



Influence of the Potato Production System in the Soil Suppressiveness to Bacterial Wilt

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Authors' contributions

This work was carried out in collaboration between all authors. Authors JZ, CFRi and CAL designed the study, performed the statistical analysis, wrote the protocol, wrote and reviewed the manuscript. Authors FAP and PCTM managed all process of study. All authors read and approved the final manuscript.

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ABSTRACT

The potato crop is highly affected by soil-borne diseases motivating its continuous migration to non-cultivated areas. Bacterial wilt caused by *Ralstonia* spp. is one of the main diseases affecting the potato crop in Brazil, since the conventional production system, used throughout the country, promotes an ideal environment for proliferation of members of this pathogen complex. Studying alternative potato production systems aiming to improve the soil biological properties is necessary in order to avoid the continuous migration of the potato crop to new areas. The objective of this work was to evaluate different potato production systems, namely, the Paces an alternative potato production system and the organic potato production systems in contrast to the conventional production system, a soil from the Paces system sterilized through autoclaving and an undisturbed Atlantic rainforest soil regarding the soil suppressiveness to bacterial wilt caused by *R. solanacearum*. The experimental design was randomized blocks with three replications, arranged

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in a 4 x 4 + 1 factorial scheme, the main factor being the production system and the secondary factor corresponding to the doses of soil contaminated with *R. solanacearum* added to each treatment (0, 25, 50 and 75%). The additional treatment corresponded to the soil of the conventional system with high incidence of *R. solanacearum*. The soil suppressiveness to bacterial wilt is a biological process inhibited by autoclaving. The Paces and the organic potato production systems have a significant potential to suppress *R. solanacearum* in infested soil.

Keywords: *Solanum tuberosum* L.; *Ralstonia* spp; sustainable production; potato crop migration.

1. INTRODUCTION

Potato (*Solanum tuberosum* L) is the third most important food source in the world, following wheat and rice [1]. In Brazil, it is the most cultivated vegetable crop, with production of above 3.5 million tons in 2017, in an area of approximately 120,000 hectares [2]. The production chain generates about 500 thousand jobs and moves R\$ 1 billion year⁻¹ in Brazil [3].

Potato bacterial wilt (BW) in Brazil is caused by *Ralstonia solanacearum* (Rs), a member of the *Ralstonia* species complex. It is one of the main potato diseases in the world, especially in tropical and subtropical regions, which present the ideal environmental conditions for the proliferation and spread of the disease [4]. The pathogen invades the plant through the roots at emergence points of secondary roots and wounds caused by nematodes, insects and mechanical damage during cultural practices [5]. Upon reaching the xylem, the bacterium multiplies and compromises the water flow to the leaves, resulting in wilting and, later, death of the plant [6]. *Ralstonia solanacearum* presents high genetic diversity as indicated by Fegan and Prior [7] and Safni et al. [8] based on molecular studies.

The Rs species complex is composed of four phylotypes (I, II, III and IV) associated with geographical distribution among continents [9,10,11]. In Brazil, potato is affected only by *R. solanacearum* phylotype II, even though differences in virulence and survival have been detected among Brazilian isolates [10]. The pathogen is able to remain in the soil for a long term due to its saprophytic ability and its ability to survive as epiphyte within the rhizosphere. These characteristics associated with a wide range of host species make the control of BW very difficult [4,12].

Successive planting of potato in areas infested with *R. solanacearum* is economically and environmentally unfeasible due to the drastic loss

of production caused by BW along with other soil diseases caused by *Rizoctonia solani*, *Streptomyces* spp., *Pectobacterium* spp., *Meloidogyne* spp. and *Pratylenchus* spp. Thus, there is a continuous search for new areas, which are free of the pathogen, for potato cultivation [13]. Nevertheless, the necessity of pathogen-free new areas could be reduced through the adoption of cultural measures, such as those which enable suppression of the main soil-borne potato diseases [14].

In the conventional potato production system in Brazil, conventional tillage through plowing and/or disk-harrowing operations (reaching up to 20 cm depth) are used for soil preparation. In this system, two to five years without potato cultivation is necessary in a given area to reduce the soil pathogen inoculum. The need to wait for such a large period results in the search for new areas for potato cultivation, giving a "nomadic" aspect to the crop [15]. As a consequence, the conventional potato production system in Brazil is a big concern for the society as far as economical and environmental sustainability are concerned [16].

