Epidemiology / Épidémiologie

Effects of potato dextrose broth and gelatin on germination and efficacy of *Phoma exigua*, a potential biocontrol agent for salal (*Gaultheria shallon*)

Shiguang Zhao and Simon F. Shamoun

Abstract: The role of a combination of four gelatin concentration levels (0.0%, 0.2%, 0.5%, and 0.8%) and eight potato dextrose broth (PDB) concentration levels (0.0%, 0.1%, 0.5%, 1.0%, 1.5%, 2.0%, 2.5%, and 3.0%) on water evaporation, water absorption, conidial germination, and biocontrol efficacy of conidia of *Phoma exigua* isolate PFC2705 for salal was evaluated. PDB and gelatin reduced water evaporation. After 3 h of evaporation at 23 °C and 22% RH, the water content of gelatin–PDB combinations increased compared with PDB alone. The relationships between the amounts of absorbed water and PDB concentration at all gelatin concentration levels were linear ($p <$ 0.001) after 60 min in a 100% RH environment. After 240 min, the mass of absorbed water was 11.8 times the dry mass of the 0.5% gelatin and 1.5% PDB combination, and this relationship did not change significantly over the following 16 h. PDB and gelatin increased conidial germination and might have provided nutrition for conidia of *P. exigua* PFC2705 at different RH environments. Conidia of PFC2705 did not germinate in sterilized ion-free water, germinated somewhat (4% to 10%) in gelatin suspensions, and germinated well (average 89%) in suspensions of different concentrations of PDB or gelatin–PDB combinations after 24 h at 23 °C. The average conidial germination at 24 h was 76% in 100% RH, 12% in 97.5% RH, and 0.0% at 92.5% RH; at 48 h it was 44.4% in 97.5% RH and 20% in 92.5% RH. No conidia germinated when RH was lower than 92.5% over 48 h. Conidia in the gelatin–PDB combinations smeared on slides stored for 4 weeks at 23 °C with 22% RH had an average conidial germination rate of 20% after 16 h incubation at 100% RH. Gelatin–PDB combinations with 0.5% gelatin and 1.5% to 2.5% PDB caused significantly larger lesions on detached young salal leaves (20 to 25 days old) than other gelatin–PDB combinations.

Key words: gelatin, potato dextrose broth, water evaporation, absorption, *Phoma exigua*, germination, salal, pathogenicity, biocontrol.

Résumé : Les effets de la combinaison de quatre concentrations de gélatine (0,0%, 0,2%, 0,5% et 0,8%) et de huit concentrations de bouillon dextrosé à la pomme de terre (BDPT) (0,0%, 0,1%, 0,5%, 1,0%, 1,5%, 2,0%, 2,5% et 3,0%) sur l'évaporation de l'eau, l'absorption de l'eau, la germination des conidies et l'efficacité des conidies de l'isolat PFC2705 du *Phoma exigua* comme agent de lutte biologique contre la gaulthérie shallon furent examinés. Le BDPT et la gélatine réduisirent l'évaporation de l'eau. Après 3 h d'évaporation à 23 °C et à une HR de 22%, le contenu en eau des combinaisons gélatine–BDPT augmenta par rapport au BDPT seul. Pour toutes les concentrations de gélatine, la relation entre les quantités d'eau absorbée et la concentration du BDPT fut linéaire ($p <$ 0,001) après 60 min dans un environnement à une HR de 100%. Après 240 min, la masse de l'eau absorbée était de 11,8 fois la masse sèche de la combinaison de gélatine à 0,5% et de BDPT à 1,5%, et cette relation ne changea pas significativement au cours des 16 h qui suivirent. Le BDPT et la gélatine augmentèrent la germination des conidies et pourraient avoir contribué à nourrir les conidies du *P. exigua* PFC2705 dans divers environnements d'HR. Après 24 h à 23 °C, les conidies du PFC2705 ne germèrent pas dans l'eau stérile déionisée, mais germèrent (4% à 10%) dans des suspensions de gélatine et germèrent bien (89% en moyenne) dans des suspensions de diverses concentrations de BDPT ou de combinaisons gélatine–BDPT. En moyenne, la germination des conidies à 24 h fut de 76% dans une HR de 100%, de 12% dans une HR de 97,5%, et de 0,0% dans une HR de 92,5%; à 48 h, elle fut de 44,4% dans une HR de 97,5%, et de 20% dans une HR de 92,5%. Après 48 h, aucune conidie ne germa à une HR inférieure à 92,5%. Des conidies, dans les

Accepted 18 February 2005.

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combinaisons gélatine–BDPT, étalées sur des lames et conservées durant 4 semaines à 23 °C et à une HR de 22% eurent un taux de germination moyen de 20% après 16 h d'incubation à une HR de 100%. Les combinaisons gélatine–BDPT avec 0,5% de gélatine et de 1,5% à 2,5% de BDPT causèrent des lésions significativement plus grandes sur de jeunes feuilles détachées de gaulthérie shallon (âgées de 20 à 25 jours) que les autres combinaisons gélatine–BDPT.

Mots clés : gélatine, bouillon dextrosé à la pomme de terre, évaporation de l'eau, absorption, *Phoma exigua*, germination, gaulthérie shallon, pouvoir pathogène, lutte biologique.

Introduction

Salal (*Gaultheria shallon* Pursh) is one of the most abundant perennial shrubs on the west coast of North America (Hitchcock et al. 1959; Fraser et al. 1993). It invades forest sites following clear-cutting and competes vigorously with the growth of conifer plantations (Weetman et al. 1989). Management of salal with herbicides (including Garlon 4E®, Vision®, Ally®, etc.) is often ineffective and may cause environmental problems (D'Anjou 1990). Screening of fungal pathogens isolated from diseased salal on Vancouver Island demonstrated that a *Phoma* sp. (isolate PFC 2705) was pathogenic on salal seedlings and had biocontrol potential (Shamoun et al. 2000). This fungus was later identified as *Phoma exigua* Desmazières by the Centraalbureau voor Schimmelcultures, Institute of Royal Netherlands Academy of Arts and Science (KNAW), Netherlands (Ref. No: det 144-2004) (S.F. Shamoun and S. Zhao, unpublished data).

It is well known that optimized formulations are essential to the development of a successful bioherbicide (Boyette et al. 1991) and that the addition of various adjuvants can enhance the performance and prolong the shelf life of biological control agents (BCAs) (Boyette et al. 1996; Prasad 1993; Sabaratnam and Traquair 2002). Adjuvants are classified as wetter-spreaders, stickers, humectants, penetration agents, and herbicide modifiers (Hazen 2000). In bioherbicide research, adjuvants may be added to enhance the activity of BCAs by prolonging water retention (humectants, anti-evaporation agents), providing nutrition, improving spore deposition (surfactants, stickers), and extending shelf life (inert carriers, antifreezing compounds, sunscreen agents) (Boyette et al. 1991; Green et al. 1998; Prasad 1993).

