Sporodex L to non-arthropod invertebrates. The request for a waiver for non-arthropod invertebrate testing is accepted.

STUDY AUTHOR'S SUMMARY AND DISCUSSION:

The applicant did not conduct studies on the effects of *P. flocculosa* on non-arthropod invertebrates. Instead, a waiver request, based on the following rationales, was submitted:

- 6. Increased exposure to *P. flocculosa*, due to use of the end-use product, Sporodex L, will be minimal:** *Pseudozyma* species are ubiquitous in nature and have been isolated from a wide variety of plant surfaces including leaf litter (Bandoni, 1985), clover, maize (Boekhout *et al.*, 1995) and cucumber (Urquhart *et al.*, 1997). No adverse effects to non-arthropod22/29

invertebrates, due to natural populations of *Pseudozyma flocculosa*, have been noted in the published literature.

Use of Sporodex L will be limited to foliar, greenhouse applications on cucumbers and roses, thus minimizing direct exposure to non-arthropod invertebrates. Roses are usually grown in containers which allow for the collection and re-use of water and run-off, while most commercial greenhouse cucumbers are grown in plastic-covered rockwool slabs which are placed on plastic ground covers. Both of these methods of production limit run-off. Any *P. flocculosa* that reaches the soil, in the form of run-off, is expected to behave as it would in the wild.

Plant material will be disposed of by composting. The applicant reasoned that *P. flocculosa* will not persist on composted vegetation because it is a parasite of powdery mildew, which in turn is an obligate parasite of plant material. No evidence or data was provided to support this claim. The inability of *P. flocculosa* to survive at high temperatures, (i.e. >37°C; Middelhoven, 1997), however, indicates that it will not persist on composted vegetation.

- 7. No evidence of adverse effects:** A literature search of the AGRICOLA, AGRIS, CAB ABSTRACTS, BIOLOGICAL ABSTRACTS, CHEMTOX (Hazardous and Regulated Chemicals Database), RTECS (Registry of the Toxic Effects of Chemical Substances) databases for the period 1980-1999 was conducted. For the purposes of this literature search, *Pseudozyma flocculosa* and other species in the genus *Pseudozyma*, as well as synonyms of *P. flocculosa* (eg. *Sporothrix flocculosa*) were used as the main search words. Searches based on the principle fungitoxic agents of *P. flocculosa* including the unsaturated fatty acids heptadecenoic acid, methyl-heptadecanoic acid, heptadecadienoic acid and methyl-heptadecadienoic acid, were also conducted. Other unrelated basidiomycetous yeasts known to be common epiphytes on plant surfaces such as *Sporobolomyces*, *Rhondotorula*, *Ustilago* anamorphs and *Tilletiopsis* ssp. were also searched.

The references were manually scanned for information related to genotoxicity, carcinogenicity, allergenicity, mutagenicity, toxicity, antibiotics, mycotoxins, mycocins, pathogenicity, environmental fate and interactions with non-arthropod invertebrates. No cases or evidence of adverse effects, due to *P. flocculosa*, were reported in the literature.

REVIEWER'S COMMENTS AND CONCLUSION:

Pseudozyma flocculosa is a saprophytic fungal epiphyte and a hyperparasite of powdery mildew and other leaf-surface moulds. *Pseudozyma flocculosa* is widely distributed in North America (Canada and USA) and in Europe on aerial plant surfaces in field or greenhouse agricultural ecosystems. Neither *P. flocculosa* nor its associated fungitoxins have been associated with adverse effects in non-arthropod invertebrates.

Sporodex L will be used in commercial greenhouses only. Non-arthropod invertebrates will not be directly exposed to the product at the time of application. Greenhouse practices designed to limit exposure to the outside environment will limit post-application exposure to non-arthropod invertebrates.

Use of Sporodex L in commercial greenhouses is not expected to result in increased exposure or adverse effects in non-arthropod invertebrates. The waiver rationale is acceptable and testing to assess the risks of Sporodex L to non-arthropod invertebrates is not required.