Since 1920, when the potato crop was introduced on a commercial scale in Brazil, the plowing and/or disk-harrowing in total area began to be used, favoring the formation of a compacted layer below the revolved layer (20 cm depth) due to the destruction of the soil structure and subsequent movement of clay particles, which are deposited below the revolved layer [17]. The mechanical resistance caused by subsoil compaction directly affects the root growth of potato and, consequently, the absorption of water and nutrients, making the plant less vigorous and more susceptible to the attack of pathogens. In addition, the compaction also makes water drainage inefficient, increasing the incidence of diseases favored by moisture, especially bacterial wilt, which presents a higher incidence and severity in flooding areas [18]. Therefore, the conventional potato production system used in Brazil needs to be reformulated

in order to provide chemical, physical and biological conditions of soil so that production is not limited by soil-borne diseases [19].

Among the systems that propose a more desirable sustainability for potato cultivation, the organic production aims at managing the natural environment, thus reducing the dependence on non-renewable energy sources and prioritizing the use of cultural and biological methods for pest and disease control. In this system, rotation / succession of crops is fundamental and the fertilization is based on the cultivation of crops used as green manures, application of cultural residues, organic composts and treated agroindustry residues [20,21].

The Paces (Designing Agriculture Committed with Sustainability) system is an experimental potato production system projected for preventing the nomadic characteristic of the crop in Brazil by maintaining a better equilibrium of chemical, biological and physical properties of the soil. Twelve cycles of rotation using this system were carried out in one and the same area in Piracicaba, São Paulo State, Brazil [15,22,23]. The Paces system is based on three principles: a) preparation of the soil to the depth of 70 cm, aiming to loosen soil compaction in depth; b) succession of crops with corn, which presents high production of aboveground and root biomass, and c) incorporation of biomass produced up to a depth of 40 cm. The deep tillage is carried out by means of machines designed specifically for the system in order to reach the depth of 70 cm for loosening the soil compaction and 40 cm for incorporating the corn residues before the planting of potato [19].

Both organic and Paces systems adopt succession (or rotation, in the case of the organic system) of crops and incorporation of plant residues aiming to increase the suppressive capacity of the soil. The suppressive effect of crops used in succession / rotation on soil-borne pathogens can be attributed to three aspects: a) the reduction of the pathogen reproduction through the use of a non-host species; b) the release of root exudates toxic to the pathogens and c) the improvement of soil microbial population through the supply of carbon [24].

The microbial population represents 70% of the living biomass of the soil and is composed of invertebrate organisms. The main function sought with the incorporation of organic material is the suppression of soil-borne pathogens,

through the increase of the population of beneficial bacteria and fungi that inhibit the action of the pathogenic organisms even in the presence of the susceptible host [25,26].

The term "soil suppressiveness" was introduced by Menzies [27], but it was popularized by Baker and Cook [28] and was defined as "the ability of the soil to keep the incidence or severity of a disease low even with the presence of the pathogen, susceptible plants and environmental conditions conducive to their development".

Several examples of naturally-occurring suppressiveness are reported in different soil and climate conditions. Therefore, suppressiveness can be considered a universal phenomenon, which can occur naturally or can be induced. Both can be rapidly altered according to soil microbial activity, which fluctuates according to the availability of nutrients, quantity and quality of plant residues and depth of the root exploration [29]. Suppressiveness depends not only on soil characteristics, but also on cultivated plants, crop sequence and crop management strategies. Generally, the soil suppressiveness to diseases requires active management for its maintenance [30].

In natural ecosystems, without human disturbance, the occurrence of diseases is rare compared to the conventional agricultural systems due to the greater biological diversity in the soil. In an experiment comparing conventional potato and eucalyptus cultivation to the natural Savannah, Silva et al. [31] reported a greater microbial activity, a higher content of total carbon and, consequently, a greater disease suppression capacity in the natural Savannah, where no soil management was carried out.