The formation of dew or free moisture on plant surfaces plays an important role during the infection process of fungal pathogens (Everts and Lacy 1990; Luo and Tebeest 1998; Pitelli and Amorim 2003; Huber and Gillespie 1992). Fulfilling dew requirements for fungi has been a challenging aspect of bioherbicide formulation (Auld and Morin 1995; Shabana, et al. 1997). To achieve the water potential needed by a BCA, adjuvants with water retention properties, such as humectants, may be added to the formulations. Humectants equilibrate the water content and increase drying time, therefore slowing evaporation. Among those investigated are liposome, guar gum, polymeric gels, gelatin, and mineral and vegetable oils (Neumann and Boland 1999; Shabana et al. 1997; Zhang et al. 2003; Connick et al. 1991; Daigle et al. 1990). Humectants can also draw moisture from the atmosphere, thus maintaining a higher level of humidity on or near the spray deposit of a BCA (Hazen 2000). However, the effect of RH on how much moisture can be drawn from the atmosphere by the biocontrol suspension is not clear, and this aspect has, to our knowledge, not been reported.

Potato dextrose broth (PDB), a combination of potato starch and dextrose, is often used as a nutritional medium for the culture of microorganisms. It was reported to be the most effective nutrient for conidia germination of *Alternaria cassiae* (Daigle and Cotty 1991) and was used as a carrier for *Phoma herbarum* Westendrop and *P. exigua* on the biocontrol of dandelion in growth-chamber as well as under field conditions (Stewart-Wade and Boland 2004, Neumann and Boland 1999). Gelatin, a heterogenous mixture of water-soluble proteins that has been widely used in food and medicine industry (Organic Materials Review Institute 2002), is also an adjuvant with adhesive and nutritional properties that has been used in other bioherbicide formulations (Morin et al. 1990; Zhang et al. 2003). The interaction of adjuvant combinations, such as PDB and gelatin, is not well known.

The objectives of this study were to (1) determine the humectant properties (evaporation resistance and moisture drawing) of PDB and gelatin under laboratory conditions; (2) evaluate the effect of PDB, gelatin, and their combinations at different concentrations on conidial germination of *P. exigua* (PFC2705) at different water potential levels; and (3) evaluate the effect of PDB and gelatin on the pathogenicity of conidia of *P. exigua* isolate PFC2705 on detached salal leaves of different ages.

Materials and methods

Production of conidial suspensions

Stock cultures of *P. exigua* PFC2705 were stored on 3.9% potato dextrose agar (PDA) (Difco Laboratories, Detroit, USA) slant tubes at 4 °C. Small pieces of mycelium from stock cultures were aseptically transferred to Petri dishes (90 mm) containing PDA, sealed with parafilm, and incubated at 20 °C in the dark for 8 days. Agar plugs (5 mm) from the margins of young, actively growing colonies were used to inoculate V8 agar media (200 mL V8 juice, 15 g agar, 3 g $CaCO_3$, 800 mL water) in Petri dishes. Plates were sealed and incubated at 25 °C with constant fluorescent light for 12 to 14 days. Conidia were harvested by flooding the plates with 10 mL of sterile deionized water containing 0.01% (*v/v*) Tween 80 (Fisher Scientific, Fair Lawn, New Jersey, USA). After gently scraping the surface of the colonies with a sterile glass slide, the resulting suspensions were filtered through two layers of sterile cheesecloth. Concentrations of the conidial suspensions were determined using of a haemocytometer and were adjusted using different concentrations of the gelatin–PDB combinations.

Production of PDB and gelatin combination solutions

To determine the effects of PDB and gelatin (Type a: Porcine Skin. Sigma Chemical Co., St Louis, Missouri, USA) on the conidial germination of *P. exigua* PFC2705, four gelatin concentration levels (gelatin level 0.0%, 0.2%, 0.5%, and 0.8%) and eight PDB concentration levels (PDB level 0.0%, 0.1%, 0.5%, 1.0%, 1.5%, 2.0%, 2.5%, and 3.0%) were used, resulting in 32 gelatin–PDB combinations. In the data presented in this paper, PDB and gelatin are abbreviated by "p" and "g", respectively, and concentrations are represented in tenths of a percentage without decimals (e.g., "05" stands for 0.5%). For example, "p01" represents 0.1% PDB; "g08" represents 0.8% gelatin; "gp0515" represents the combination 0.5% gelatin and 1.5% PDB. "Water" represents the combination 0.0% gelatin and 0.0% PDB.

To prepare the gelatin–PDB combinations, stock solutions of 5.0% PDB and deionized water were autoclaved at 121 °C for 25 min; 2.0% gelatin was prepared by adding gelatin in cool deionized water and heating the solution in a microwave until completely dissolved. Ten millilitres of each combination solution was prepared in a 50-mL sterilized plastic tube under sterile conditions using the stock solutions.

Effect of PDB and gelatin on evaporation

Two hundred milligrams of each gelatin–PDB combination was evenly spread on preweighed slides (14 cm^2; SuperFrost, Fisher Scientific, Pittsburgh, Pennsylvania, USA), which were placed in a dark chamber set at 23 °C with RH 22%. Slides were weighed every 15 min until 180 min after placement. Each treatment had three replicates, and the experiment was repeated once. Uncoated slides served as the control.

Effect of PDB and gelatin on water absorption

To compare water absorption among different combinations, 50 µL of each gelatin–PDB combination was evenly spread onto preweighed slides, dried in the laboratory for 24 h, and subsequently transferred into a chamber at 23 °C with 100% RH. Mass change was determined 60 min after placement. Each treatment had three replicates, and the experiment was repeated once. The slide net mass change represented the water content of each combination.

To further study the water absorption process, a combination of 0.5% gelatin and 1.5% PDB (pg0515) was used to assess the slide mass change over time. The experiment was conducted as described above, except slides were weighed every 10 min immediately after removal from the 100% RH chamber for the first 90 min, and then at 4 and 20 h. Once a slide was removed from the chamber and weighed, it was discarded. Each treatment had six replicates, and the experiment was repeated once.

Effect of PDB and gelatin on conidial germination

Conidial germination in suspension

Three millilitres of a 10^5 conidia/mL suspension of *P. exigua* PFC2705 for each gelatin–PDB combination was placed in a Petri dish (65 mm), sealed with parafilm, and incubated at 23 °C in the dark. The number of germinated conidia was determined using a microscope at 16 and 24 h with the aid of a digital camera. Each treatment had three replications, and the experiment was repeated once.