References Cited

- Bandoni, R.J. (1985). On an undescribed, pleomorphic hyphomycete from litter. *Botanical Journal of the Linnean Society* **91**:37-43.
- Boekhout, T., Fell, J.W. and O'Donnell, K. (1995). Molecular systematics of some yeast-like anamorphs belonging to the Ustilginales and Tilletiales. *Studies in Mycology* **38**:175-183.
- Middelhoven, W.J. (1997). Identity and biodegradative abilities of yeasts isolated from plants growing in an arid climate. *Antonie van Leeuwenhoek* **72**:81-89.
- Urquhart, E.J., Sun, L.J., and Punha, Z.K. (1997). Identification of species of *Tilletiopsis* using random amplified polymorphic DNA analysis. *Canadian Journal of Plant Pathology* **19**:380-389.
- Urquhart, E.J., Sun, L.J., and Punha, Z.K. (1997). Identification of species of *Tilletiopsis* using random amplified polymorphic DNA analysis. *Canadian Journal of Plant Pathology* **19**:380-389.



Reviewer: Esther Seto , Date Dec. 7, 2001

Peer Review: Zigfridas Vaituzis, PhD
Microbial Pesticides Branch
Biopesticides and Pollution Prevention Division
U.S. Environmental Protection Agency

STUDY TYPE: Microorganisms, Tier I
PMRA DATA CODE M9.7

TEST MATERIAL (PURITY): Sporodex L (EP)

SYNONYMS: *Pseudozyma flocculosa* (DAOM 196992 - National Collection of Fungus Cultures)

CITATION: Traquair, J., Hale, J. and Brown, W. (2000). Sporodex WP Waiver in Support of Not Submitting Microorganism Data. January 24, 2000.

SPONSOR: Plant Products Co. Ltd.

EXECUTIVE SUMMARY: In place of submitting microorganism studies, Plant Products Co. Ltd. submitted a summary of the host range and mode of action for *Pseudozyma flocculosa*. This information has been reviewed for Part M2 *Product Characterization and Analysis*. The applicant has also based a waiver rationale on the natural occurrence and limited additional exposure which will be expected due to the proposed uses of Sporodex L.

A potential exists, particularly in a greenhouse environment where conditions are optimal for growth (eg. high relative humidity, controlled temperatures), for *P. flocculosa* to adversely affect non-target microorganisms. Based on the natural occurrence, limited exposure outside of a greenhouse, mode of action and host range of *P. flocculosa*, however, non-target microorganism testing with Sporodex L will not be required.

STUDY AUTHOR'S SUMMARY AND DISCUSSION:

The applicant did not conduct studies on the effects of *Pseudozyma flocculosa* on non-target microorganisms or microbial processes. The following factors were discussed by the applicant in their waiver request:

8.Mode of action and pest host range: *Pseudozyma flocculosa* antagonizes a number of different powdery mildew fungi (*Sphaerotheca pannosa* var. *rosae*, *Sphaerotheca fulginea*, *Erysiphe graminis* var. *tritici* and *Erysiphe polygoni*) on many different plants (eg. cucumber,

rose, wheat and tomato) in greenhouse and field environments when the relative humidity is 70% or more (Jarvis *et al.*, 1989; Hajlaoui and Bélanger, 1991). This fungus is a necrotroph mycoparasite that kills susceptible target host cells upon contact or in close proximity. Rapid death and collapse of host cells without penetration is brought about by the secretion of three fungitoxic unsaturated fatty acids (9-heptadecenoic acid, 6-methyl-9-heptadecenoic acid and 4-methyl-7-11-heptadecadienoic acid) and an acyclic norterpene (2, 6, 10, 14, 18-pentamethyl-2, 6, 8, 10, 12, 14, 17-nonadecaheptene-1, 19-diol) (Benyagoub *et al.*, 1996; Choudhury *et al.*, 1994). These diffusible inhibitory compounds have been detected in the culture filtrates and produce the same symptoms of antagonism when tested alone on mildews and other fungi *in vitro* (Hajlaoui *et al.*, 1994).