A comparison between organic and conventional systems carried out by Bruggen et al. [32] indicated a lower severity of soil-borne diseases in the organic system. According to the author, the higher suppressive potential observed in the organic system was related to the rotation of crops, the regular application of organic material in the soil and the absence or reduction in the application of agrochemicals, stimulating the natural biological control and avoiding imbalances in the microbiota. Results obtained by Gorissen et al. [33] indicate a reduction in the population of *R. solanacearum* biovar 2 and in the number of infected potato plants by adding 17 t ha⁻¹ of liquid pig manure in a compaction-free soil. Moreover, Montenegro-Coca et al. [34]

observed a reduction in the incidence of soil-borne diseases of potato with the application of chicken manure.

The objective of this work was to evaluate the soil suppressiveness to potato bacterial wilt in alternative potato production systems (namely Paces) and organic production systems in comparison to the conventional system, undisturbed soil under natural vegetation and sterilized soil.

2. MATERIALS AND METHODS

2.1 Experimental Area

The experiment was carried out at the Embrapa Vegetables facilities in Brasília, Federal District, Brazil, in a greenhouse with controlled temperature of 18-40°C throughout the experiment, from July to November, 2018.

2.2 Experimental Design

A randomized block design was used with three replications and a factorial scheme 4 x 4 + 1. The main factor was the production system, the secondary factor corresponded to the dose of soil contaminated with *R. solanacearum* added to each treatment (0, 25, 50 and 75%) and the additional treatment corresponded to the soil of the conventional potato production system with high incidence of *R. solanacearum*.

2.3 Characterization of Treatments

Soil samples that comprised the treatments were collected from four areas corresponding to different potato production systems, as follows: a) soil from a conventional system with more than 20 years of potato cultivation in Brazilian Savannah biome, in Brasília, Federal District, historically presenting a high incidence of the bacterial wilt pathogen, identified as *Ralstonia solanacearum* race 1, biovar 1, phylotype II through biochemical tests and multiplex PCR analysis, performed at Embrapa Vegetables [35]. The soil was classified as Oxisol; b) soil from an area under organic production for more than 15 years with the same edapho-climatic conditions and soil classification of the conventional system; c) soil from an undisturbed area with native vegetation in the Brazilian Atlantic rainforest biome from the city of Piracicaba, São Paulo State and d) an area cultivated with potato for 12 years through the Paces alternative potato production system, contiguous to the previously mentioned undisturbed Atlantic rainforest area, in

Piracicaba. Both the systems “c” and “d” have the soil classified as Ultisol. Additionally, a replication of the samples collected in the Paces alternative potato production system was sterilized by autoclaving (60 min at 121°C) and comprised the treatment “e” named “sterilized soil”. The samples from treatments “b”, “c”, “d” and “e” were mixed to the soil “a” at doses of 0, 25, 50 and 75% soil “a” in order to evaluate suppressiveness at different doses of the pathogen.

2.4 Collecting of Soil

The soil samples were collected manually with the aid of spades and shovels and were placed in permeable plastic bags and stored in a ventilated place without direct incidence of sunlight, in order to keep temperature and humidity stable. The samples collected in Piracicaba (treatments “c”, “d” and “e”) were transported to Brasília, Federal District, one day after collection, remaining stored for five days until the collection of soils of treatments “a” and “b” as well as the autoclaving of the treatment “e”.

2.5 Conduction and Evaluation of the Experiment

In order to evaluate the soil suppressiveness, the methodology of Cardoso and Echandi [36] was followed with modifications for inclusion of the different doses of soil contaminated with *R. solanacearum*. Plastic trays (0,40 x 0,20 x 0,15 meters) with capacity for 8 liters were used as containers for transplanting 8 sprouted potato tubers of the cultivar Agata with about 30 mm diameter. Each tray corresponded to an experimental unit.

Irrigation was carried out daily in order to maintain the soil with high humidity. The percentage of diseased plants was evaluated every five days from the appearance of symptoms, at 25 days after planting (DAP), until the stabilization of the number of symptomatic plants (50 DAP).

2.6 Statistical Analysis

Percentage data were transformed into $\arcsin(\sqrt{x})$. Then, the analysis of variance and regression were carried out in order to establish the dose of the mixture between evaluated and contaminated soil that best expressed the

suppressive capacity of each soil. Using the most representative dose, analysis of variance and comparison of means by contrasts among the five treatments were carried out.