Germination at different RH

For each gelatin–PDB combination, 20 µL containing 10^6 conidia/mL of *P. exigua* PFC 2705 was smeared onto a 14-cm^2 slide with a glass slide cover (approximately 1400 conidia/cm^2). The slides were dried at 23 °C in the dark for 24 h under sterile conditions and subsequently transferred onto grills in airtight glass containers (23 cm in diameter, 5.7 L in volume) with 400 mL of water or saturated salt solutions at the bottom. The containers remained in the laboratory at 23 °C (±0.2 °C) with 22% (±1%) RH (temperature and RH were monitored using a HOBO Data Logger in the description followed). Germination was determined at 16, 24, or 48 h on three slides, and the experiment was repeated once.

The RH in the glass containers was adjusted using salts in saturated solutions, and their theoretical RH values associated with them at 25 °C were K_2SO_4 (97.5%), KNO_3 (92.5%), KCl (85.5%), and NaCl (75.5%) (Winston and Bates 1960). Temperature and RH in the containers were measured at 15-min intervals with HOBO Pro RH/Temp Data Loggers (Onset Computer Corp., Bourne, Massachusetts, USA), which were fastened on the lid inside each container at the start of all experiments and provided a precision on RH determinations of ±3% (±4% in condensing environment) and of ±0.2 °C for temperature. The measured RH values in containers were often 2%–6% higher than the theoretical values at 25 °C. Temperatures in the containers were around 23 °C, and the theoretical values of RH changed slightly between 20 and 25 °C, so the theoretical value of RH at 25 °C was used as the standard.

Effect of different dry periods on germination

This experiment was designed to investigate the effect of alternating dry and wet periods on conidial germination. For each of the gelatin–PDB combinations, 20 µL of a 10^6 conidia/mL suspension was smeared onto a slide and left to dry in a sterile flow hood for 24 h. Subsequently, slides were transferred into a sterile box (23 °C, dark, 22% RH) for a dry period of 1, 2, 3, and 4 weeks. After dry incubation, slides were transferred to a Petri dish (90 mm) containing moist filter paper (100% RH), sealed with parafilm, and incubated for 8 h at 23 °C in the dark, followed by exposing the slides in the laboratory (22% RH) under sterile conditions for 16 h. This process was repeated three times followed by a continuous incubation at 100% RH for 24 h and then assessed for germination. The conidia were stained with 25 µL lacto-phenol cotton blue, and a minimum of 100 spores per slide were arbitrarily examined with the aid of a microscope (100×). A spore was considered germinated if the length of the germ tube exceeded the minor diameter of a spore. Each treatment included three slides and was repeated once.

Effect of PDB and gelatin on lesion size

Salal seedlings (approximately 8 months old) grown at the Pacific Forestry Centre, Victoria, British Columbia, Canada, were moved from a shaded greenhouse (9–15 °C with natural daylight) to a growth chamber (Conviron, model

E-15, Controlled Environments, Winnipeg, Manitoba) with a RH of 55%–79% and a temperature regime of 12 °C : 19 °C (dark:light) with a 12 h/day photoperiod (180–200 $\mu E \cdot m^{-2} \cdot s^{-1}$). After 1 week, new shoots began to emerge, and 4 or 7 weeks later, 20- to 25-day-old or 40- to 45-day-old leaves were collected, respectively. The detached leaves were surface sterilized and inoculated by placing 20 µL of a 10^6 conidia/mL suspension in different gelatin–PDB combinations on the abaxial surface of each salal leaf. The inoculated leaves remained in a flow hood for 30 min to allow conidial suspension drops to dry. Subsequently, leaves were incubated in sealed Petri dishes with moistened filter paper to maintain 100% RH in a chamber at 23 °C with 12 h light. The diameter of the necrotic lesions was measured 5 days after inoculation, and as a measure of disease severity, lesion areas were calculated. Control treatments consisted of pg0515 without conidia and sterilized water without conidia. Each treatment had eight leaves, with each leaf as a replication. The experiment was conducted twice.

Data analysis

All experiments were performed using a completely randomized block design (Little and Hills 1978). The data for the two trials were pooled after an equal variance (homogeneity) test indicated that it was valid to do so. Analyses were performed using the SigmaStat (version 2.03, Systat Software Inc. Richmond, California, USA) statistical software package. The data collected were analyzed via ANOVA (one-way or two-way) to determine the effect of the concentrations of PDB, gelatin, and their combinations on evaporation, water absorption, conidial germination, and lesion size. Differences among mean values were determined using a Student–Newman–Keul's test at $p \leq 0.05$. Linear regression and correlation were performed for the analysis of the relationship between concentrations of PDB, gelatin, and water evaporation or absorption.

Results

Effect of PDB and gelatin on evaporation

Water evaporation rate changed with different gelatin–PDB concentration combinations over time within the 180 min of observation (Fig. 1). More water evaporated in gelatin levels 0.0% (with PDB only; Fig. 1a), 0.2% (Fig. 1b), and 0.5% (Fig. 1c) than in gelatin level 0.8% (Fig. 1d) in the first 30 min ($p < 0.05$). From 30 to 60 min, evaporating rates in gelatin level 0.0% were significantly higher than those in gelatin levels 0.2%, 0.5%, and 0.8% ($p < 0.05$). From 60 to 120 min, differences in water evaporation rates between different gelatin levels were significant ($p < 0.05$). More water evaporated in higher concentrations of gelatin combinations. From 120 to 180 min, evaporation slowed down in all gelatin concentrations (Figs. 1a, 1b, 1c, and 1d), and the differences in evaporation rates were not significant among different gelatin levels ($p > 0.146$). Unlike gelatin–PDB combinations, the water control treatment evaporated slowly (Fig. 1a).

The water had evaporated completely in gelatin level 0.0% after 150 min. No further evaporation was observed from 150 to 180 min on some combinations within gelatin level 0.0% (p01, p05, and p10 in Fig. 1a). Water content was determined for all the gelatin–PDB combinations after 180 min (Table 1). A two-way analysis of variance showed that water content was influenced by both PDB and gelatin concentrations. PDB concentration significantly affected water content ($p < 0.001$). The relationship between PDB concentration and water content was significantly positive ($R = 0.95$) at gelatin level 0.0% (PDB alone). With the presence of gelatin (gelatin levels 0.2%, 0.5%, and 0.8%), water content was significantly increased ($p = 0.030$) compared with gelatin alone (gelatin level 0.0%) (Table 1), but differences among those three gelatin levels were not significant ($p = 0.888$), nor was the relationship between gelatin concentration and water content (gelatin level 0.2%: $p = 0.601$; gelatin level 0.5%: $p = 0.474$; and gelatin level 0.8%: $p = 0.068$). No significant interaction was observed between different levels of PDB and gelatin combinations ($p = 0.16$) with the presence of both PDB and gelatin.

