Phytoprotection

Effect of different salts on the development of *Fusarium solani* var. *coeruleum*, a causal agent of potato dry rot

Effet de différents sels sur le développement du *Fusarium solani* var. *coeruleum*, un agent pathogène responsable de la pourriture sèche de la pomme de terre

Mélanie R. Mecteau, Joseph Arul and Russell J. Tweddell

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Article abstract

The objectives of this study were (1) to evaluate the effect of different salts on the *in vitro* development of *Fusarium solani* var. *coeruleum*, and (2) to evaluate the efficacy of the salts for reducing dry rot severity caused by the pathogen in potato tubers. The study showed that several salts significantly inhibited the mycelial growth of *F. solani* var. *coeruleum*. Aluminium acetate, aluminium chloride, sodium benzoate, sodium metabisulfite, potassium sorbate and trisodium phosphate completely inhibited mycelial growth. Exposure of *F. solani* var. *coeruleum* conidia to aluminium acetate, potassium sorbate, sodium benzoate, sodium metabisulfite or trisodium phosphate at 0.2 M resulted in 100% mortality of the conidia after 1 h while aluminium chloride and aluminium lactate caused 100% mortality after an exposure of 24 h. In order to evaluate the effect of salts on potato dry rot development, *F. solani* var. *coeruleum*-inoculated tubers were treated with the different salts and disease severity was evaluated following an incubation period of 7 d. Among the test salts, only aluminium chloride caused a significant reduction in potato dry rot compared with the control. The study points out the possibility of using aluminium chloride to control potato dry rot.

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Keywords: Aluminium, benzoate, dry rot, fungitoxicity, *Fusarium solani* var. *coeruleum*, metabisulfite, phosphate, potato, salt, sorbate, tuber.

[Effet de différents sels sur le développement du *Fusarium solani* var. *coeruleum*, un agent pathogène responsable de la pourriture sèche de la pomme de terre]

Les objectifs de cette étude étaient (1) d'évaluer l'effet de différents sels sur le développement *in vitro* du *Fusarium solani* var. *coeruleum* et (2) d'évaluer l'efficacité des sels testés à réduire le développement de la pourriture sèche de la pomme de terre causée par cet agent pathogène. Les travaux réalisés montrent que plusieurs sels utilisés à une concentration de 0.2 M ont réduit significativement la croissance mycélienne du *F. solani* var. *coeruleum*. L'acétate d'aluminium, le chlorure d'aluminium, le benzoate de sodium, le métabisulfite de sodium, le sorbate de potassium et le trisodium phosphate ont complètement inhibé la croissance mycélienne du champignon. L'exposition de conidies du *F. solani* var. *coeruleum* à l'acétate d'aluminium, au benzoate de sodium, au métabisulfite de sodium, au sorbate de potassium et au trisodium phosphate a causé la mortalité complète des conidies après 1 h. Le chlorure d'aluminium et le lactate d'aluminium ont pour leur part causé la mortalité complète des conidies après 24 h d'exposition. Afin d'évaluer l'effet des sels sur le développement de la pourriture sèche de la pomme de terre, des tubercules inoculés avec *F. solani* var. *coeruleum* ont été traités avec les différents sels et la sévérité de la maladie a été évaluée après 7 j d'incubation. Parmi les sels testés, seul le chlorure d'aluminium a permis, comparativement au témoin, une réduction significative de la pourriture sèche de la pomme de terre. Cette étude suggère la possibilité d'utiliser le chlorure d'aluminium pour lutter contre la pourriture sèche de la pomme de terre.

Mots clés : aluminium, benzoate, fongitoxicité, *Fusarium solani* var. *coeruleum*, métabisulfite, phosphate, pomme de terre, pourriture sèche, sel, sorbate, tubercule.

Centre de recherche en horticulture, Pavillon de l'Envirotron, Université Laval, Québec (Québec), Canada G1V 0A6; corresponding author e-mail: russell.tweddell@ssaa.ulaval.ca

INTRODUCTION

The use of synthetic pesticides remains the most widely used disease control measure in the ongoing struggle against plant pathogens, even though they have shown major drawbacks such as their lack of long-term efficacy due to the development of resistance by plant pathogens (Avis 2007). In the present context of safe and sustainable disease control, great strides toward the use of alternative means of disease control have been made (Avis 2007). However, there remains an urgent need for disease control measures that present novel, efficient modes of action as well as reduced risks for the environment.