3. RESULTS AND DISCUSSION

The number of wilted plants increased homogeneously among the treatments, accelerated especially after 40 days after planting (DAP) (Fig. 1), when the potato plantlets became totally independent of the seed tuber (35 to 42 DAP), after initial root development. The presence of wilted plants was enhanced after root contact with the pathogen in the soil [4]. The average percentage of wilted plants ranged from 0 to 72.2% considering all treatments.

A significant interaction between the treatments and the doses was verified as indicated by

regression curves built for each treatment. All treatments presented linear adjustment, indicating that the incidence of bacterial wilt increased proportionally to the doses of infested soil used (Fig. 2). Similar results were found by Costa and Costa [37] and Reis et al. [38].

A significant effect of soil treatments was verified only at the dose of 75% of infested soil. For this reason, the comparison of means by contrasts was carried out among treatments at this dose. This dose was established by presenting statistically significant difference in the test, and it may be considered the ideal dose for the study of the behavior of the different treatments in relation to the suppression to BW. The conventional system presented the highest incidence of BW (Fig. 3) and was distinguished from all but sterilized Paces soil.

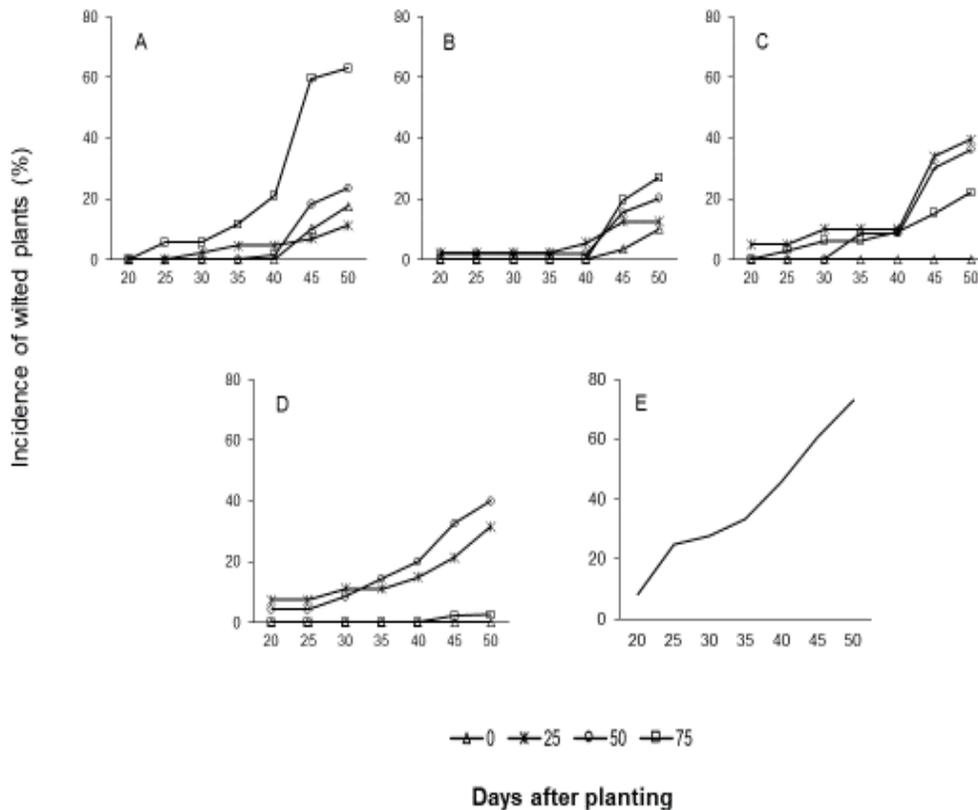


Fig. 1. Bacterial wilt progress curves in potato cultivar Agata cultivated in five soil treatments, namely sterilized soil (A), organic potato production system (B), undisturbed forest (C), Paces alternative potato production system (D) and conventional system contaminated with *Ralstonia solanacearum* (E). The values from 0 to 75 correspond to the percentage of soil contaminated in mixture with the studied soil treatment

