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Efficacy of Stickers for Seed Treatment with Organic Matter or Microbial Agents for the Control of Damping-off of Sugar Beet

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ABSTRACT

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Sticker solutions, used to coat sugar beet seeds with biocontrol agents (organic matter or bacterial), were assessed for their effect on the control of damping-off caused by *Pythium* sp. "group G". Of the ten stickers tested, polyvinyl alcohol 10%, methyl cellulose 1%, alginate 1%, and carrageenan 0.5% were most effective in binding organic matter to sugar beet seeds. The efficacy of flax straw powder to protect sugar beet seeds against *Pythium* damping-off was dependent on the amount coated on the seeds. More than 6.5 mg of flax straw powder per seed was required to significantly reduce incidence of *Pythium* damping-off compared to the untreated control. *Erwinia rhapontici* strain 980 is an effective agent against *Pythium* damping-off at concentrations above 10^6 colony forming units (CFU)/seed. None of the four stickers showed detrimental effects on survival of the bacteria coated on the seeds or beneficial effects in improving efficacy of biocontrol agents against the pathogen. Storage temperature was the important factor affecting survival and efficacy of the bacterial agent coated onto the seeds. Coated seeds stored at room temperature (20 ±2℃) showed a ten-fold decrease or more in bacterial counts over an 8-week storage period bringing the bacterial concentration below 10^6 CFU/seed, which resulted in the loss of biocontrol activity. On the other hand, bacterial survival on coated seeds stored at 5℃ remained constant and retained biocontrol activity after 8 weeks of storage.

Key words: Sugar beet, Damping-off, *Pythium* sp. "group G", Biocontrol, Seed treatment, Stickers

INTRODUCTION

Seed rots and seedling damping-off caused by soil-borne pathogen *Pythium* spp., can seriously affect stand establishment of sugar beet (*Beta vulgaris* L.) [(2)], resulting in substantial economic loss [(30)]. *Pythium* sp. "group G", a form of *Pythium ultimum* Trow, is widespread in southern Alberta and is highly virulent to sugar beet and other field crops and vegetables [(6)]. Fungicide seed treatment is the most effective and least expensive method for control of damping-off of sugar beet [(5,16)]. Because of the detrimental effect of numerous chemical pesticides, biological control has been investigated as possible alternative for the control of the disease in sugar beet. Previous studies showed the seed treatment with *Erwinia rhapontici* strain 980 was effective in controlling damping-off of sugar beet (L. Liu and H. C. Huang, unpublished) and safflower [(8)] caused by *Pythium* sp. "group G". In addition to bacterial seed treatment, preliminary studies showed that some organic substances such as flax straw powder coated on sugar beet seeds were effective in reducing damping-off in *Pythium*-infested soil while others such as chitin has no beneficial effect (S.D. Bardin and H.C. Huang, unpublished).

One of the limiting factors for commercial application of biological agents to control plant diseases is the lack of consistency in effectiveness. This has often been related to poor survival and establishment, and low antagonistic activity of the introduced agents [(17,24)]. Developing formulations that provide high densities of inoculant, and high survival rates during storage of the microbial agents constitute an important step in the development of effective biocontrol products [(11,26)].

Seed coating is a general term for the application of finely ground solids or liquids containing dissolved or suspended solids to form a layer covering the seed coat [19]. This enables materials to be applied in such a way that they affect the seed or soil at the seed-soil interface and is the most reliable way to apply the biocontrol agent in close proximity with the germinating seed [7,14]. This method is therefore well suited for the control of *Pythium* damping-off diseases. In addition, because *Pythium* spp. have a poor competitive ability for nutrients [9,22], coating an effective agent on the seeds may prevent the establishment of the pathogen and therefore plant disease. The process of seed coating usually involves the use of adhesives, also known as stickers, to bind material to the surface of the seed. Adhesives used for binding biocontrol agents to seeds includes, methyl cellulose, gelatine, dextran, gum arabic, polyvinyl and polyethylene derivatives and casein [19,21]. The aim of this study is to investigate the efficacy of ten stickers to coat organic matter (flax straw powder and chitin) and a bacterial agent (*Erwinia rhapontici* strain 980) onto sugar beet seeds. The effects of stickers on the shelf-life of the bacterial agent coated on the seeds, the control of *Pythium* damping-off of sugar beet by the coated seeds, and their effects on seed germination were also determined.