An interesting alternative to fungicide application involves the use of the antimicrobial properties of some organic and inorganic salts that are widely used in the food industry as preservatives and antimicrobial agents (Russell and Gould 1991) to control plant diseases. These compounds have shown broad-spectrum antimicrobial activity with low mammalian toxicity (Olivier *et al.* 1998), possess biocompatibility (Horst *et al.* 1992), and are generally recognized as safe (GRAS). Moreover, they are less sensitive to environmental conditions than other alternative control measures such as biological control agents, which may make them particularly useful for the efficient control of plant pathogens. Several salts were shown to reduce the development of plant diseases. Bicarbonate salts, applied by spraying, provided good control of gummy stem blight (*Didymella bryoniae* (Auersw.) Rehm) and Alternaria leaf blight (*Alternaria cucumerina* (Ellis & Everh.) J.A. Elliot) in greenhouse-grown muskmelon (Ziv and Zitter 1992). Horst *et al.* (1992) showed that powdery mildew (*Sphaerotheca pannosa* (Wallr.:Fr.) Lév. var. *rosae* Woronichin) and black spot of roses (*Diplocarpon rosae* Wolf) could be controlled by spraying an aqueous solution of sodium bicarbonate. Post-harvest application of salts has also been shown to control different diseases of fruits and vegetables. Post-harvest application of ammonium bicarbonate, sodium bicarbonate, potassium carbonate, calcium propionate and potassium sorbate reduced black root rot on carrots caused by *Chalara elegans* Nag Raj & Kendrick (Punja and Gaye 1993). Aluminium chloride, aluminium lactate, ammonium acetate, calcium chloride, potassium sorbate, sodium benzoate, sodium carbonate, sodium metabisulfite and trisodium phosphate applied on potato (*Solanum tuberosum* L.) tubers as post-harvest treatments were shown to markedly reduce the severity of silver scurf (*Helminthosporium solani* Durieu & Mont.) (Hervieux *et al.* 2002).

Dry rot is one of the most important post-harvest diseases of potato tubers, causing significant economic losses worldwide (Stevenson *et al.* 2001). The disease is caused by different species of *Fusarium*, including *Fusarium solani* (Mart.) Sacc. var. *coeruleum* (Lib. ex Sacc.) C. Booth and *Fusarium sambucinum* Fuckel. Control of dry rot has been achieved primarily by post-harvest applications of thiabendazole, a benzimidazole fungicide (Secor and Gudmestad 1999). However, many strains of *F. sambucinum* have become resistant to thiabendazole (Desjardins *et al.* 1993; Holley and Kawchuk 1996; Platt 1997), thus resulting in increased incidence and severity of dry rot (Secor and Gudmestad 1999). Although cultural practices such as crop rotation, use of disease-free seed, wound-healing of stored potatoes and minimizing wounds during harvesting and handling can help to reduce dry rot (Secor and Gudmestad 1999), alternative control strategies are needed.

The objectives of this study were (1) to evaluate the effect of different salts on the *in vitro* development of *F. solani* var. *coeruleum*, and (2) to evaluate the efficacy of the salts for reducing dry rot severity caused by the pathogen in potato tubers.

MATERIALS AND METHODS

Fungal isolate

The isolate of *F. solani* var. *coeruleum* was obtained from the Laboratoire de diagnostic en phytoprotection (MAPAQ, Québec, QC). The fungus, isolated from a potato tuber, was maintained on Potato Dextrose Agar slants (PDA; Difco Laboratories, Becton Dickinson, Sparks, MD). The agar slants were stored at 4°C and served as stock cultures.

Chemicals

All salts were purchased from Sigma Chemical Co. (St. Louis, MO, USA) with the exception of ammonium acetate and sodium bicarbonate (BDH Inc., Toronto, ON, Canada).