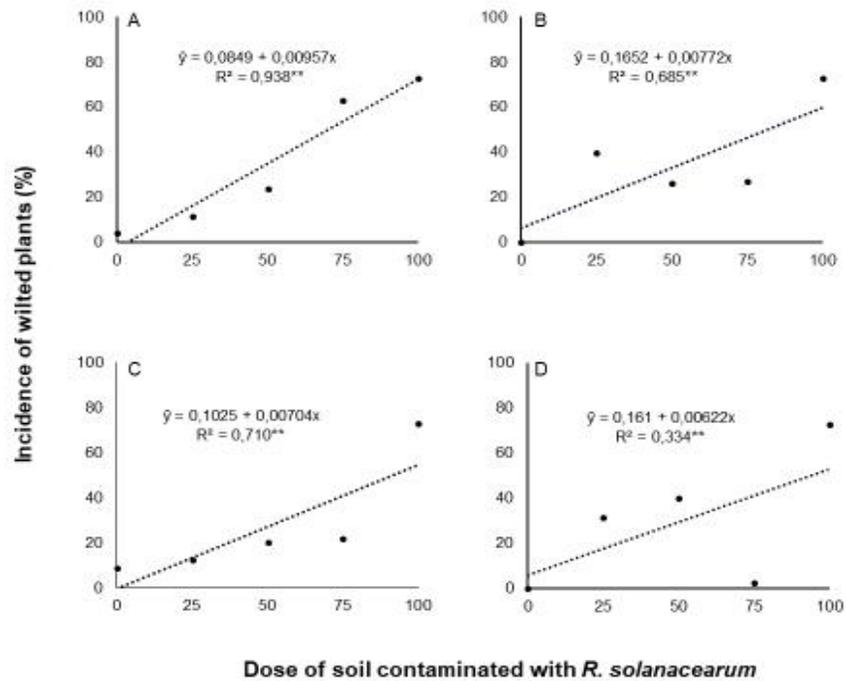


Fig. 2. Incidence of wilted plants of potato cultivar Agata as a function of the dose of contaminated soil used in mixture with four soil treatments, namely sterilized soil (A), organic potato production system (B), undisturbed forest (C) and Paces alternative potato production system (D). Coefficient of variation of 63.32%

NOTE: The dose 100 corresponds the treatment containing only the conventional production system with *Ralstonia solanacearum* contamination

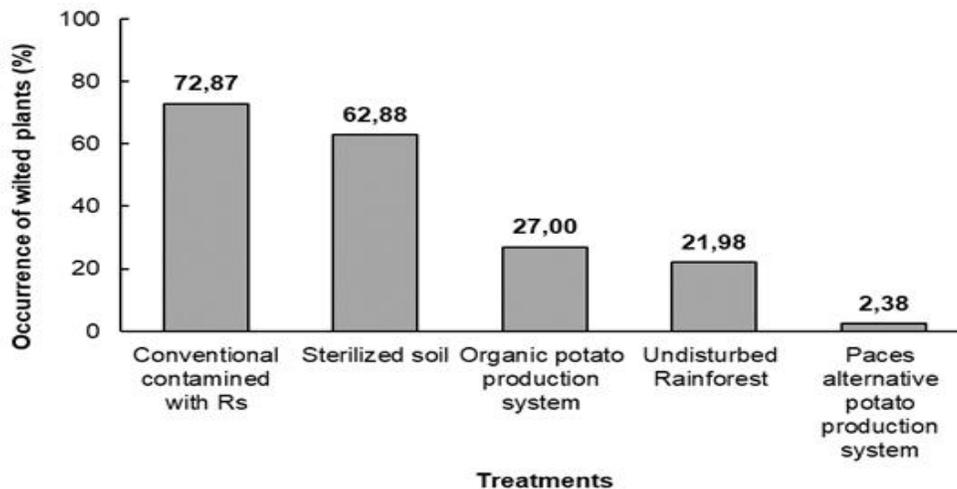


Fig. 3. Incidence of plants with bacterial wilt on potato cv. Agata under different treatments

Note: P = .005

The results suggest that the suppressiveness is only attainable due to soil microbiota, a fact already described in the literature by [39,40,41,42,43]. Thus, undisturbed forest, organic and Paces alternative potato production systems presented biological conditions more

favorable to the suppression of bacterial wilt than the conventional production system.