MATERIALS AND METHODS

Preparation of sticker solutions and seed coating

The 10 stickers were prepared as water-based solutions and stored at room temperature (20±2℃). The stickers, alginic acid (ALG) (sodium salt, medium viscosity; Sigma Chemical Co., USA), carrageenan (CAR) (type I, Sigma Chemical Co., USA), dextrin (DIN) (Type I, Sigma Chemical Co., USA), dextran (DAN) (industrial grade MW = 60,000-90,000, Sigma Chemical Co., USA), pelgel (PEL) (LiphaTech, Inc., Milwaukee, Wisconsin, U.S.A.), polyethylene glycol (PEG) (MW = 8,000, Aldrich Chemical Corp., USA) and polyvinyl pyrrolidone (PVP) (MW = 40,000, K value 26-35, Sigma Chemical Co., USA) were dissolved in cold water. Methyl cellulose (MC) (MW = 86,000, Aldrich Chemical Corp., USA), polyvinyl alcohol (PVA) (MW = 70,000-100,000, Sigma Chemical Co., USA) and gelatine (GEL) (type B, approx. 225 bloom, Sigma Chemical Co., USA) were dissolved in boiling water. The stickers DIN, DAN, PEL, PEG, PVP, and PVA were tested at concentrations of 1%, and 10% solution (w/v). The stickers MC, ALG and GEL were tested at 1% only as they did not completely dissolve at a concentration of 10%. CAR dissolved poorly at a 1% concentration and was only tested at a concentration of 0.5%.

To investigate the ability of stickers to bind organic matter to the seed, sugar beet seeds cultivar HM7006 (Norvatis Seed, Inc., Co, USA) were coated with chitin (from crab shells powder, Practical grade, Sigma Chemical Co, USA) or flax straw powder (cv. MacGregor). Flax straw was ground using a Thomas-Wiley Laboratory Mill, Model 4 grinder (Thomas Scientific, USA) equipped with a 1 mm mesh screen. Seeds were soaked for 15 min in the sticker solution (3 ml/100 seeds), scooped out and mixed with the organic matter (1.5 g/100 seeds) in a plastic bag. Seed coating was performed by vigorously shaking the inflated bag for 2 min. To assess the amount of organic matter coated onto the seeds, 100 sugar beet seeds were divided into 5 lots of 20 seeds/lot (replicate). Each lot was weighed and coated with the organic matter as described above using the various stickers. The seeds were dried over night (16 h) at room temperature. The unbound and loosely bound material was removed through a 2 mm mesh sieve (The W.S. Tyler Company of Canada LTD, St. Catherines, ON, Canada) and the coated seeds in each lot were weighed. The amount of organic matter coated per seed in each lot was determined by subtracting the weight of the coated seeds by the weight of the uncoated seeds and dividing by 20. The amount was then averaged over the five replications.

The ability of the stickers to coat bacteria on sugar beet seeds was investigated by using the biocontrol agent *E. rhapontici* strain 980. The bacterial slurry was prepared by flooding a 24 h- old PDA grown bacterial culture, spread in a 9 cm in diameter Petri plate, with 5 ml of a sticker solution and gently scraping the plate to resuspend the bacterial culture. A slurry control was also prepared by making a bacterial suspension with 5 ml of water. The amount of bacteria in the slurries was determined by plating serial dilutions on Luria-Bertani agar (LBA) [10] and was reported as Colony Forming Units (CFU) per ml. Sugar beet seeds were soaked in the bacterial slurry (3 ml/100 seeds) for 15 min, scooped out and dried overnight at room temperature on a metallic mesh which was placed on a paper towel to absorb the excess slurry. The CFU/seed was determined by vortexing 5 coated seeds in 5 ml of sterile water for 30 sec and plating serial dilutions on LBA. No difference in bacterial counts was observed when the seeds were vortexed in water or in water containing 1% fine sand, suggesting that water alone was efficient in removing bacteria from the coated seeds regardless of the sticker used. Each bacterial count was performed in duplicate and averaged. Each experiment was done three times. CFU counts on sugar beet seeds were determined after the coated seeds were dried (T-0), and after eight weeks (T-8) of storage in paper envelopes at room temperature or at 5℃.