Effect of salts on *in vitro* growth and development of *F. solani* var. *coeruleum*

Effect of salts on mycelial growth

Fusarium solani var. *coeruleum* was grown in a Petri dish on PDA unamended (control) or amended with test salts (0.2 M) at 24°C. PDA agar disks (6 mm diam) of actively growing mycelium of *F. solani* var. *coeruleum* were used to inoculate the plates. For each plate, diam of the colony was determined after a 10-d incubation period. Colony diam was measured as the average of the longest diam and the shortest diam. Inhibition of mycelial growth was calculated as follows: [(control radial growth - salt-amended radial growth) / control radial growth] x 100. The experimental design was a completely randomized block with three replicates.

ED_{50} values of salts

The effective doses of salts that can cause 50% inhibition (ED_{50}) of mycelial growth were determined using the SAS Probit procedure (SAS Institute Inc., Cary, NC, USA) (Mecteau *et al.* 2002). Mycelial growth was determined, as previously described, in PDA containing different concentrations (0-0.2 M) of each test salt.

Fungitoxicity of salts

Conidia of *F. solani* var. *coeruleum* (9×10^5) were suspended with agitation (150 rpm; 24°C) in 50 mL triplicate flasks containing 20 mL of each salt solution (0.2 M) or double-distilled water (control). Samples (600 µL) were withdrawn at intervals (1, 3, 6, 9, 12 and 24 h) from the flask and placed into microtubes.

Conidia were recovered by centrifugation as described by Mecteau *et al.* (2002) and incubated in 500 µL of Malt Extract Broth (Difco Laboratories) at 24°C with agitation to favour germination. After 24 h of incubation, an aliquot of the conidia suspension was examined with a light microscope (Olympus, Tokyo, Japan) using a hemacytometer in order to rate the germination of conidia; non-germinated conidia were considered as non-viable. The level of mortality was based on 100 conidia and expressed as a percentage: {[germinated conidia (control) – germinated conidia (salt solution)] / germinated conidia (control)} x 100.

Effect of salts on potato dry rot development

Potatoes

Tubers of the cultivar Dark Red Norland were obtained from Propur Inc. (St-Ambroise, QC, Canada). Potatoes were stored in the dark at 4°C until use. Selected tubers were washed as previously described by Mecteau *et al.* (2002). Four wounds (4 mm deep) were performed on each tuber using a cork borer.

Inoculation of tubers with F. solani *var.* coeruleum *and application of salts*

Twenty-five µL of a suspension of *F. solani* var. *coeruleum* conidia (5 x 10^5 conidia mL^{-1}) prepared from a 10-d-old culture grown on PDA were injected into each wound. Tubers were incubated in the dark at 24°C for 24 h. They were then dipped (10 min) in the different salt solutions (0.2 M) or in double-distilled water (control) and incubated individually in the dark at 24°C in plastic chambers containing a humidified towel. Disease severity was evaluated after 7 d of incubation. The experimental design was a completely randomized block with three replicates.

Disease severity assessment

Dry rot severity was assessed by lesion area (cm^2) as described by Satyaprasad *et al.* (1997). Lesion area is a mean of four inoculation sites per tuber.

Data analysis

Analysis of variance (ANOVA) was performed using the GLM (General Linear Models) procedure of SAS. When significant ($P < 0.1$), treatment means were compared using the test of Tukey and contrasts for *in vitro* and *in vivo* experiments, respectively.

RESULTS

Several salts significantly inhibited mycelial growth of *F. solani* var. *coeruleum* (Table 1). Aluminium acetate, aluminium chloride, sodium benzoate, sodium metabisulfite, potassium sorbate and trisodium phosphate completely inhibited mycelial growth. Aluminium lactate, sodium carbonate, sodium citrate and sodium propionate inhibited growth to a smaller extent (54.2-88.7%). For the salts that completely inhibited mycelial growth, ED_{50} values were determined (Table 2).

Table 1. Effect of salts on *Fusarium solani* var. *coeruleum* mycelial growth[a]