Lantz et al. [40] compared the biological activity of the soil in the southwestern Amazon under four different soil management, namely native forest, pasture, deforested native vegetation and agriculture, they showing a greater biological activity for the native forest soil. Ferreira et al. [44] found that in undisturbed soils the microbial population exists in perfect dynamic equilibrium, so the native environments are the best models of suppressive soils. In the present study, the occurrence of wilted plants in the treatment containing undisturbed forest soil was not significantly different from the organic and the Paces systems (Table 1). Therefore, both treatments have potential suppressiveness to *R. solanacearum*.

The higher incidence of bacterial wilt in the conventional system in comparison to the organic system was possibly due to the different use of agricultural inputs. In the conventional system, fertilization is restricted to inorganic fertilizers and the control of diseases, pests and weeds relies upon synthetic products. In the organic system studied, fertilization is carried out with organic compost [45], magnesium thermophosphate and Bokashi [46] and no phytosanitary control is carried out except for mechanical weed control. According to D'andrea et al. [47], the microbial biomass is intensified in the presence of organic fertilizers in comparison to the inorganic ones, due to the increase in the proportion of labile carbon and nitrogen added to the soil.

As examples of inputs usually carried out in conventional production systems with influence in soil microbial activity, Vivian et al. [48] evaluated the influence of the sulfametrazone herbicide on the microbial activity in an Oxisol and verified toxic action of the herbicide in the soil microbial community. In a similar way, when evaluating the influence of the residual effect of the herbicide sulfametrazone on the microbiological activity, Galon et al. [49] observed a decrease in the metabolic quotient of the soil as a function of time, concluding that the toxic effect of the herbicide is able to alter the long-term soil microbiota. Tironi et al. [50] reported a decrease in soil respiratory rate as a function of the application of an herbicide of the chemical group Sulfonylurea compared to a treatment without herbicide application. Dallman et al. [51] evaluated the influence of the application of glyphosate herbicide at doses of 0, 0,960 and 1,920 kg active ingredient ha⁻¹ on the production of CO₂ and soil microbial biomass, and concluded that the higher the herbicide dose, the lower was the quality of the soil. The same result was found by Braga et al. [52] when evaluating the herbicides paraquat, fomasafen and fluzifop-p-butyl. Thus, as the action of synthetic agricultural inputs adversely affects the microbial activity of the soil, possibly the greater suppressiveness of the organic system is related to the greater use of organic inputs and the absence of synthetic products such as herbicides and fungicides. Besides that, Fontenelle et al. [53] when evaluating the addition of organic fertilizer "Bokashi", used in the organic system, deduced that the addition of this organic compost is the main suppressing agent to *R. solanacearum*.

Table 1. Contrasts between different soil management for the variable incidence of bacterial wilt in potato cultivar Agata

	Conventional potato production contaminated with Rs	Sterilized soil	Undisturbed forest	Organic potato production system
Sterilized soil	0,3333			
Undisturbed Forest	0,0004**	0,0020**		
Organic potato production system	0,0009**	0,0046**	0,6223	
Paces alternative potato production system	0,0001**	0,0001**	0,0753	0,0318*
C.V. (%)	32,71			

Note: *P = .044 **P < .001

The Paces system presented a lower occurrence of plants with bacterial wilt symptoms than the conventional and organic systems. Possibly, this was due to the incorporation of organic material in depth, which activates the soil microbiota. In order to demonstrate the positive correlation between organic matter addition in soil and biological activity, Silva et al. [54] evaluated the addition of different sources of organic material to the soil and found a higher respiration rate and soil metabolic quotient for treatments with organic residues addition in comparison to the control without addition of plant material.

Ragassi et al. [19] did not observe incidence of bacterial wilt in the Paces System, attributing the cause to the management adopted. In addition, Zucolotto et al. [55], when evaluating the respirometric activity for the soil layers from 0 to 20 and 20 to 40 cm in the Paces system compared to the conventional system, observed a higher average of the microbiota activity in the Paces system, the CO₂ production by the organisms of the soil being significantly higher in the 20 to 40 cm layer. This factor possibly gave the Paces system a greater potential for suppression of diseases when compared to the treatments without deep soil tillage.

4. CONCLUSION

The soil suppressiveness to potato bacterial wilt can be considered a biological process highly dependent on the soil management adopted. Both Paces alternative potato production system and organic potato production system have a potential of suppressing potato bacterial wilt.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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