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Kouamé Kohi Alfred1,2*, Yapi Yapi Eric1,3, N’Cho Mathias2, Bouatenin Koffi Maïzan Jean-Paul2, Coulibaly Wahauwoulé Hermann4, W.A.Mireille Alloue-Boraud2 and Djé Koffi Marcellin2

Abstract: The aim this work was to determine some biochemical and organoleptic characteristics of the juices obtained after mango preservation using bacterial biopesticides. For this purpose, a sample of 12 Brooks mangoes collected at the fruit quay of Adjamé (Abidjan, Côte d’Ivoire) was divided into 3 batches of 4 mangoes and used for the preservation test. Two batches of mangoes were preserved respectively with Bacillus subtilis GA1 and Bacillus sp while the other batch served as a control. After 15 days of storage at room temperature, the mangoes were transformed into juice and were subjected to microbiological and physicochemical analyses to demonstrate the effect of the biopesticides. The results of the physicochemical analyses revealed that the juices had a pH between 4.86 and 5.34, a titratable acidity between 0.81 and 1.17% and a brix degree between 9.25 and 11.75. In terms of biochemical parameters, the contents of ash, protein, total sugars and reducing sugars were respectively between 0.24% ± 0.05 and 0.32% ± 0.17; 2.58% ± 0.74 and 3.06% ± 1.44; 56.48% ± 6.62 and 70.6% ± 3.68 and 22.28% ± 3.95 and 29.28% ± 3.8. From the mango juices obtained the presence of Mesophilic Aerobic Germs (MAG), fungi and coliforms was recorded with the highest loads being respectively 2.8.103 CFU/mL, 3.1.102 CFU/mL and 5.1.101 CFU/mL. The use of bacterial biopesticides in mango preservation has little influence on the biochemical and organoleptic characteristics and preserves the sanitary quality of mangoes.

Subjects: Microbiology; Biotechnology; Food Biotechnology

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PUBLIC INTEREST STATEMENT
Mangoes are nutrient-rich fruits. However, they are climacteric fruits that deteriorate rapidly. Several methods of preservation of these fruits (chemical, pesticides) are used but these methods pose a public health problem. The use of certain microorganisms as bio-pesticides is necessary. This study highlights the impact of bio-pesticide on the quality of mangoes during storage.
Keywords: mango; biopesticides; Bacillus subtilis GA1; Bacillus sp; nutritional and organoleptic quality

1. Introduction
The mango (Mangifera indica) is the most consumed tropical fruit after the banana. It is cultivated in many tropical and subtropical countries. The world production of mangoes was estimated at more than 33 million tons in 2007 (Gagnon, 2007). According to FAOSTAT, Nigeria, Kenya and Tanzania are the largest mango producing countries in Africa mainly for their domestic market and export to neighboring countries. Nigeria is the leading producer in the subregion and alone accounts for 56% of ECOWAS production. In Côte d’Ivoire, production exceeds 100,000 tons per year (PACIR, 2013). Mango is the third most important export fruit after banana and pineapple (K. M. Koffi, 2000). In addition, Côte d’Ivoire is the first African mango exporting country and the third largest supplier to the European market after Brazil (65,000 t) and Peru (29,000 t) (Gerbaud, 2007). In Côte d’Ivoire, the main mango production region is located in the north of Côte d’Ivoire, in the Poro region (Braz, 2004). The marketing of mangoes has played an important role in the economic development of this region, which until then had focused mainly on the cultivation of cotton and cashew nuts. Mango production was previously intended for local consumption, but today it is resolutely oriented towards export. In 1999, Côte d’Ivoire was the 2nd supplier to the European market with more than 10,200 tons of mangoes exported (Koffi, 2000). However, there has been a decline in mango production at the local level due to competition from other markets and post-harvest losses caused by fungal diseases including anthracnose, particularly noticed in 2007 on exported fruit (Gerbaud, 2008). Post harvest infection depreciates the marketable quality of mangoes during marketing (Diedhiou et al., 2007). To mitigate this problem, several conservation techniques, including modified atmospheres, the addition of antioxidants (ascorbic acid, citric acid) and firming agents (calcium) have been developed (Tassadit, 2012). However, these techniques do not really satisfy the producers who rather use synthetic pesticides. Yet, the use of chemical pesticides in mango preservation could have adverse effects on the environment and consumer health. Research was then conducted in the direction of biological control. Indeed, the provision of biological material to producers to protect and preserve the fruit without having side effects on the consumer and the environment would be an adequate solution for the scientific and agricultural community. In this context, studies have been carried out on pineapple and mango in particular. Indeed, the work of Koffi et al. (2017a) has made it possible to preserve pineapple over a period of two weeks (15 days) using compound bacterial biopesticides. Also, Koffi et al. (2017a) were able to preserve mango over a 15-day period with Bacillus subtilis GA1. In spite of all the advantages of biological control, the organoleptic and nutritional characteristics of the preserved fruit could be altered. This could be a real obstacle to the marketing, processing and consumption of these fruits. In fact, previous studies have focused on the preservation and without considering their influence on the organoleptic characteristics.