Effect of stickers on seed germination

The effects of the stickers on germination of sugar beet seeds was investigated on water agar plates and in soil. Seeds were coated with the sticker solutions without bacteria as described above. The plate assay consisted of plating 10 sticker-coated seeds per replicate and five replicates per

treatment on water agar (15 g/L; Difco Detroit, MI, USA) in Petri plates. The plates were kept at room temperature and seed germination was recorded after 7 days. Germination of sticker-coated seeds was compared to an untreated control and to a water control in which the seeds were soaked in water for 15 min and dried on a metallic mesh. The soil assay was performed by planting 18 sticker-coated seeds per replicate and three replicates per treatment in root trainer (Spencer-Lemaire Industries, Edmonton, AB, Canada) containing non-sterile soil made of 3 parts top soil (dark brown chernozemic loam), 1 part sand and 1 part peatmoss (Premier Horticulture Inc., Red Hill, PA, USA). One seed was planted per root trainer cell (cell size: 1.5 cm^2 wide, 15 cm long) at a depth of 1.5 cm. The soil was watered by putting the root trainer in water until the top of the soil was moist and the root trainers were placed in growth chambers under controlled light and temperature conditions (16 h day at 20℃ and 8 h night at 15 ℃). Further watering was performed as needed. Seedling emergence was recorded 14 days after seeding. Each experiment was done three times.

Biocontrol activity of the coated seeds

Sugar beet seeds coated with the sticker alone, the sticker and bacterium *E. rhapontici* strain 980 or the sticker and flax straw powder were tested in soil artificially infested with *Pythium* sp. "group G", strain 2105 [6]. The *Pythium* inoculum, was prepared by inoculating a wheat bran/corn meal substrate with 20 plugs (0.8 cm in diameter) of a 48 h-old PDA culture of the fungus. The substrate was made of 150 g of wheat bran (Ellison Milling, Lethbridge, AB, Canada) and 150 g of corn meal (McCormick Inc., London, ON, Canada) mixed with 300 ml of distilled water and autoclaved twice for 30 min at 121℃. After two weeks of incubation at room temperature in the dark, the bran-corn meal mix colonised by *Pythium* sp. "group G" was dried at room temperature for four days and ground using a Thomas-Wiley Laboratory Mill, Model 4 grinder equipped with a 1 mm mesh size. Soil experiments in root trainers were performed as described above except the soil used to fill root trainer books was artificially infested with *Pythium* sp. "group G" at a concentration of 0.2% (g of *Pythium* grown on bran-corn meal per g of soil). The watered root trainers were kept in propagator trays (The Stewart Company, Croydon, Surrey, UK) to maintain high moisture and therefore required no further watering during the 14 days of the experiment. Each experiment was performed with 18 coated seeds per replicate and three replicates per treatment. Seedling emergence was recorded 14 days after sowing, and emergence of coated seeds was compared to the untreated control. Each experiment was done three times.

Statistical analysis

The amount of organic matter coated onto the seeds was expressed as mean ± standard error (SE). The viable counts of bacterial agents were expressed as mean CFU per ml or CFU per seed ± standard error (SE). Treatments for all seedling emergence experiments in growth cabinet were arranged in completely randomised block designs. Emergence data were analysed statistically using the SAS statistical package (SAS institute Inc., Cary, NC, U.S.A.). Treatment means were separated using the least significant difference (LSD) test at P=0.05 level.

