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PLANTSKYDD® ANIMAL REPELLENT: EFFECTS ON FUNGAL COLONIZATION OF STYROBLOCK CONTAINERS AND STORAGE MOLD ON CONTAINER-GROWN WESTERN RED CEDAR SEEDLINGS

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ABSTRACT

Plantskydd® is a very effective animal repellent when applied topically to young conifer seedlings prior to outplanting. Treating western red cedar seedlings at the USDA Forest Service Nursery, Coeur d'Alene, Idaho while they were within containers prior to extraction and cold storage resulted in very high levels of molding following storage. Many seedlings had to be culled because of the extensive molding. Plantskydd® was not fungicidal on a treated styroblock container. High levels of potentially-pathogenic *Fusarium* and *Botrytis* survived treatment. The major *Fusarium* species surviving on the treated container was *F. proliferatum*; *F. avenaceum* and *F. sporotrichioides* were also commonly isolated. The most common fungi isolated from superficial mycelial growth on molded seedlings were *Aspergillus, Mucor*, and *Phoma* spp.; those frequently isolated from necrotic stem lesions were *Phoma* spp. Seedling treatments with Plantskydd® at the nursery prior to storage results in high levels of molding and poor seedling quality. Treatments should be made either after storage or in the field during outplanting.

INTRODUCTION

Animal damage of outplanted seedlings on forest regeneration sites is a serious problem in the Northern Region. Various techniques have been evaluated to try to reduce damage, which is especially severe during the first year after outplanting. Recently, a material made of bloodmeal, water, common salt, and vegetable fat (Plantskydd®) manufactured in Sweden, was evaluated for its

ability as an animal repellent on conifer seedlings produced at the USDA Forest Service Nursery, Coeur d'Alene, Idaho. This repellent provided excellent results in the field and was especially effective on recently-planted western red cedar (*Thuja plicata* Donn) seedlings, which are usually heavily damaged by animals. One treatment is usually effective for the entire first growing season in the field.

Plantskydd ® is an EPA exempted product/minimum risk pesticide that is exempt from the Federal Insecticide, Fungicide, and Rodenticide Act requirements. The material is mixed with water to make a solution within which seedlings are dipped; it can also be applied as a foliar spray. Foresters want the repellent to be applied to seedlings at the nursery prior to shipment for outplanting. The easiest application procedure was to dip seedlings just prior to extraction from containers, before seedlings were placed in cold storage. However, treated western red cedar seedlings removed from cold storage for outplanting were extensively molded (figure 1). This was in contrast to non-treated seedlings that were essentially mold-free.

Growers at the Coeur d'Alene Nursery were interested to know if Plantskydd® had potential fungicidal activity within treated containers, i.e., were potentially-pathogenic fungi, which routinely inhabit styroblock containers following seedling extraction, adversely affected by the repellent. In addition, growers wanted to know the identity of fungi associated with seedling molding and their potential effects following out-planting. Therefore, an evaluation was conducted to help answer these questions

MATERIALS AND METHODS

Analysis of repellent effects on fungi residing within containers was conducted on one styroblock 12 container, which was dipped in an aqueous solution of Plantskydd® using standard label concentration rates. The container was dipped, topside down, to a level about midway down the length of cells, i.e., the bottom of cells were not treated. Assays were taken from 40 randomly-selected cells at the top, middle [about 7.5 cm from the top] and bottom of sampled cells. Two pieces of styrofoam from each sample location per cell were aseptically extracted and placed on a selective agar medium for *Fusarium* and closely-related fungi (Komada 1975). Plates were incubated under diurnal cycles of cool, fluorescent light at about 24°C for at least 7 days. Selected emerging fungi were transferred to potato dextrose agar (PDA) and carnation leaf agar (Fisher et al. 1982) for identification using the taxonomy of Barnett and Hunter 1988 and Nelson et al. 1983. The percent of sampled cells colonized by a particular fungus was calculated as "infection"; the percent of sampled styrofoam pieces colonized by particular fungi was calculated as "colonization."

Figure 1. Container-grown western red cedar seedlings that had been treated with the animal repellent Plantskydd® prior to cold storage at the USDA Forest Service Nursery, Coeur d'Alene, Idaho. Seedlings were extensively molded following removal from cold storage.

In addition, residual roots from the previous seedling crop that adhered to styrofoam cell walls, were sampled. A total of 14 root pieces were sampled from the top of cells; 13 and 25 root pieces were sampled from the middle and bottom of cells, respectively. Roots were aseptically extracted, cut into pieces about 5 mm long, surface sterilized in a 10% bleach solution (0.525% aqueous sodium hypochlorite), rinsed in sterile water, blotted dry and placed on Komada's medium. Plates were incubated and associated fungi identified as described above. Percentage of root pieces colonized by particular fungi was calculated.