The present study was carried out with the aim of highlighting the influence of biopesticides on some organoleptic and biochemical characteristics of mangoes.

2. Materials and methods

2.1. Biological materials
Sixty samples of unaltered mango fruits, Brooks variety, were collected from fruit markets of Abidjan Plateau, Cote d’Ivoire. A purified and well identified B. subtilis strain GA1, Bacillus sp, respectively from the collection of Walloon Center of Industrial Biology (CWBI), University of Liege Gembloux AgroBio Tech (Belgium) and the collection of the Laboratory of Biotechnology and food Microbiology, University of Nangui Abrogoua, Abidjan, Côte d’Ivoire was used for different tests. Mangoes were obtained from a commercial warehouse in Abidjan-Plateau, Côte d’Ivoire.
2.2. Microbial inocula preparation for in vivo assays
The production of bacterial biopesticide supernatant was carried out according to the method of Koffi et al. 2017b. Sixty milliliters (60 mL) of yeast extract-peptone-dextrose (YPD) medium contained in two Erlenmeyer flask were each inoculated with an 18-hour old pre-culture of Bacillus sp and Bacillus subtilis GA1. These cultures were incubated at 30°C for 8 hours under 105 rpm agitation. At the end of incubation the bacterial load in each flask was 107 CFU/ml. The cultures were used to inoculate 340 mL of YPG medium contained in 1-liter Erlenmeyer flasks. The media inoculated with the cultures were incubated at 30°C under 100 rpm agitation for 48 hours. After incubation, both media were centrifuged at 6000 rpm for 10 minutes and the supernatants were collected in sterile jars and stored at 4°C for the 3 conservation tests.

2.3. Conservation of mangoes by immersion
The mango preservation test by immersion in the supernatant was carried out according to the technique described by Cissé (2012). This technique consists of immersing the mangoes in the supernatant contained in containers. To do this, the mangoes were carefully washed with tap water and rinsed three times with sterile distilled water. They were then disinfected using toilet paper soaked in ethanol at 70°C and divided into three batches of twenty (20) mangoes. The first batch of mangoes was immersed for 3 minutes in the supernatant of Bacillus subtilis GA1, the second batch immersed in the supernatant of Bacillus sp and the third batch was untreated. After immersion, the mangoes were stored at room temperature in sterile, perforated cartons for 15 days.