RESULTS

Adhesiveness of the stickers

The 10 stickers used in this study varied in their ability to coat organic matter onto sugar beet seeds. The ability of a given sticker to coated organic matter onto sugar beet seeds was not dependent on the coating material as the sticker coated chitin and flax straw powder to a similar extent. Preliminary experiments indicated that sugar beet seeds should be coated with ≧ 5 mg of organic matter per seed to evenly coat the seeds and formed a hard shell (Fig. 1) that did not deteriorate after storage for several months at room temperature (20±2℃). Of the concentration tested using the ten stickers, only methyl cellulose (MC) 1%, alginate (ALG) 1%, carrageenan (CAR) 0.5%, and polyvinyl alcohol (PVA) 10% bound enough chitin or flax straw powder on sugar beet seeds (Table 1). The amount of organic matter coated on sugar beet seed ranged from 9.0 mg/seed of chitin and flax straw powder by PVA 10% to 5.0 mg/seed of chitin by ALG 1% and 6.2 mg/seed of flax straw powder by CAR 0.5% (Table 1). The stickers polyvinyl pyrrolidone (PVP), dextrin (DIN), polyethylene glycol (PEG), and pelgel (PEL) at a concentration of 1% did not stick any organic matter onto sugar beet seeds and the "coated seeds" looked like the water treated seeds (Fig. 1). When increasing the concentration of these stickers to 10%, they were able to coat some organic matter onto the seeds but its distribution was patchy and the amount coated was less than 1mg/seed. The distribution of organic matter on sugar beet seeds was more uniform when the sticker gelatine (GEL) 1% and dextran (DAN) 10% was used and the amount of organic matter coated onto the seeds reached 2.9±0.6 mg/seed for GEL 1% and 2.6±0.4 mg/seed for DAN 10%. However, the coating was friable and deteriorated to dust during storage.

Effect of stickers on seed germination and control of damping-off

Germination of sugar beet seeds treated with ALG 1%, CAR 0.5%, MC 1%, and PVA 10% was similar to the untreated control when plated on water agar and the germination rate reached 93 to 100% after 4 days incubation at room temperature. Coating the seeds with the sticker solutions slightly increased the emergence of sugar beet seedlings in soil experiments from 58% for the untreated

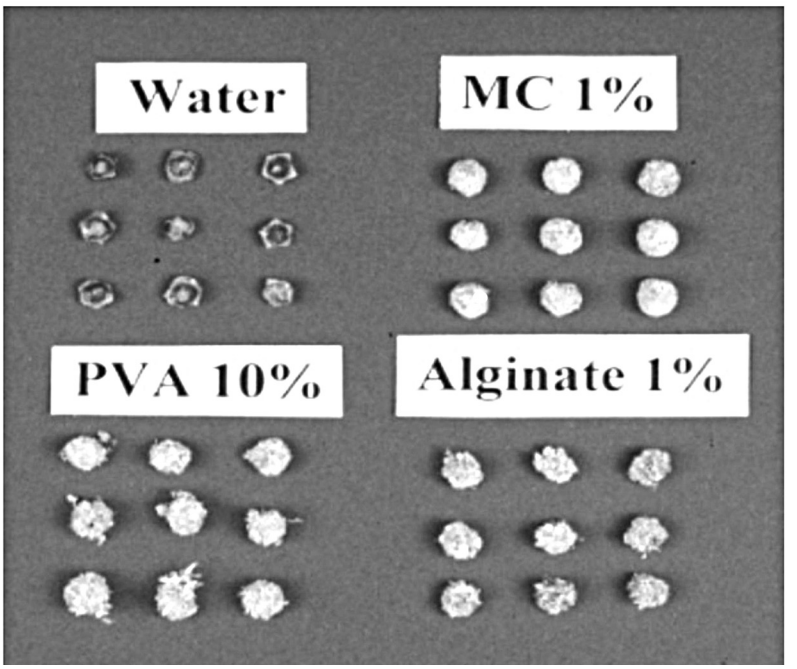

Fig. 1. Sugar beet seeds coated with chitin powder using water (control), MC (methyl cellulose) 1%, PVA (polyvinyl alcohol) 10% and alginate 1% as sticker. Note the difference in seed shapes and sizes, the evenly coated seeds when chitin was bound to the seeds with MC 1%, PVA 10% and alginate 1% and the absence of coating of chitin when water was used.