Effect of PDB and gelatin on water absorption

Differences in absorbed water (AW) among different concentrations of PDB within each gelatin level were significant ($p < 0.001$) after a 60-min exposure in 100% RH, and the relationship between PDB concentration and absorbed water was positively related (Fig. 2). The effect of interaction between gelatin and PDB on moisture absorption was significant ($p < 0.001$) at gelatin levels 0.2% and 0.5%, but no interaction existed at gelatin levels 0.0% and 0.8%. Linear regression showed that gelatin had only a minor effect on water absorption (AW = 0.197 + 0.045gelatin; $R^2 = 0.003$).

The amount of absorbed water in the combination of 0.5% gelatin and 1.5% PDB (gp0515) increased over time in the first 4 h (Fig. 3). During the first 90 min, water was absorbed until the mass of absorbed water was 5.4 times that of the dried gelatin–PDB combination (the dry mass of 50 µL gp0515 = 1 mg). At 4 and 20 h, the mass of absorbed water was 11.8 and 11.9 times the dry mass of gelatin–PDB combination, respectively.

Effect of PDB and gelatin on conidial germination

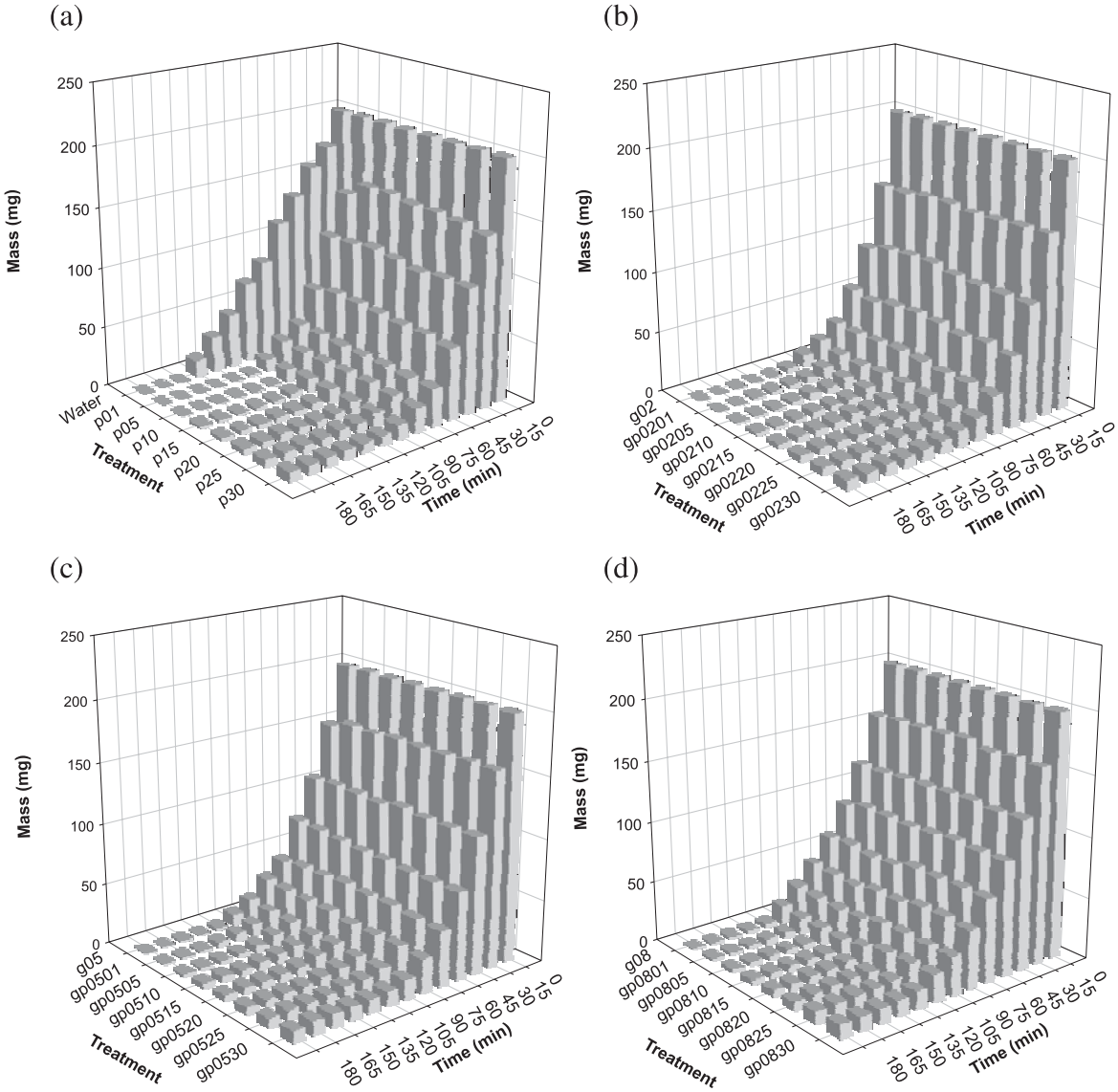
Germination in gelatin–PDB suspension

Conidia began to germinate after 11 h in suspension at 23 °C in the dark. About 60% to 70% of conidia had germinated in PDB or gelatin–PDB combinations after 16 h. Germination was 91% to 96% in all concentrations of PDB or gelatin–PDB combinations after 24 h, except for gp0201 (83%), gp0501 (84%), and gp0801 (80%). Germination in gelatin alone (g02, g05, and g08) was low (from 3.5% to 9.8%), and no conidia germinated in deionized water (Fig. 4a). Average germination at 16 h in PDB alone (p01 to p30) was higher ($p < 0.001$) than that in gelatin–PDB combinations, but these differences disappeared at 24 h.

Germination at 100% RH

Conidial germination in gelatin–PDB combinations after drying and exposure to 100% RH for 16 h was lower (Fig. 4b) than that in gelatin–PDB suspensions (Fig. 4a). Conidial germination in PDB alone (average 20.4%) was significantly lower ($p < 0.001$) than that in gelatin–PDB combinations (average 55%). Within gelatin–PDB combina-

Fig. 1. Effect of PDB and gelatin concentrations on the mass change of solutions over time. Glass slides (14 cm^2) were coated with 200 mg of each treatment at the gelatin concentration levels of 0.0% (a), 0.2% (b), 0.5% (c), and 0.8% (d); placed in a chamber at 23 °C with 22% RH; and weighed every 15 min. The data shown are means of two trials each with three replicates. For the treatment descriptors, PDB and gelatin are abbreviated by "p" and "g", respectively, and concentrations are represented in tenths of a percentage without decimals (e.g., "05" stands for 0.5%).



tions, there was no significant difference in germination between gelatin levels 0.2% and 0.5% (p = 0.391), but germination in gelatin level 0.8% was less (p = 0.002) than germination in gelatin levels 0.2% and 0.5%. Germination in gelatin levels 0.2% and 0.5% was higher than that in gelatin levels 0.8% and 0.0% at 24 h (p = 0.006). No differences were observed between gelatin levels 0.2% and 0.5% (p = 0.383), and between gelatin levels 0.8% and 0.0% (p = 0.594).