The fungitoxins disrupt susceptible plasma membranes and cytoplasmic organelles within 30 minutes of exposure. The inhibitory response includes a loss of proteins and electrolytes (Hajlaoui *et al.*, 1994). After 24 hours, the host cells rapidly collapse and die as a result of the activity of the fungitoxins on the host cell's membranes and lipids (Hajlaoui *et al.*, 1992). The unsaturated C-17 fatty acids increase the fluidity of the host plasma membrane. Since many metabolic reactions occur in association with the plasma membrane, the modification of membrane fluidity is extremely detrimental to the host organism.

Benyagoub *et al.* (1996) showed that the major responses of sensitive hosts to sub-lethal doses of antifungal fatty acids were a decrease in total lipids, an increase in fatty acid unsaturation, an increase in free fatty acids and phosphatidic acid and decrease in total phospholipids, and an increase in sterol/phospholipid ratio. It appears that the fungitoxins stimulate the breakdown of phospholipids and inhibit the incorporation of fatty acids into acyl lipids and/or stimulate *de novo* synthesis of fatty acids. Free fatty acids are known to be toxic to fungi in high concentrations (Sisler and Ragsdale, 1977). The increase in fatty acid unsaturation further disrupts the membrane which leads to its deterioration. Cell-wall degrading exoenzymes, such as chitinase, are not involved in the initial stage of cellular disruption (Hajlaoui *et al.*, 1992).

Fungal sensitivity to unsaturated C-17 free fatty acids is related to a high degree of unsaturation of phospholipid fatty acids and a low proportion of sterols. Hajlaoui *et al.* (1994) demonstrated that methylene chloride extracts completely inhibited *Cladosporium cucumerinum*, *Botrytis cinerea*, *Rhizoctonia solani*, *Pythium aphanidermatum*, *Pythium ultimum* and *Phytophthora infestans* at a concentration of 132 mg/mL within 72 hours in 50 mL of potato-dextrose broth cultures. *Fusarium* f. sp. *radicis-lycopersici* and *Fusarium graminearum*, however, were only moderately inhibited. Similarly, Choudhury *et al.* (1994) showed that *Trichoderma viride*, *Fusarium oxysporum* f.sp. *lycopersici* are moderately inhibited by 4-methyl-7,11-heptadecadienoic acid (C-17 unsaturated fatty acid) at concentrations ranging from 0.5 to 5 mg/mL within 48 hours in agar-diffusion bioassays. Furthermore, Choudhury *et al.* (1997) demonstrated the antifungal property of 2, 6, 10, 14, 18-pentamethyl-2, 6, 8, 10, 12, 14, 17-nonadecaheptene-1,19-diol using agar diffusion

bioassays. It was shown that *Trichoderma viride*, *Fusarium tricinctum* and *Trichoderma viride* were inhibited within 48 hours by 5 mg/mL of the acyclic norterpene. Similarly, *Bacillus subtilis* and *Xanthomonas campestris* were inhibited by 10 mg/mL of the acyclic norterpene. Very little of the metabolite, however, is secreted outside the cell hence it has very limited antifungal potential.

The applicant claims that the bioactive compounds in *Pseudozyma flocculosa* are not stable on the plant leaf surface under greenhouse conditions, but no data was provided as support.

Pseudozyma flocculosa kills mildew *in situ* but its efficacy on other soil- and foliage-borne phytopathogenic fungi in the greenhouse and the field is unclear. The applicant claims that preliminary evidence with soil-borne fungal pathogens such as *Trichoderma viride*, *Fusarium oxysporum*, *Fusarium graminearum*, *Pythium* ssp., *Phytophthora infestans* and *Rhizoctina solani* and selected Gram-negative (*Xanthomonas campestris*) and Gram-positive (*Bacillus subtilis*) bacteria indicate a moderate to weak antimicrobial activity.

9. Increased exposure to *P. flocculosa*, due to use of Sporodex L, will be minimal:

Pseudozyma species are ubiquitous in nature and have been isolated from a wide variety of plant surfaces including leaf litter (Bandoni, 1985), clover, maize (Boekhout *et al.*, 1995) and cucumber (Urquhart *et al.*, 1997).