Table 1. Efficacy of stickers to coat chitin and flax straw powder onto sugar beet seeds

Sticker	Chitin (mg/seed) [1]	Flax (mg/seed) [1]
PVA 10%	9.0 ±1.0	9.0 ±0.5
MC 1%	6.5 ±0.5	7.9 ±0.4
ALG 1%	5.0 ±0.3	6.5 ±0.4
CAR 0.5%	5.2 ±0.3	6.2 ±0.4
None (water)	0.0	0.0

[1.] Amount of chitin and flax straw powder coated per seed averaged over five replications ± standard error.

control to 75% for the seed treated with CAR 0.5%, however these increases were not statistically significant ($P > 0.05$) (Table 2).

The amount of flax straw powder on sugar beet seeds needed to improve seedling emergence in *Pythium*-infested soil was investigated using four stickers, ALG 1%, CAR 0.5%, MC 1%, and PVA 10%. Only seeds coated with flax straw and MC 1% or PVA 10% increased emergence ($P < 0.05$) to 55% for MC 1% and 51% for PVA 10% compared to 27% for the untreated control in *Pythium*-infested soil in growth chamber experiments (Table 2). These two sticker solutions coated the largest amount of flax straw onto the seeds (Table 1). Seeds treated with flax straw and ALG 1% or CAR 0.5% increased emergence of sugar beet seedling to 38% and 39%, respectively, however the increase was not significant ($P > 0.05$) compared to the untreated control (Table 2). The amount of flax straw coated onto the seeds, rather than the sticker used was responsible for the increase in seedling emergence in *Pythium*-infested soil because none of the sugar beet seeds coated with the stickers alone significantly ($P < 0.05$) improved emergence of sugar beet compared to the untreated control (Table 2). Also, none of the stickers increased incidence of damping-off as emergence of the sticker coated seeds did not show significant ($P > 0.05$) decrease in sugar beet emergence compared to the untreated control (Table 2).

Efficacy of stickers to coat bacteria on the seeds.

The four stickers, ALG 1%, CAR 0.5%, MC 1%, and PVA 10% were tested for their efficacy in coating the bacterial agent *E. rhapontici* strain 980 onto sugar beet seeds. The concentration of *E. rhapontici* in the sticker slurries, averaged over the three experiments, ranged from (2.8 ±0.3) x 10^9 CFU/ml for PVA 10% to (4.1 ±0.8) x 10^9 CFU/ml for ALG 1%, (5.0 ±0.9) x 10^9 CFU/ml for CAR 0.5% and (5.7 ± 1.9) x 10^9 CFU/ml for MC 1%. Bacterial counts in the stickers slurries were not significantly ($P > 0.05$) different from the one in the water slurry (3.0 ±0.9) x 10^9 CFU/ml. Bacterial counts on the seed at T-0 ranged from (1.8 ±0.8) x 10^6 CFU/seed with the PVA 10% slurry to (6.5 ±1.1) x 10^6 CFU/seed with the CAR 0.5% slurry (Fig. 2). These were not significantly different ($P > 0.05$) from the bacterial count obtained on the seeds when the water slurry was used (4.2 ± 0.8) x 10^6 CFU/seed (Fig. 2).

Table 2. Effect of stickers on emergence of sugar beet seedlings in non-infested and in *Pythium*- infested soil in presence or absence of flax straw powder coated onto the seeds

Treatment	% Emergence [1]		
		Pythium-infested soil	
	Non-infested soil	Without Flax	With Flax
Untreated	58 a [2]	28 a	27 c
PVA 10%	67 a	26 a	51 ab
MC 1%	65 a	26 a	55 a
ALG 1%	68 a	21 a	38 bc
CAR 0.5%	75 a	33 a	39 bc

[1.] Emergence of sugar beet seeds 14 days after planting in soil infested with *Pythium* sp. "group G" strain 2105.