Germination at lower RH

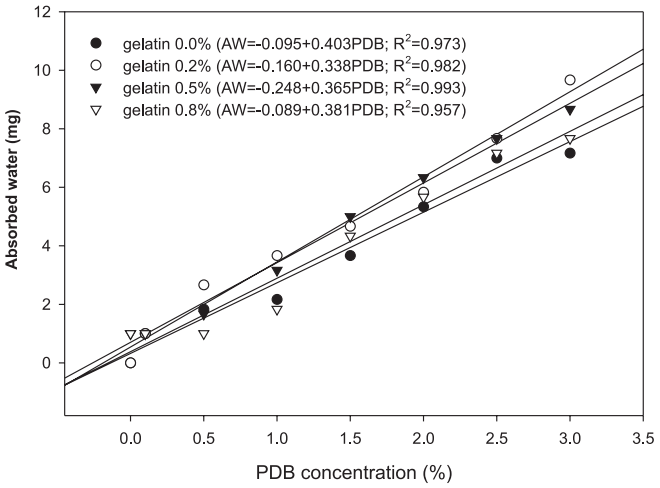
Germination at different levels of gelatin–PDB combinations increased with incubation time at 97.5% RH (Fig. 4c). No conidium germinated at 97.5% RH after 16 h. Conidial germination in gelatin levels 0.2%, 0.5%, and 0.8% was 14%, 16.7%, and 11.5%, respectively, at 24 h. This was significantly higher than germination in gelatin level 0.0% (PDB alone) (6.6%) ($p < 0.003$). At 48 h, conidial germination in gelatin levels 0.2%, 0.5%, 0.8%, and 0.0% was 62.6%, 54.7%, 40%, and 22.6%, respectively. The differences among different gelatin levels were significant ($p < 0.002$), except between levels 0.2% and 0.5% (p = 0.083). At 92.5% RH, conidia did not germinate at 16 and 24 h. The average conidial germination after 48 h at 92.5% RH in gelatin levels 0.2%, 0.5%, 0.8%, and 0.0% was 32%, 26.4%, 10.8%, and 9%, respectively, (Fig. 4d). Germination in gelatin levels 0.2% and 0.5% was significantly higher than that in gelatin levels 0.8% and 0.0% ($p < 0.001$). Dif-

Table 1. Effect of gelatin–PDB combinations on percent water content after evaporation at 23 °C, RH 22% for 3 h.

	Percent water content for the following gelatin levels:			
PDB (%)	0.0%	0.2%	0.5%	0.8%
0.0	0.0±0.0 b	20.0±12.6 a	8.3±8.3 b	6.7±3.3 b
0.1	0.0±0.0 b	33.3±6.7 a	30.0±10.0 a	30.0±6.3 a
0.5	8.3±8.3 ab	18.3±11.7 a	27.8±5.6 a	27.8±4.6 a
1.0	16.7±7.4 a	23.3±3.3 a	35.0±3.2 a	25.0±3.0 a
1.5	20.8±4.2 a	29.2±2.8 a	31.1±2.2 a	28.1±4.4 a
2.0	22.2±2.2 a	24.2±2.4 a	26.6±2.0 a	29.6±2.3 a
2.5	26.6±2.0 a	20.2±3.6 a	27.4±3.1 a	37.8±1.9 a
3.0	26.0±2.9 a	21.5±1.5 a	26.8±3.4 a	33.1±3.3 a

Note: Data (±SE) represent the mean of 2 trials × 3 replicates. A two-way analysis of variance was performed. Means followed by the same letter in each column are not significantly different ($p = 0.05$) according to the Student–Newman–Keul's test.

Fig. 2. Effect of PDB, gelatin, and their combinations on the amount of absorbed water (AW). Slides with 50 µL of each gelatin–PDB combination were dried at 23 °C for 24 h, weighed, then exposed to 100% RH for 60 min and reweighed. The net mass increase (mg) was equal to the amount of water absorbed. The data shown are the average of two trials each with three replicates.



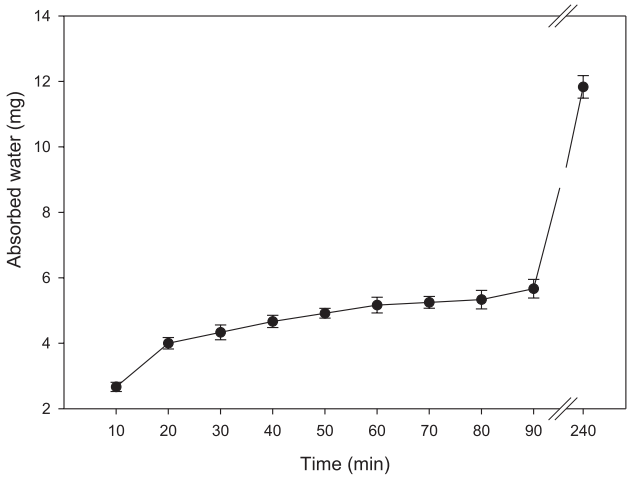
ferences were not significant between gelatin levels 0.2% and 0.5% ($p = 0.149$) or 0.8% and 0.0% ($p = 0.644$) at 92.5% RH.

No conidium germinated at 85% and 75% RH after 48 h of incubation (data not shown).

Effect of different dry periods on germination

The effect of dry periods on conidial germination was significant (Fig. 5). The average conidial germination at all combinations on slides with a dry period of 1 week was 53%. There were no significant differences on germination between a dry period of 1 or 2 weeks (49.3%) ($p = 0.375$), while the difference on germination rate between 2 and 3 weeks dry period (23.9%) was significant ($p < 0.001$). Compared with the 3-week dry period, germination rate

Fig. 3. Water absorption by dried 0.5% gelatin – 1.5% PDB combination (gp0515) over time. Slides coated with 50 µL of gp0515 were dried at 23 °C for 24 h, weighed, and exposed to 100% RH. The net mass increase (mg), which was equal to the amount of water absorbed, was measured every 10 min. The data shown are the average of two trials each with six replicates, and the bars represent standard error of the means.



dropped significantly to 12.6% ($p = 0.008$) after a 4-week dry period.