Salt (0.2 M)	Mycelial growth inhibition[b] (%)
Aluminium acetate ($C_2H_5O_4Al$)	100.0 a[c]
Aluminium chloride ($AlCl_3 \cdot 6H_2O$)	100.0 a
Aluminium lactate ($[C_3H_5O_3]_3Al$)	85.3 ab
Ammonium acetate ($NH_4C_2H_3O_2$)	-2.2 cd
Ammonium chloride (NH_4Cl)	-3.2 d
Calcium chloride ($CaCl_2 \cdot 2H_2O$)	42.4 abc
Diammonium phosphate ($(NH_4)_2HPO_4$)	0.6 cd
Disodium phosphate ($Na_2HPO_4 \cdot 7H_2O$)	9.0 bcd
Potassium chloride (KCl)	39.3 abcd
Potassium sorbate ($C_6H_7O_2K$)	100.0 a
Sodium acetate ($C_2H_3O_2Na$)	-1.2 cd
Sodium benzoate ($C_7H_5O_2Na$)	100.0 a
Sodium bicarbonate ($NaHCO_3$)	12.5 bcd
Sodium carbonate (Na_2CO_3)	88.7 ab
Sodium citrate ($C_6H_5O_7Na_3 \cdot 2H_2O$)	83.7 ab
Sodium formate (HCOONa)	-1.2 cd
Sodium lactate ($C_3H_5O_3Na$)	3.6 bcd
Sodium metabisulfite ($Na_2S_2O_5$)	100.0 a
Sodium propionate ($C_3H_5O_2Na$)	54.2 abcd
Sodium succinate ($C_4H_4O_4Na_2 \cdot 6H_2O$)	13.2 bcd
Sodium tartrate ($C_4H_4O_6Na_2 \cdot 2H_2O$)	12.8 bcd
Trisodium phosphate ($Na_3PO_4 \cdot 12H_2O$)	100.0 a

[a] *Fusarium solani* var. *coeruleum* was grown on PDA unamended (control) or amended with test salts (0.2 M) at 24°C. Colony diam was determined after a 10-d incubation period.

[b] Inhibition of mycelial growth is expressed as percentage: [(control radial growth – salt-amended radial growth) / control radial growth] x 100. Each value represents the mean of three replicates.

[c] Values followed by the same letter are not significantly different ($P < 0.1$) according to Tukey's test.

Table 2. Salt dose needed to cause a 50% reduction in *Fusarium solani* var. *coeruleum* mycelial growth (ED_{50})

Salt	Mycelial growth ED_{50} (mM)
Potassium sorbate	1.8
Aluminium chloride	3.7
Sodium metabisulfite	4.2
Sodium benzoate	9.8
Aluminium acetate	18.6
Trisodium phosphate	36.1

ED_{50} values were determined using the SAS Probit procedure.

The effect on spore viability of salts causing a mycelial growth inhibition superior to 50% was also evaluated (Fig. 1). The results showed that an exposure of 1 h to aluminium acetate, potassium sorbate, sodium benzoate, sodium metabisulfite or trisodium phosphate caused 100% mortality. Aluminium chloride and aluminium lactate caused 100% mortality after an exposure of 24 h. Mortality of the conidia exposed to sodium citrate was approximately 50% after 24 h. Sodium propionate exhibited a lag phase in mortality of 12 h, reaching 20% mortality at 24 h. Sodium carbonate showed no mortality.

In order to evaluate the effect of salts on potato dry rot development, *F. solani* var. *coeruleum*-inoculated tubers were treated with the different salts and disease severity was evaluated following an incubation period of 7 d. Among the test salts, only aluminium chloride caused a significant ($P < 0.1$) reduction in potato dry rot compared with the control (Fig. 2). The application of aluminium chloride was shown to decrease disease severity by 40% compared with the control.

DISCUSSION

Dry rot is an important potato tuber disease caused by different species of *Fusarium*, including *F. solani* var. *coeruleum* and *F. sambucinum*. With the appearance of resistant strains of *F. sambucinum* to thiabendazole, a fungicide that made it possible to control potato dry rot, increased incidence and severity of the disease were observed. In an attempt to develop alternative strategies for the control of potato dry rot, Mecteau *et al.* (2002) tested several organic and inorganic salts for their effect on dry rot caused by *F. sambucinum*. They showed that specific salts, when applied on potato tubers, made it possible to reduce disease severity, thus suggesting that salts may eventually be used in the control of potato dry rot.