[2.] Means within each column followed by the same letter are not significantly different at 0.05 level (LSD test).

"group G" all treatments significantly improved sugar beet emergence compared to the untreated control at T-0 (Table 3). None of the stickers affected the biocontrol activity of *E. rhapontici* compared to the water control (Table 3). Bacterial coated seeds stored at room temperature for 8 weeks, that showed a decrease in bacterial counts (Fig. 2), did not retain biocontrol activity against *Pythium* sp. "group G" as seedling emergence of *E. rhapontici*-coated seeds was not significantly different ($P > 0.05$) from emergence of the untreated control (Table 3). Only the seeds stored at 5℃, showing no decrease in bacterial counts (Fig. 2), retained biocontrol activity after storage for 8 weeks with all treatments, except PVA 10%, increasing emergence of sugar beet compared to the untreated control (Table 3). None of the stickers were beneficial or

Shelf-life and biocontrol activity of bacterial agents on coated seeds

Temperature was the important factor influencing bacterial survival on the seeds. When *E. rhapontici* strain 980 was coated on sugar beet seeds with stickers CAR 0.5%, MC 1%, ALG 1%, or PVA 10% and stored at room temperature, bacterial counts on the seeds decreased by 10 fold or more after 8 weeks of storage (T-8) (Fig. 2). On the other hand, bacterial counts on seeds stored at 5℃ showed no significant changes over the 8 week storage period compared to the bacterial counts at T-0, except for PVA 10% that showed a 4-fold decrease (Fig. 2). None of the stickers showed beneficial or detrimental effects to the bacterial agent as bacterial counts from seeds coated with the stickers did not differ from bacterial counts from seeds coated with water (Fig. 2).

When the seeds, coated with *E. rhapontici* strain 980 and the stickers CAR 0.5%, MC 1%, ALG 1%, or PVA 10%, were tested in soil artificially infested with *Pythium* sp.

Table 3. Effect of stickers and seed storage temperature on emergence of sugar beet seeds coated with *Erwinia rhapontici* strain 980 in *Pythium*-infested soil

Treatment	% Emergence [1]		
	T-0	T-8; 5℃	T-8
Untreated (control I)	30 b [2]	47 b	46 a
E. rhapontici in PVA 10%	51 a	64 ab	58 a
E. rhapontici in MC 1%	51 a	74 a	38 a
E. rhapontici in ALG 1%	53 a	76 a	53 a
E. rhapontici in CAR 0.5%	50 a	76 a	55 a
E. rhapontici in WATER (control II)	50 a	79 a	50 a

[1.] Emergence data were collected 14 days after sowing sugar beet seeds coated with various stickers and the biocontrol agent *E. rhapontici* strain 980 in soil artificially infested with *Pythium* sp. "group G" strain 2105 at day 0 (T-0) and after 8 weeks storage at 5℃ (T-8; 5℃) or at room temperature (T-8; RT).

[2.] Data showing the % emergence of sugar beet plants, averaged over three replications. Means within each column followed by the same letter are not significantly different at 0.05 level (LSD test).