Samples of western red cedar seedlings thawed from cold storage and sent to the North Zone of the Idaho Panhandle National Forest for outplanting were evaluated for associated mold organisms. Many seedlings were almost totally necrotic with extensive damage to foliage, main stem and branches. Extensive superficial molding was evident on the sampled seedlings. Isolations were made from 15 molded seedlings; superficial mycelium was aseptically transferred onto 2% water agar (WA) with sterile forceps. After incubation for 2-4 days at 24°C, emerging fungi were transferred to PDA for identification. Isolations were also made from stem lesions. The location of lesion margins was found by dissecting stems with a sterile razor blade. Thin sections on the edge of lesions (where healthy and necrotic tissues met) were

aseptically extracted, surface sterilized, placed on WA and incubated as described above. A total of 17 different fungal isolates were obtained from superficial mycelial growth, whereas 12 different isolates were obtained from necrotic stem lesions. Isolates of *Pythium* and *Phoma* spp. were identified using the taxonomy of Waterhouse (1968) and Dorenbosch (1970), respectively.

RESULTS

Effects of Plantskydd® on infection and colonization of a treated styroblock container are summarized in tables 1 and 2, respectively. The bottoms of container cells were not treated and bottom colonization can be contrasted with that of the top of cells. Samples from the middle of cells may or may not have been treated. There was no apparent effect of Plantskydd® on survival of potentially-pathogenic groups of fungi (*Fusarium, Botrytis*, and *Cylindrocarpon*) or common saprophytes (*Trichoderma* and *Penicillium*). There was also no fungicidal effect on unidentified "other" fungi, most of which were probably saprophytes. Effects of the repellent on colonization of residual seedling roots within the treated container are summarized in table 3. No definitive effects of Plantskydd® on fungal colonization of these roots were found.

Seven *Fusarium* species were isolated from the sampled styroblock container (table 4); a total of 66 *Fusarium* isolates were examined. The most commonly isolated species was *F. proliferatum* (Matsushima) Nirenberg. Other fairly common species included *F. avenaceum* (Fr.) Sacc. and *F. sporotrichioides* Sherb. The other isolated fusaria included *F. acuminatum* Ell. & Ev., *F. oxysporum* Schlecht., *F. culmorum* (W.G. Smith) Sacc., and *F. sambucinum* Fuckel.

Seventeen different fungi were isolated from superficial mycelium on necrotic foliage from cold-stored western red cedar seedlings (table 5). The most frequently isolated fungi were *Aspergillus, Mucor*, and *Phoma* spp. Other fungi isolated from foliage included *Cylindrocarpon didymium* (Hartig) Wollenw., *F. oxysporum*, *Penicillium* spp., and *Pythium ultimum* Trow. The most common fungi isolated from necrotic stem lesions were *Phoma* spp. (*P. eupyrena* Sacc. and *P. pomorum* Thum. and *P. ultimum*. *Alternaria* spp. and *Aureobasidum* were isolated infrequently from stem lesion tissues.

Table 1. Effects of an aqueous solution of Plantskydd® on infection of a styroblock container by selected fungi - USDA Forest Service Nursery, Coeur d'Alene, Idaho[1].

Sample Position[2]	Fungal Genus					
	Fusarium	*Botrytis*	*Cylindrocarpon*	*Trichoderma*	*Penicillium*	Other[3]
Top	52.3	27.5	2.5	20.0	52.5	87.5
Middle	12.5	7.5	0	7.5	37.5	97.5
Bottom	67.5	15.0	5.0	32.5	70.0	62.5

[1] Values in table are percentages of sampled cells infected with particular fungi.
[2] Samples of stryrofoam extracted from the top, middle (about 7.5 cm from the top) and bottom of each sampled cell.
[3] Unidentified, probably saprophytic fungi.

Table 2. Effects of an aqueous solution of Plantskydd® on colonization of a styroblock container by selected fungi - USDA Forest Service Nursery, Coeur d'Alene, Idaho[1].

Sample Position[2]	Fungal Genus					
	Fusarium	*Botrytis*	*Cylindrocarpon*	*Trichoderma*	*Penicillium*	Other[3]
Top	33.8	15.0	2.5	11.3	37.5	82.5
Middle	6.3	5.0	0	5.0	21.3	87.5
Bottom	42.5	8.8	2.5	21.3	57.5	51.3

[1] Values in table are percentages of sampled cells colonized (two styrofoam pieces sampled per cell) with particular fungi.
[2] Samples of stryrofoam extracted from the top, middle (about 7.5 cm from the top) and bottom of each sampled cell.
[3] Unidentified, probably saprophytic fungi.

Table 3. Effects of an aqueous solution of Plantskydd® on colonization of residual seedlings roots within a styroblock container by selected fungi - USDA Forest Service Nursery, Coeur d'Alene, Idaho[1].