The present study showed that *F. solani* var. *coeruleum* development is strongly affected by several organic and inorganic salts. Indeed, the mycelial growth of the fungus was completely inhibited by aluminium acetate, aluminium chloride, potassium sorbate, sodium benzoate and sodium metabisulfite at 0.2 M. These results are in agreement with those of Mills *et al.* (2004) who reported that these salts strongly inhibited *F. solani* var. *coeruleum* mycelial growth. Among the test salts, trisodium phosphate was also shown to completely inhibit mycelial growth. Mycelial growth appeared particularly sensitive to potassium sorbate, aluminium chloride, sodium metabisulfite, sodium benzoate and aluminium acetate with ED_{50} lower than 20 mM. Moreover, the results showed that exposure to aluminium acetate, potassium sorbate, sodium benzoate, sodium metabisulfite, trisodium phosphate, aluminium chloride and aluminium lactate was toxic to *F. solani* var. *coeruleum* conidia. The toxicity of these salts against *F. sambucinum* conidia had previously been observed by Mecteau *et al.* (2002).

The application of aluminium chloride by dipping significantly decreased dry rot severity in infected tubers. Of particular interest is the fact that aluminium chloride was previously shown to reduce the development of other important potato tuber pathogens, namely *F. sambucinum* (Mecteau *et al.* 2002) and *H. solani* (Hervieux *et al.* 2002). This salt, which is the active ingredient in the fungicide Synermix™, was also shown to be effective against Botrytis mold (*Botrytis cinerea* Pers.:Fr.) on grapevines (*Vitis* spp.) (Jeandet *et al.* 2000).

Several salts strongly inhibited the *in vitro* development of *F. solani* var. *coeruleum*, but only aluminium chloride was able to reduce dry rot severity in potato tubers, thus suggesting that some salts lose their inhibitory effect when applied on potato tubers (Mecteau *et al.* 2002). Application of salts on tubers, on the other hand, may involve biochemical reactions such as defense mechanisms contributing to disease control. Aluminium chloride is in fact known to induce mechanisms of defense in plants (Jeandet *et al.* 2000; Mecteau *et al.* 2002; Mucharromah and Kuc 1991).

In this study, several salts were shown to affect the *in vitro* development of *F. solani* var. *coeruleum*. Among them, aluminium chloride was particularly interesting since it showed efficiency in reducing potato dry rot infection caused by both *F. solani* var. *coeruleum* and *F. sambucinum* (Mecteau *et al.* 2002) when applied on tubers. This indicates the possibility of using aluminium chloride to control dry rot on either seed tubers or warehouse potatoes for human consumption. However, additional research is required before we can consider industrial application of salts, including the evaluation of salts efficacy at a larger scale and the evaluation of potential health and environmental risks associated with their application.