2.4. Mango juice production
After 15 days of storage, the mangoes from each batch were sorted, washed with tap water containing liquid soap to which a few drops of NaCl (sodium hypochlorite) were added. The washed mangoes were peeled and cut into small pieces using a sterile, stainless steel knife. The mango pulp was ground in a Binatone blender (Binatore Industries Ltd. London SE10 OER Mill BLG-555). The resulting shredded material was collected in stomacher bags. In order to obtain mango juice, the grind was pressed and filtered through a sieve and a white. The mango juice was formulated with 20% mash and 80% water then centrifuged and filtered with a clean cloth. This formulation was in accordance with international legislation since the name of the fruity water was therefore attributed to beverages whose fruit content was between 12% and 20%. The juice of each batch of mangoes contained in 1.5 liter bottles was pasteurized at 70°C for 10 minutes to eliminate all vegetative forms of microorganisms. Then, these juices were cooled and stored at 4°C in these bottles for the rest of the study.

2.5. Analytical determinations
Value of the pH was determined with a pH-meter (Hanna Instruments; HI 8010) after calibration with KCl buffer. Titratatable acidity was determined through titration with 0.1N NaOH. The titratatable acidity was expressed as% meq of lactic acid. Density of each juice was determined by using of densimeter (Mettler Toledo). The total soluble solids, expressed as °Brix value were determined in using a hand refractometer (Atago N-20E, Japan) (Kasse, 2015). Two independent measurements were made on each sample. The percentage of moisture was determined using the methods of (Djeni et al., 2011). The method used for the determination of ash content is that described by AOAC (1990) which consists of incinerating a sample until white ash is obtained. Thus, 5 g of mango juice was placed in an incineration capsule of mass m0. Then, the whole was placed in a muffle furnace (BioBase WGL-125B) with automatic regulation and incinerated at 550 ± 15°C for 12 hours. The capsule is then removed and weighed after cooling in the desiccator. The total protein was determined from the total nitrogen determination according to the AOAC kjeldhal method (AOAC, 1990). It consists of a mineralization phase, followed by a distillation phase and a sulphuric acid titration phase. while the reducing sugars were quantified as described by Djeni et al. (2011) and expressed in mg/100 g of fresh matter. Water-soluble carbohydrates were determined by the phenol sulphuric acid method according Djeni et al. (2011) and the values were expressed in g/100 g of fresh matter. Preparation of stock solutions, inoculation of agar plates, cultivation and quantification of microorganisms were carried out according to Djeni et al. (2011). Aerobic mesophiles were enumerated on plates of Plate Count Agar (PCA Oxoid LTD,
Basingstore, Hampshire, UK) and incubated at 30 °C for 2 days. Enumeration of total coliforms was carried out using plates of Violet Red Bile Lactose agar (VRBL, Merck 10,660, Merck, Darmstadt, Germany) which were incubated for 24 h at 30 °C for total coliforms. Yeasts were enumerated on plates of Sabouraud-chloramphenicol agar (Fluka, Biochemica 89,579; Sigma-Aldrich Chemie GmbH, SaintLouis, MO, USA) incubated at 30°C for 2 days.

2.6. Statistical analysis
Statistical analyses of the results obtained were carried out using Excel 2013 software for the calculation of means and standard deviations. On the other hand, the R software was used to perform the analyses of variance with one factor (ANOVA 1) to highlight the statistical differences between the means obtained.

Figure 1. Internal and external appearance of canned mangoes.
(A: with Bacillus sp.; B: with Bacillus subtilis GA 1, C: control mango)
3. Results

3.1. Mango conservation
The results obtained after mangoes preserved with the supernatants of the biopesticides are shown in Figure 1. After 15 days of storage, the fruit showed spots on the outside without affecting the quality of the pulp. On the other hand, control mangoes that were not preserved or inoculated with biopesticide supernatants showed signs of alteration from the 7th day of storage, resulting in loss of pulp coloring.