Dry period also significantly affected conidial germination at different gelatin levels with the presence of PDB (Fig. 5). Differences in germination among different gelatin levels were not significant ($p = 0.346$) (Fig. 5a) after a 1-week dry period. After a 2-week dry period, significant differences appeared only between gelatin levels 0.8% (gp0801 to gp0830) and 0.2% (gp0201 to gp0230) ($p = 0.004$) (Fig. 5b). After a 3-week dry period, average germination in gelatin level 0.2% was 13%, lower than at the gelatin levels 0.5%, 0.8%, and 0.0% (33%, 35%, and 25%, respectively) ($p < 0.048$) (Fig. 5c). After a 4-week dry period, germination at gelatin level 0.8% was 28.9%, significantly higher ($p < 0.001$) than at gelatin levels 0.2% (9.9%), 0.5% (15.1%), or 0.0% (2.1%) (Fig. 5d). Germination rates in gelatin level 0.0% (PDB alone, p01 to p30) dropped dramatically down to 2.1% after a dry period of 4 weeks from 25.5% after a 3-week dry period (Figs. 5c and 5d).

The overall germination rates in gelatin alone (g02, g05, and g08) were low (from 2.7% to 4.3%) and did not change substantially with a 4-week dry period ($p = 0.845$) (Fig. 5). No conidia germinated in water.

Effect of PDB and gelatin on lesion size

The interaction between lesion size, gelatin–PDB concentrations, and salal leaf age was significant. Lesions on young leaves were generally larger than on old leaves (Figs. 6a and 6b). For young leaves, different PDB and gelatin concentrations had significant effects on the lesion size (Fig. 6a), and there was a strong interaction between PDB and gelatin level ($p < 0.001$). The average lesion size at gelatin level 0.5% (g05 to gp30) was larger than that at gelatin levels 0.0% (PDB alone, p01 to p30), 0.2% (g02 to gp30), or 0.8% (g08 to gp30). A two-way analysis of variance showed that lesion at PDB levels 1.5% and 2.0% within the

Fig. 4. Effect of PDB, gelatin, and their combinations on percent germination of conidia of *Phoma exigua* PFC2705 at 16, 24, or 48 h in water suspensions (a), in RH 100% (b), in RH 97.5% (c), and in RH 92.5% (d). The data shown are average percent conidial germination rate of two trials each with three replicates, and the bars represent the standard error of the means.

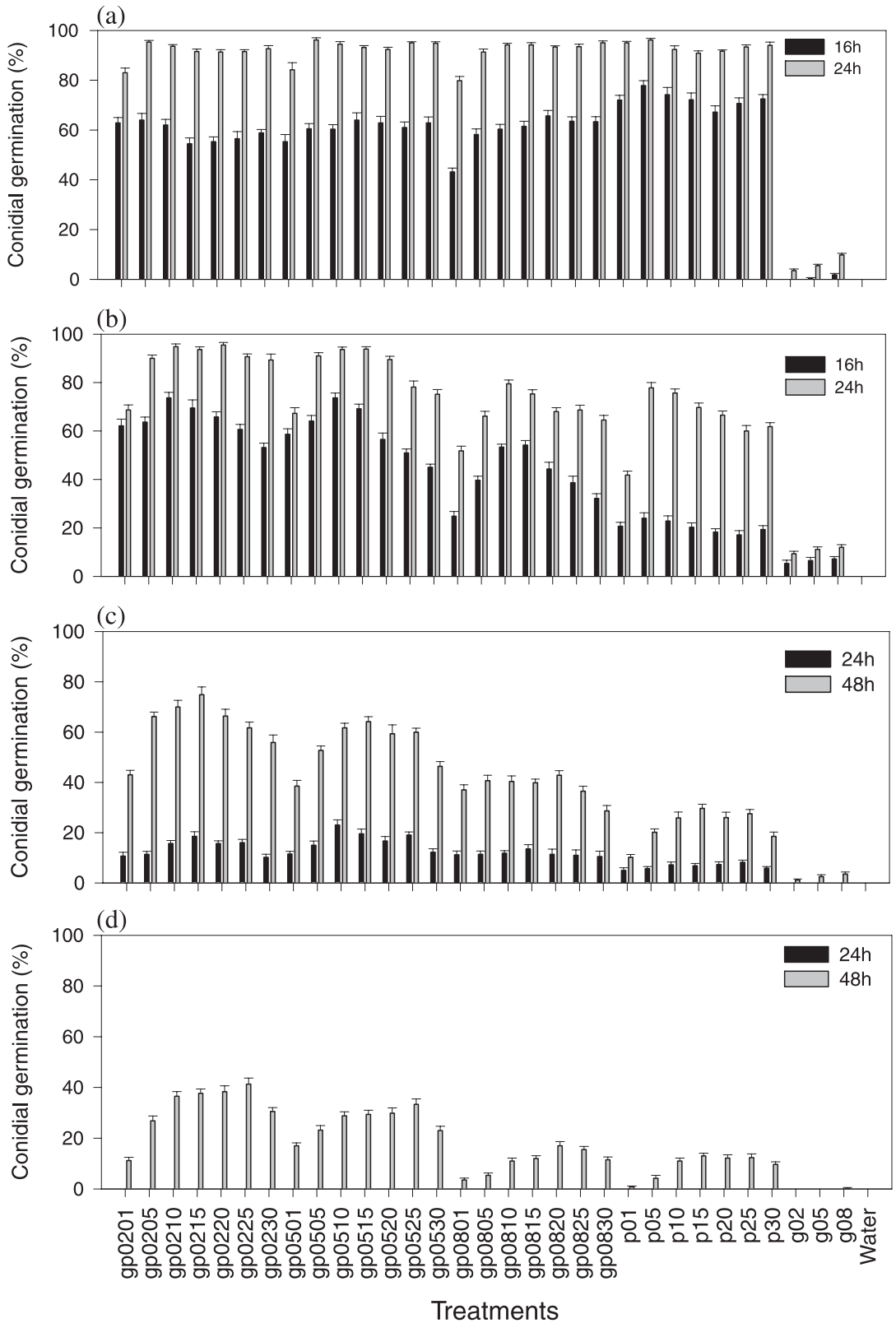


Fig. 5. Effect of dry period on germination of conidia of *Phoma exigua* PFC2705 in different gelatin–PDB combinations. Slides with 10 µL of conidial suspensions in each gelatin–PDB combination were dried (at 23 °C with RH 22%) for 1 week (a), 2 weeks (b), 3 weeks (c), and 4 weeks (d), germinated with three cycles at 23 °C in a dark chamber with RH 100% for 8 h followed by 16 h at RH 22%, and then incubated at RH 100% for 24 h. The data shown are the average germination rates of two trials each with three replicates, and the bars represent the standard error of the means.