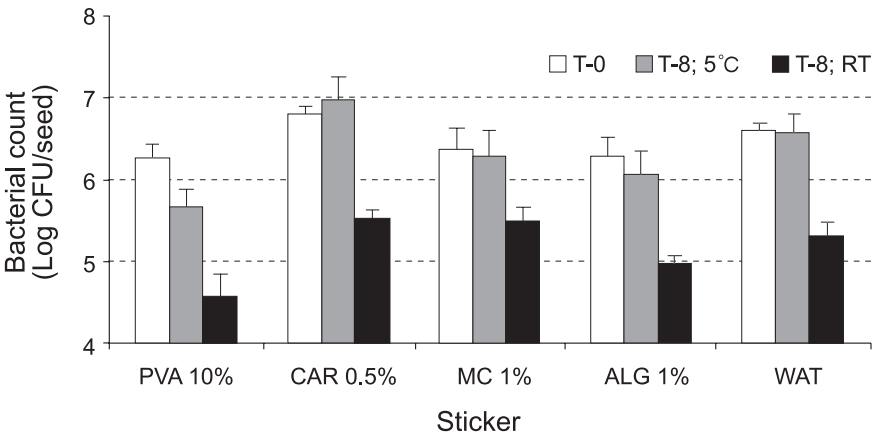

Fig. 2. Effect of four stickers and storage temperature on survival of *Erwinia rhapontici* strain 980 coated on sugar beet seeds. Bacterial counts were expressed as Log number of CFU per seed, after the seeds were coated and dried (T-0) and after storage for 8 weeks at 5℃ (T-8; 5℃) and at room temperature (20±2℃; T-8; RT). Each bar represent the average of three experiments + standard error (error bar). Abbreviation: PVA (polyvinyl alcohol); MC (methyl cellulose); ALG (alginate); CAR (carrageenan); WAT (water).

detrimental towards the survival of *E. rhapontici* on sugar beet seeds as the biocontrol ability of the strain against *Pythium* sp. "group G" was similar whether stickers or water were used to coat the bacteria to the seeds (Table 3).

DISCUSSION

A good sticker is essential to bind organic matter to seeds and thereby enhance control of *Pythium* damping-off by the organic material. Among the 10 stickers investigated, CAR 0.5%, ALG 1%, MC 1% and PVA 10% were the best in increasing affinity between the seed coat and the coating material and possessed qualities such as strength and plasticity that prevented deterioration of the coated materials on the seeds. These stickers also have appropriate viscosity for ease of application. The study reveals that seed coating with flax straw powder is effective in the control of sugar beet damping-off but the effectiveness of control is determined by the amount of flax straw coated on the seed. Of these four stickers, only MC 1% and PVA 10% coated sufficient flax straw powder to protect sugar beet from *Pythium* damping-off. This suggests that more than 6.5 mg of flax straw powder must be coated on each sugar beet seed to be efficient and improving emergence of this crop in *Pythium* contaminated soils. Because an increase in the concentration of sticker solution increased the amount of organic matter on the seed, increasing the concentration of CAR and ALG sticker solutions is likely to increase the amount of flax coated on the seeds and therefore improve protection of the coated seeds against *Pythium* damping-off.

The binding abilities of stickers, characterized by the amount of organic matter they bound onto sugar beet seeds, did not translate into increased numbers of bacteria *E. rhapontici* coated on the seeds. On the contrary, seeds treated with bacterial slurries made with the sticker PVA 10% averaged the lowest bacterial counts on seeds even though it was one of the best stickers to bind organic matter to the seed. None of the stickers tested significantly increased bacterial counts on the seeds compared to water, possibly because sugar beet seeds can be saturated with bacteria applied as a film-coat without the help of stickers due to the rough surface of the seeds. Seed treatment with water slurries have also been used to coat biocontrol bacteria onto seeds of tomato and oilseed rape and this coating procedure was effective in controlling wilt diseases [13].

None of the four stickers, PVA 10%, CAR 0.5%, MC 1%, and ALG 1%, showed any toxicity towards sugar beet seeds as seed germination was slightly increased by these stickers. ALG slurry was however previously shown to significantly reduce germination of lettuce seeds, while not affecting germination of cucumber seeds [1]. This suggests that the toxicity of stickers vary with plant seed. Previous reports showed that cellulose [21] and methyl cellulose [29] stimulated growth of *Pythium ultimum*, causing an increase in the severity of *Pythium* damping-off of tulips [29]. However such negative effect of MC was not observed in our experiments. None of the four stickers tested stimulated the pathogenic activity of the fungus, nor did they provide protection of sugar beet seeds from infection by *Pythium* sp. "group G". MC has frequently been reported as a sticker in formulations of biocontrol agents [12,27], probably due to its low cost and the low concentration of sticker solution needed. Our study indicates that MC also exhibits superior ability to bind organic matter to sugar beet seeds and likely other seeds. Alginate, another frequently reported sticker, is mostly used as beads that provided protection to microbial agent when added to soils [18,23,25].