3.2. Physico-chemical parameters of mango juices
The physico-chemical parameters of the analyzed juices have been grouped in Table 1. The mango juices analyzed are characterized by an acid pH ranging from 4.86 ± 0.17 to 5.34 ± 0.04. The pH of the juice obtained from mango preserved with Bacillus subtilis GA1 is statistically different from that of the other two juices. The titratable acidity and dry matter content of the juices did not show a significant difference at the 5% threshold with values ranging from 0.81% ± 0.05 to 1.17%± 0.05; 11.97%± 0.23 and 13.23%± 1.63 respectively. However, statistical analyses revealed a significant difference between the brix degree of the control juice and that of the mango juice preserved with Bacillus sp. However, no difference was observed between the brix degree of the mango juice preserved with the supernatant of Bacillus subtilis GA1, and that of the control batch of mango.

On the same line the numerical values with the same alphabetical letters are not statistically different at the 5% threshold.

3.3. Biochemical parameters of mango juices
Table 2 shows the different levels of ash, protein, reducing sugars and total sugars in the mango juice samples preserved with the bacterial biopesticides and the unpreserved control mangoes. The highest ash content (0.32%) was observed in the juice of the control mango, and the lowest in the juice of mangoes preserved with Bacillus subtilis GA1 (0.19%). However, no significant difference (p>0.05) was observed between the different ash contents of the different mango juice samples.

Protein levels in the mango juices ranged from 2.58% to 3.06%. The control mango sample appears to be the richest in protein 3.06%. The mango juices preserved with Bacillus sp (2.95%)

| Table 1. Physico-chemical parameters of mango juices |
|-----------------------------------------------------|
| physico-chimical parameters | Mango control | Mango preserved with Bacillus subtilis GA1 | Mango preserved with Bacillus sp. |
| pH                              | 4.86 ± 0.17\textsuperscript{a} | 5.34 ± 0.04\textsuperscript{b} | 5.02 ± 0.05\textsuperscript{a} |
| Titratable acidity (%)          | 1.17 ± 0.05 \textsuperscript{a} | 0.81 ± 0.05 \textsuperscript{a} | 0.9 ± 0.1\textsuperscript{a} |
| Dry matter (%)                  | 11.97 ± 0.23 \textsuperscript{a} | 13.23 ± 1.63 \textsuperscript{a} | 13.03 ± 0.16 \textsuperscript{a} |
| Brix degree                     | 11.75 ± 0.39 \textsuperscript{b} | 10.5 ± 0.7 \textsuperscript{ab} | 9.25 ± 0.35 \textsuperscript{a} |

| Table 2. Biochemical parameters of mango juices |
|------------------------------------------------|
| Biochemical parameters | Mango control | Mango preserved with Bacillus sp | Mango preserved with Bacillus subtilis (GA1) |
| Ash (%)                | 0.32 ± 0.17 \textsuperscript{a} | 0.24 ± 0.05 \textsuperscript{a} | 0.29 ± 0.09 \textsuperscript{a} |
| Proteins (%)           | 3.06 ± 1.44 \textsuperscript{a} | 2.95 ± 0.77 \textsuperscript{a} | 2.58 ± 0.74 \textsuperscript{a} |
| Reducing sugars (%)    | 29.28 ± 3.8 \textsuperscript{b} | 22.28 ± 3.95 \textsuperscript{a} | 25.95 ± 2.23 \textsuperscript{ab} |
| Total sugars (%)       | 70.6 ± 3.68 \textsuperscript{b} | 56.48 ± 6.62 \textsuperscript{a} | 58.31 ± 7.48 \textsuperscript{a} |
and *Bacillus subtilis* GA1 (2.58%) were lower in protein. However, no significant difference (*p* > 0.05) was observed between the protein contents of the different mango juice samples.

The levels of reducing sugars and total sugars ranged from 22.28(%) ± 3.95 to 29.28(%) ± 3.8 and 56.48(%) ± 6.62 to 70.6(%) ± 3.68 respectively. Juice from the control mangoes had the highest levels of reducing sugars (29.28 ± 3.8) and total sugars (70.6 ± 3.68). In general, significant differences were observed between the levels of reducing and total sugars in the juice obtained after mango storage and the juice obtained from stored mangoes (control).