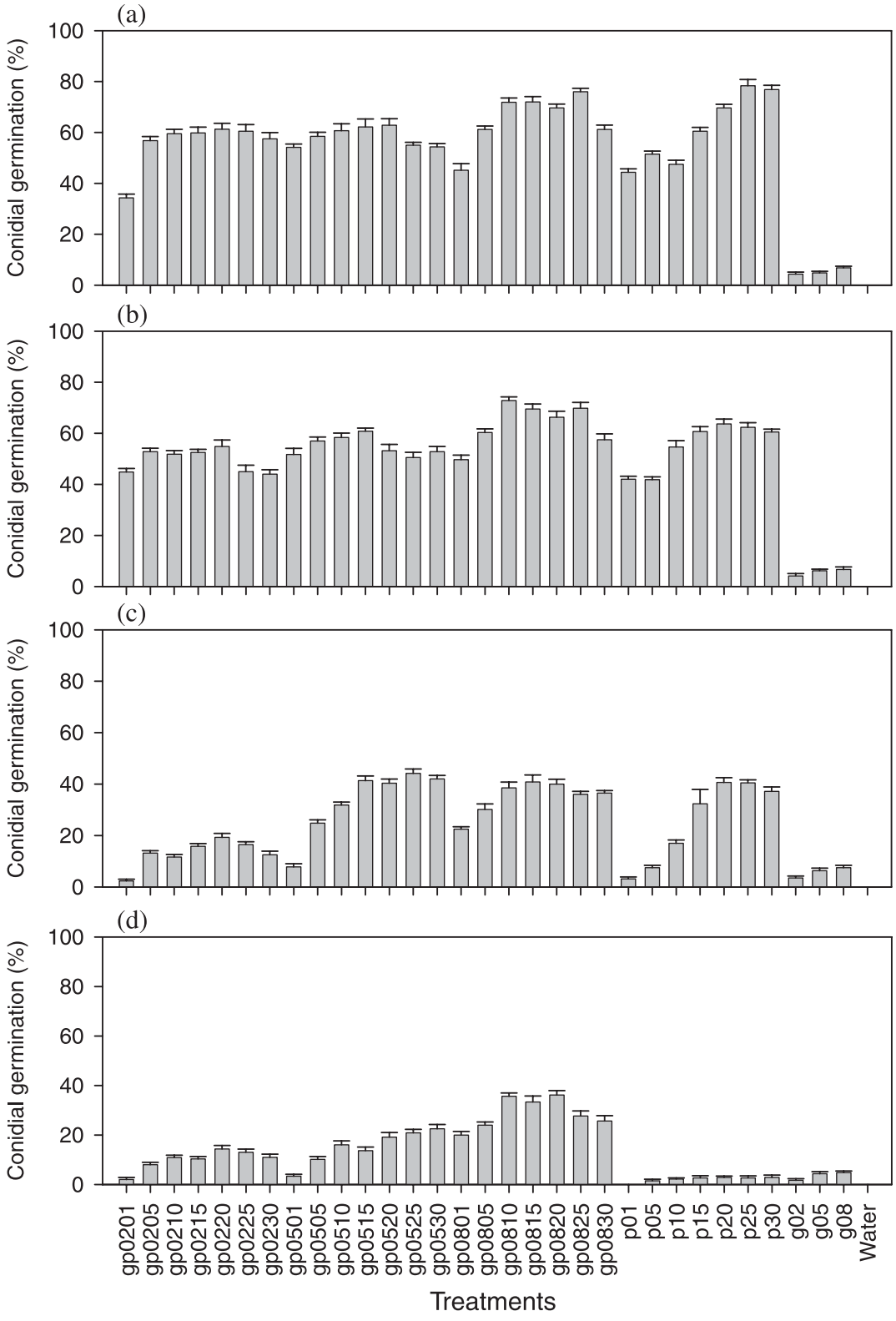
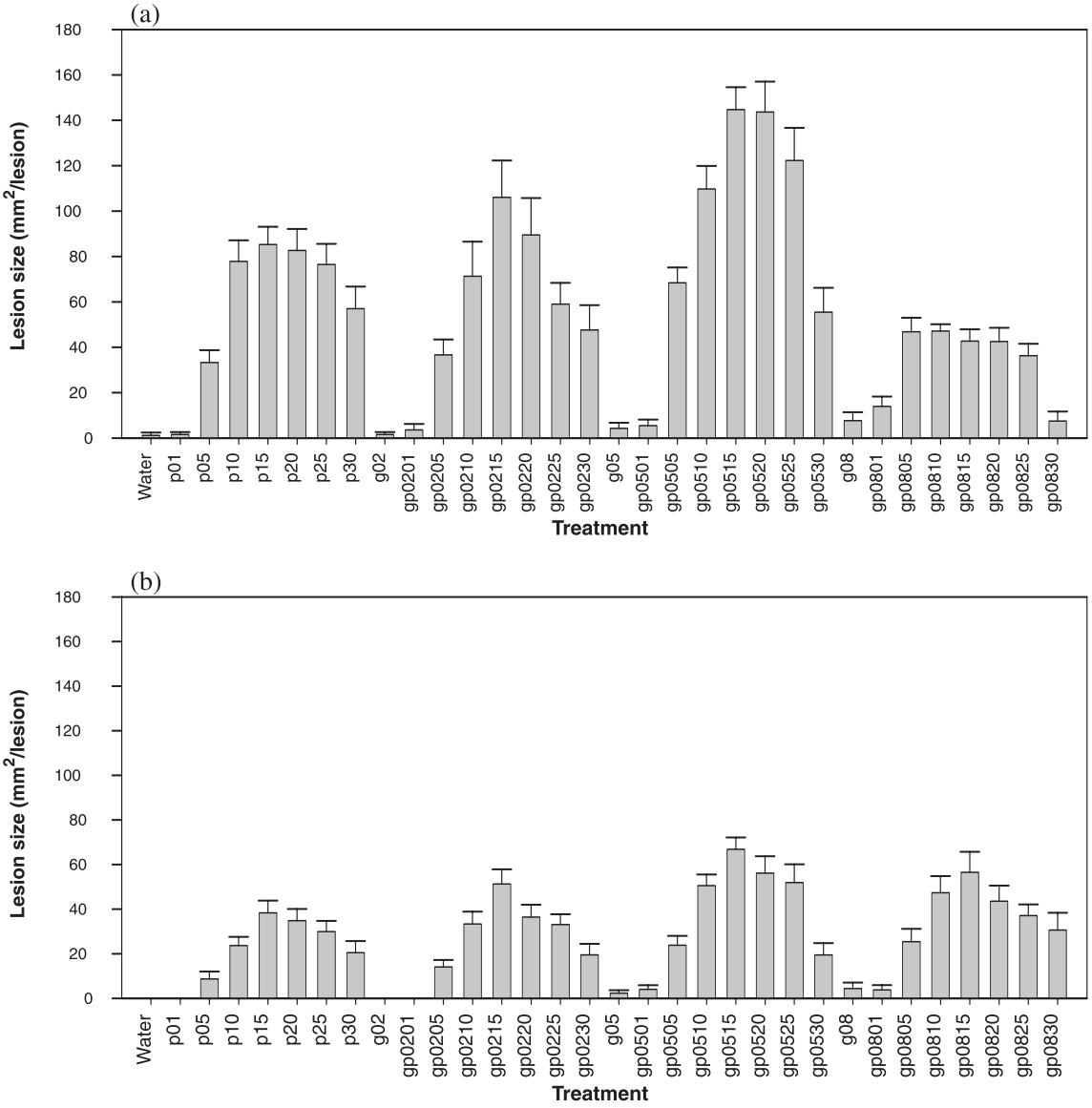