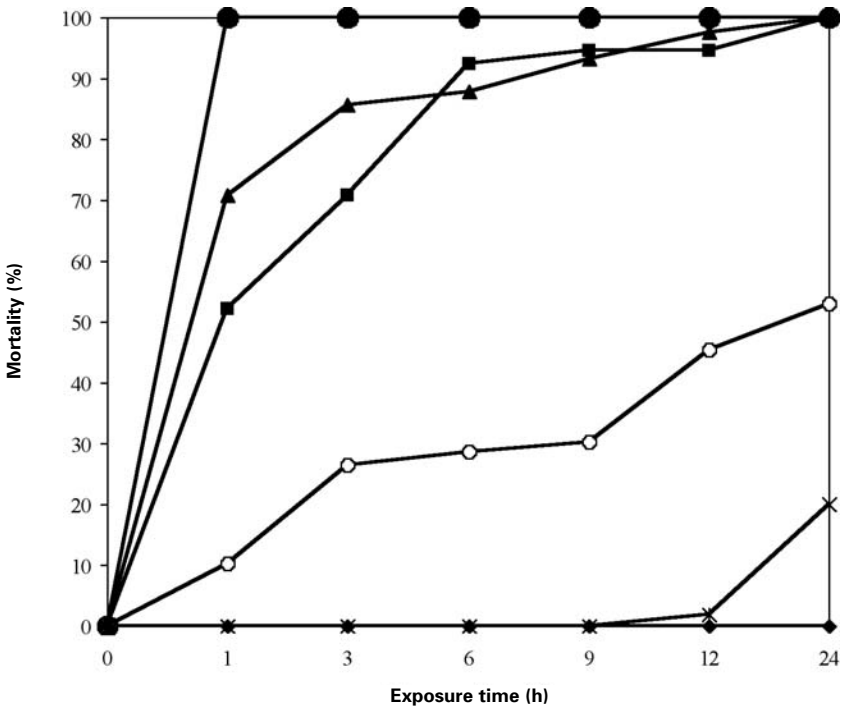

Figure 1. Effect of salts and exposure time on *Fusarium solani* var. *coeruleum* conidia viability. Conidia of *F. solani* var. *coeruleum* were suspended with agitation in 20 mL of each salt solution (0.2 M) or double-distilled water (control) for 1, 3, 6, 9, 12 and 24 h of incubation. They were then recovered by centrifugation and rated for their viability. Mortality was expressed as percentage: {[germinated conidia (control) – germinated conidia (salt solution)] / germinated conidia (control)} x 100. Each value represents the mean of three replications. (●) Aluminium acetate; (▲) aluminium chloride; (■) aluminium lactate; (●) potassium sorbate; (●) sodium benzoate; (♦) sodium carbonate; (○) sodium citrate; (●) sodium metabisulfite; (×) sodium propionate; (●) trisodium phosphate.

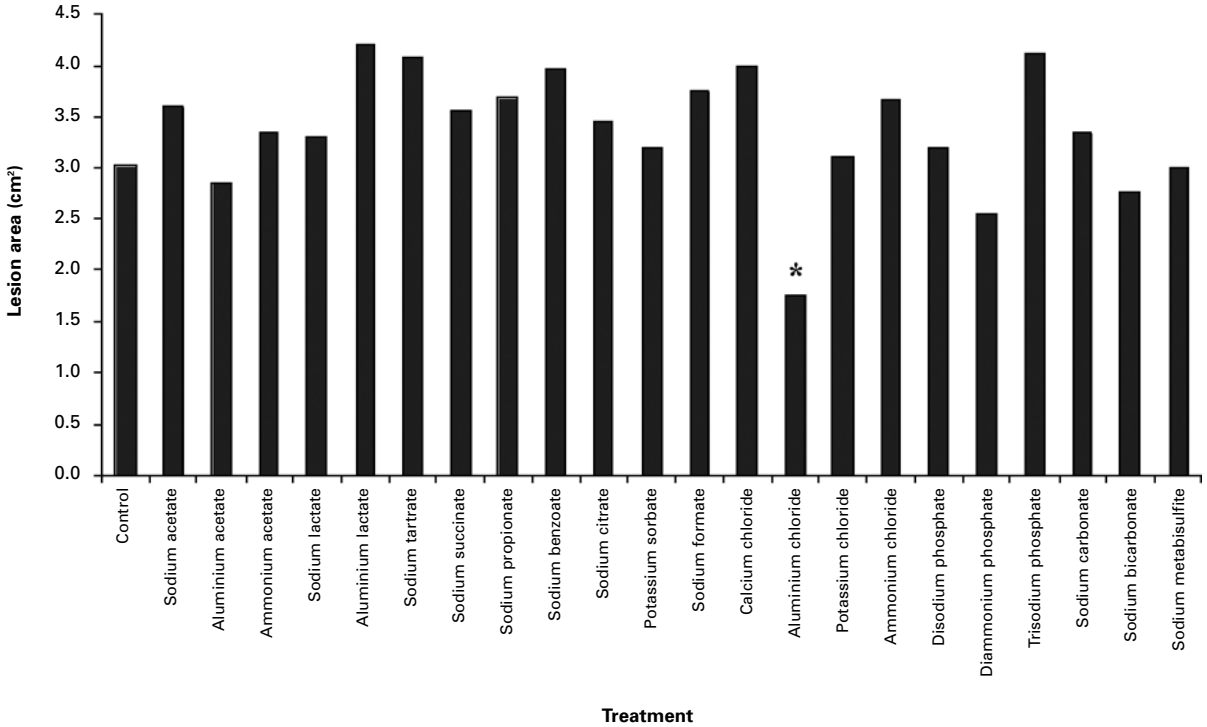

Figure 2. Effect of salts on the development of potato dry rot caused by *Fusarium solani* var. *coeruleum*. Tubers inoculated with *F. solani* var. *coeruleum* were dipped (10 min) in the different salt solutions (0.2 M) or in double-distilled water (control), and subsequently incubated in the dark at 24˚C. Disease severity was evaluated after 7 d of incubation. Each value represents the mean of three replicates. *Significantly different from the control ($P < 0.1$).

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