Sample Position[2]	Fungal Genus					
	Fusarium	*Botrytis*	*Cylindrocarpon*	*Trichoderma*	*Penicillium*	Other[3]
Top	14.3	0	0	14.3	21.4	50.0
Middle	15.4	7.7	0	0	30.8	46.2
Bottom	24.0	12.0	4.0	4.0	52.0	4.0

[1] Values in table percentage of sampled root pieces colonized by the appropriate fungi; sample sizes: top [14], middle [13], bottom [25].
[2] Samples of root extracted from the top, middle (about 7.5 cm from the top) and bottom of each sampled cell.
[3] Unidentified, probably saprophytic fungi.

Table 4. *Fusarium* species isolated from a styroblock container treated with Plantskydd® - USDA Forest Service Nursery, Coeur d'Alene, ID.

Fusarium Species	No. Isolates Examined	Percent of Isolates
F. proliferatum	37	56.1
F. avenaceum	15	22.7
F. sporotrichioides	7	10.6
F. acuminatum	4	6.1
F. oxysporum	1	1.5
F. culmorum	1	1.5
F. sambucinum	1	1.5
Totals	66	100.0

Table 5. Fungi isolated from molded western red cedar seedlings treated with Plantskydd® prior to storage - USDA Forest Service Nursery, Coeur d'Alene, Idaho[1].

Isolated Fungus	Percent of Isolates	
	Necrotic Foliage[2]	Stem Lesions[3]
Alternaria	0	8.3
Aspergillus	41.2	0
Aureobasidium	0	8.3
Cylindrocarpon didymium	5.9	0
Fusarium oxysporum	5.9	0
Mucor	17.6	0
Penicillium	5.9	0
Phoma eupyrena	11.7	41.7
Phoma pomorum	5.9	16.7
Pythium ultimum	5.9	25.0

[1] Fifteen seedlings sampled
[2] Seventeen total isolates obtained.
[3] Twelve total isolates obtained.

DISCUSSION

Plantskydd® is a very effective animal repellent that can be used to protect outplanted conifer seedlings during their first growing season on forest sites. Its non-toxic, persistent nature makes handling treated seedlings less potentially dangerous than other chemicals, such as fungicides. Treatment costs are low and the resulting protection of seedlings make use of Plantskydd® very desirable.

Plantskydd® is composed of organic materials that provide an excellent food

source for fungi. When foliage is covered with the repellent, a wide variety of mold fungi are able to utilize the added nutrients for rapid growth. This occurs readily while seedlings are in cold storage, even when storage temperatures are near 0°C. There was much more severe molding of treated than non-treated seedlings following cold storage. Western red cedar was especially prone to damage. Many of the associated mold fungi were general saprophytes that are able to infect seedling tissues when sufficient nutrients are present. When seedlings are stored under conditions of high humidity, inoculum build up and growth of mold fungi are enhanced. Two groups of potential pathogens (*Phoma* spp. and *Pythium ultimum*) were frequently isolated from molded seedlings. These fungi were isolated from necrotic stem tissues, indicating they successfully penetrated woody host tissues. Both groups of fungi are frequently associated with conifer seedling diseases (Dorenbosch 1970; James 1982, 1983, 1984b, 1984c; James and Hamm 1985). Seedlings with stem lesions infected with *Phoma* or *Pythium* would likely not survive after outplanting, due to the pathogenic potential of these two fungi. Therefore, heavy culling of molded seedlings is necessary prior to outplanting.

Containers used to grow conifer seedlings within greenhouses are important sources of potential pathogens when they are reused for several seedling crops (James et al. 1988). Reused containers are adequately sterilized by hot water immersion (James and Woolen 1989; Peterson 1990; Sturrock and Dennis 1988), which has become routine at many nurseries. Unfortunately, Plantskydd® did not have apparent fungicidal action on the treated styroblock container sampled in this evaluation. Levels of most fungal groups were similar in treated and non-treated portions of cells and on pieces of residual roots left within the container following seedling extraction. Isolated fungi of major concern from a disease potential standpoint were *Fusarium* spp. (particularly *F. proliferatum*) and *Botrytis cinerea*. *Fusarium proliferatum* can be a very aggressive pathogen on several conifer species grown in containers (James et al. 1997). *Botrytis* is also an important pathogen on container-grown conifer seedlings, but is confined mostly to foliage (James 1984a). These and other fungi were probably able to use Plantskydd® applied to containers as a food base.

Application of Planskydd® to seedlings prior to storage caused significant molding problems, resulting in extensive culling. It is preferable to treat seedlings after storage to prevent this molding problem. Seedlings could be treated shortly before or after outplanting. Although this might be more expensive, it would alleviate molding problems and concerns about handling Plantskydd®-treated seedlings. Freshly-applied Plant-skydd® has a highly offensive odor and the consistency of blood. However, about 24 hrs. after treatment, the odor is mostly gone and the material has dried on foliage exposed to ambient air. Therefore, treatments should be made when the repellent can rapidly dry. This would be a few days before or shortly after planting. Because of the great efficacy of Plantskydd® as an animal repellent, cost-effective application procedures must be developed so the

material can be more widely used on outplanted conifer seedlings.

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