Fig. 6. Effect of PDB, gelatin, and their combinations on lesion size (mm^2) caused by conidia of *Phoma exigua* PFC2705 on detached young (a) and old (b) salal leaves. Detached salal leaves (young: 20–25 days old and old: 40–45 days old) were inoculated on the lower surface with 20 µL of 10^6 conidia/mL suspension of each gelatin–PDB combination and transferred to chambers with RH 100% at 23 °C with 12 h light. The sizes of the necrotic lesions were measured 5 days postinoculation. The data shown represent the average lesion area of 16 replications.



combinations were larger than lesions at any other PDB concentrations; the lesions at PDB level 0.0% (gelatin alone) and 0.1% were smaller, and gelatin did not contribute to the lesion development at these levels. The interaction between gelatin and PDB was not significant for old leaves (p = 0.271). Treatment control (gp0515 without conidia) and systematic control (water without conidia) did not cause any damage on salal leaves.

Discussion

The role of PDB and gelatin in water retention and absorption and their effects on conidial germination and lesion size of *P. exigua* PFC2705 on salal were evaluated in the present study. When PDB and gelatin are used as adjuvants, they possess more than one function. First, they acted as spreaders. Water is the most common carrier for crop production sprays. When a wetting agent is added, it reduces the surface tension within a spray droplet and allows the droplet to lie flat in a thin layer on the surface of the crop. Consequently, this thin layer tends to evaporate rapidly (Hazen 2000). In our evaporation experiment, water evaporated more quickly at all concentration levels of PDB, gelatin, or their combinations than did deionized water, especially within the first 2 h. This suggests that both gelatin and PDB acted as wetting agents or spreaders by reducing the surface tension. Second, PDB and gelatin acted as humectants. Humectants possess water retention properties; they increase the equilibrium water content and therefore the drying time of an aqueous spray deposit, and they may

draw moisture from the atmosphere to maintain a higher humidity level on or near the spray deposit (Hazen 2000). In our evaporation experiment, deionized water evaporated completely after 150 min, while the addition of PDB, gelatin, and their combinations helped retain water, and the water content was from 10% to 40% at 180 min. PDB absorbed more water than gelatin. After 60 min at 100% RH, the amount of water absorbed by dried PDB or gelatin–PDB combinations on slides showed a linear relationship with PDB concentrations at all gelatin concentrations ($R^2 > 0.957$), but the relationship between water absorption and gelatin concentration was weak ($R^2 = 0.003$). Hence, in all gelatin–PDB combinations, water absorption was mainly determined by PDB concentrations.

PDB, as a humectant, may have reduced the dew period needed for conidial germination. In this study, because of the moisture absorption of PDB, conidial germination reached as high as 40% (gp0225) after 48 h, even at RH 92.5%. In other words, as a result of moisture absorption, the dew period was most likely prolonged. This corresponds with Daigle and Cotty's (1991) conclusion that the dew period requirement for a given mycoherbicidal activity can be extensively shortened by proper formulation. As a humectant, PDB absorbed water from the environment with high RH and kept itself in liquid form, which made nutrients accessible to the germinating conidia and growing germ tubes. Third, PDB and gelatin acted as nutrients. *Phoma* species live usually as saprophytic fungi (von Arx 1987). Most saprophytes require additional nutrients in the process of germination, including inorganic salts, carbon sources, specific amino acids, and vitamins (Griffin 1981). No conidia germinated in deionized water in our germination experiment, even after 48 h. Conidia started to germinate after 11 h at 23 °C, and germination reached 60% after 16 h in the presence of PDB. Even though the germination rate in gelatin was rather low (below 10%) after 24 h, it still suggests that conidia may use gelatin as a nutrient source. Zhang et al. (2003) reported that germination of five *Phoma* isolates (including *P. exigua*) increased to nearly 100% after addition of gelatin into 1.5% water agar at a concentration of 5%. This is substantially higher than the germination rate of isolate PFC2705 of *P. exigua* and is likely caused by differences between individual *Phoma* isolates.

PDB and gelatin also acted as stickers. This was especially true for gelatin, which formed a film around conidia and so adheres the conidia onto the target (data not shown). Gelatin may also prolong the viability of the conidia. After a 4-week dry period, germination rates dropped close to zero without gelatin, while germination rates were higher in the presence of gelatin and increased significantly at higher gelatin concentrations.

In this paper, we have elucidated some functions of PDB and gelatin as adjuvants. PDB not only provided nutrients to conidia, but it also acted also as a wetter, sticker, and humectant. Gelatin, although acting to some extent as a nutrient source during germination, acted mainly as a sticker (S. Zhao and S.F. Shamoun, unpublished data). Unlike PDB, gelatin did not draw moisture from the environment. However, in gelatin–PDB combinations, gelatin kept water absorbed by PDB and inhibited evaporation. In appropriate concentrations, gelatin increased the germination of conidia and enhanced the lesion size on detached young salal leaves. On older leaves, the effect of gelatin on lesion size was not significant. To better understand the effects of PDB and gelatin on the pathogenicity of conidia of *P. exigua* PFC2705 on salal and to explore the potential of this pathogen as a biocontrol agent, experiments using intact plants need to be conducted.

Acknowledgements

We are grateful to Weyerhaeuser Canada Ltd., and the Canadian Forest Service Enhanced Timber Production and Protection Working Group for funding this project. Special thanks are due to Dr. Susanne Vogelgsang (Swiss Federal Research Station for Agroecology and Agriculture, Switzerland) and Dr. Karen Bailey (Agriculture and Agri-Food Canada, Saskatoon Research Centre, Canada) for reviewing an early version of the manuscript.

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