Use of Sporodex L will be limited to foliar, greenhouse applications, thus minimizing direct environmental exposure. Roses are usually grown in containers which allow for the collection and re-use of water and run-off, while most greenhouse cucumbers are grown in plastic-covered rockwool slabs which are placed on plastic ground covers. Both of these methods of production limit run-off. Any *P. flocculosa* that reaches the soil is expected to behave as it would in the wild.

REVIEWER'S COMMENTS AND CONCLUSION:

The articles included in the submission indicate that a number of organisms are more sensitive to the fungitoxins than the applicant claims. Hajlaoui *et al.* (1994) showed the *Pythium* ssp., *Phytophthora infestans* and *Rhizoctina solani* were completely inhibited by 132 µg/mL of *Pseudozyma flocculosa* methylene chloride medium extracts. Given the intended use of Sporodex L for control of powdery mildew and the mode of action of *P. flocculosa*, there is a potential for other beneficial microorganisms, particularly fungi, to be adversely affected. However, greenhouse practices limiting exposure of the outside environment to Sporodex L are adequate and non-target microorganism testing will not be required.

References Cited

Bandoni, R.J. (1985). On an undescribed, pleomorphic hyphomycete from litter. Botanical Journal of the Linnean Society **91**:37-43.

- Benyagoub, M., Willemot, C. and Bélanger, R. R. (1996). Influence of subinhibitory dose of antifungal fatty acids from *Sporothrix flocculosa* on cellular lipid composition in fungi. *Lipids* **31**:1077-1082.
- Boekhout, T., Fell, J.W. and O'Donnell, K. (1995). Molecular systematics of some yeast-like anamorphs belonging to the Ustilaginales and Tilletiales. *Studies in Mycology* **38**:175-183.
- Choudhury, S. R., Traquair, J. A. and Jarvis, W. R. (1994). 4-Methyl-7,11-heptadecadienal and 4-methyl-7,11-heptadecadienoic acid: new antibiotics from *Sporothrix flocculosa* and *Sporothrix rugulosa*. *J. Natural Products* **57**(6):700-704.
- Choudhury, S. R., Traquair, J. A. and Jarvis, W. R. (1997). A new polyenediol from *Sporothrix flocculosa* and *S. rugulosa*. Unpublished.
- Hajlaoui, M. R. and Bélanger, R. R. (1991). Antagonism of the yeast-like phylloplane fungus *Sporothrix flocculosa* against *Erysiphe graminis* var *tritici*. *Biocontrol Science and Technology* **3**:427-434.
- Hajlaoui, M. R., Benhamou, A. and Bélanger, R. R. (1992). Cytochemical study of the antagonistic activity of *Sporothrix flocculosa* on rose powdery mildew, *Sphaerotheca pannosa* var. *rosae*. *Phytopathology* **82**:583-589.
- Hajlaoui, M. R., Traquair, J. A., Jarvis, W. R. and Bélanger, R. R. (1994). Antifungal activity of extracellular metabolites produced by *Sporothrix flocculosa*. *Biocontrol Science and Technology* **4**:229-237.
- Jarvis, W. R., Shaw, L. A. and Traquair, J. A. (1989). Factors affecting antagonism of cucumber powdery mildew by *Stephanoascus flocculosus* and *S. rugulosus*. *Mycological Research* **92**(2):162-165.
- Middelhoven, W.J. (1997). Identify and biodegradative abilities of yeasts isolated from plants growing in an arid climate. *Antonie van Leeuwenhoek* **72**:81-89.
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- Starmer, W.T., Ganter, P.F., Aberdeen, V., Lachance, M.A. and Phaff, H.J. (1987). The ecological role of killer yeasts in natural communities of yeasts. *Canadian Journal of Microbiology* **33**:783-796.
- Traquair, J.A., Shaw, L.A., and Jarvis, W.R. (1988). New species of *Stephanoascus* with *Sporothrix* anamorphs. *Canadian Journal of Botany* **66**:926-933.

Urquhart, E.J., Sun, L.J., and Punha, Z.K. (1997). Identification of species of *Tilletiopsis* using random amplified polymorphic DNA analysis. Canadian Journal of Plant Pathology **19**:380-389.