Biocontrol activity of *E. rhapontici* strain 980 was observed when the number of bacteria on the seed ranged between 1.0×10^6 - 1.0×10^7 CFU/seed. At concentrations below 1.0×10^6 CFU/seed, insufficient numbers of viable cells were present on the seeds to show a significant biocontrol effect against *Pythium* sp. "group G". Other studies have correlated the CFU of biocontrol agents on the seed to their biocontrol activity [3,4,15,20]. Callan *et al.* [3] demonstrated that at least 1×10^7 CFU/seed of *P. fluorescens* AB254 was needed to achieve maximum protection of sweet corn damping-off by *Pythium ultimum*. Although none of the stickers affected the shelf-life of the biocontrol agent *E. rhapontici* coated on sugar beet seeds over a 8- week storage period, storing the coated seeds at low temperature (5℃) was crucial for maintaining viability and therefore biocontrol effectiveness of the bacterial agent against the pathogen. Extended bacterial survival under low temperature was shown to be due to a reduction in metabolic activity in the bacteria [28]. Cliquet and Scheffer [4] reported that low storage temperature (9℃) was more critical to survival of *Trichoderma* spp. spores coated on radish and cucumber seeds than the substrate used for spore production. Shah-Smith and Burns [20] also showed that survival of Pseudomonas putida 40RNF in film-coats was temperature dependent. The biocontrol efficacy of bacteria coated seeds stored at 4℃ was correlated to the decrease in CFU over a 52-week period, but the efficacy of film-coated seeds stored at room temperature was independent of the number of CFU of *P. putida*. Biocontrol activity of the coated-seeds stored at room temperature for 16 and 52 weeks was not reduced even though the density of *P. putida* was below 100 cells/pellet [20]. The biocontrol mechanism behind this phenomenon has not been characterized.

In summary, our study showed that organic amendment or bacterial seed treatments were efficient in the control of sugar beet damping-off if a threshold level of the antagonist was present. For organic amendment, this threshold level can be obtained by coating the seeds with a suitable sticker such as MC 1% or PVA 10%. For bacterial agent, the threshold level was obtained by coating the seeds with high inoculum and was maintained by storing the bacterium-coated seeds at low temperature. In this case the presence of a sticker was not required.

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摘 要

Sylvie D. Bardin [1]、黃鴻章 [1,2]. 2003. 不同粘著劑對甜菜種子處理有機物或生防細菌的效果及其對猝倒病的防治. 植病會刊 12:19-26. ([1.] 加拿大農部 Lethbridge 研究中心；[2.] 聯絡作者：電子郵件 huangh@agr.gc.ca，傳眞：+0021-403-382-3156)

本研究旨在比較不同粘著劑做種子處理有機物或生物防治用拮抗菌的效果及其防治猝倒病的可行性。在十個供試黏著劑中，以Polyvinal alcohol 10%, methyl cellulose 1% , alginate 1% 及carrageenan 0.5% 等對甜菜種子之黏著有機物或拮抗細菌的效果良好。每粒甜菜種子表面如黏著6.5 mg 以上的亞麻植株粉末，可以顯著減低猝倒病 (*Pythium* damping-off) 的發病率。每粒甜菜種子表面好粘著 10^6 菌落數 (cfu) 的拮抗細菌 *Erwinia rhapontici*，也有顯著減低猝倒病的效果。這種黏著劑本身不會影響拮抗細菌的存活，也不具有增強拮抗菌防病效果的能力。又黏著在種子表面細菌之存活是受貯藏溫度的影響。經細菌處理的種子存放於室溫 (22±2℃) 8 週後，細菌存活率降低十倍以上，並且消失防病效果。但是經細菌處理的種子存放於低溫(5℃) 8 週，則能保存良好的防病效果。

關鍵詞：甜菜、猝倒病、*Pythium* sp. "group G"、生物防治、種子處理、黏著劑