In one line, the mean values followed by a different alphabetical letter are statistically different (*P* = 0.05) (DUNCAN multiple t-test).

### 3.4. Microbiological parameters of mango juices

Microbiological analyses of mango juices revealed the presence of mesophilic aerobic germs (MAG), the highest loads of which were observed in juices obtained from mangoes preserved with biopesticides. The loadings are respectively 6.5.10³ CFU/mL for AMG, 3.1.10² CFU/mL for yeast moulds and 5.1.10¹ CFU/mL for coliforms (Table 3).

### 4. Discussion

Mangoes are fruits that are highly vulnerable to microbial contamination from the harvesting phase to processing and storage. Indeed, according to Gerbaud (2008), the decline in production observed is due to competition from other markets and, primarily, to the quality of mangoes, which has been greatly depreciated by the presence of round spots characteristic of fungal diseases, particularly anthracnose, which was particularly noticeable in 2007 on exported fruit. Also, Diedhiou et al. (2007) indicated that post-harvest infection depreciates the presentation quality of mangoes at the time of marketing. These fungal activities can also lead to mycotoxin contamination, and could represent a health risk for consumers (Koffi-Nevry & Gohou, 2011).

Based on this analysis, it is therefore necessary to limit or even inhibit the action of these pathogenic germs. The use of biopesticides in this study showed the capacity of these agents to inhibit the main fungal or bacterial strains responsible for the deterioration of mangoes. The good conservation of mangoes over 15 days by the supernatant of *Bacillus subtilis* GA1 and *Bacillus* sp is explained by the presence of substances produced by the microorganisms during their metabolism in their respective growth media. Indeed, the presence of these substances would more or less limit the establishment of pathogenic germs on the mango skin. The production of molecules of a lipopeptide nature by *Bacillus subtilis*, notably fengycine, surfactins and iturins, would promote the bursting of the cell wall of fungi (Ongen'a, 2014). These results are in agreement with those of Koffi et al., (2017a) on the conservation of Kent mangoes with *Bacillus subtilis* GA1. However, the signs of deterioration observed on mangoes after 15 days of storage could be related to the presence of natural mango flora. According to Koffi (2018), this natural flora adapts more easily to the product unlike biopesticides which need time to adapt in order to optimize their fruit preservation activities. Thus Koffi (2018), has obtained the same protection both in the fruits treated with the culture supernatant and with the cells themselves. The mango

### Table 3. Microbial loads (CFU/mL) of mango juices

|                     | MAG                  | Yeasts and moulds     | Coliforms          |
|---------------------|----------------------|-----------------------|--------------------|
| Mango preserved with *Bacillus sp* | (6.5 ± 2.1).10³<sup>a</sup> | (1.2 ± 0.6).10³<sup>a</sup> | (5.2 ± 3.3).10<sup>3</sup> |
| (Mango control)     | (3.4 ± 1.5).10³<sup>b</sup> | (9 ± 5).10³<sup>b</sup> | (1.1 ± 0.2).10<sup>3</sup> |
| Mango preserved with *Bacillus subtilis* GA1 | (5.6 ± 2.3).10³<sup>a</sup> | (3.1 ± 1.8).10<sup>2</sup> | (2.4 ± 2.1).10<sup>3</sup> |

Notes: In one column, the mean values followed by a different alphabetical letter are statistically different (*P* = 0.05) (DUNCAN multiple (t-test))., MAG: mesophilic aerobic germs.
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Juices analyzed are characterized by low dry matter content. This could be explained by the fact that most tropical fruits would contain high moisture content. This was confirmed by Koffi (2018), who obtained moisture contents of 83–85% after an analysis of pineapple produced in Côte d’Ivoire. According to CNUCED (2016), the moisture content of mango juice is 86%, indicating a low dry matter content.

The mango juices analyzed have an acidic pH. This could indicate the presence of several organic acids in the mangoes. The more acidic nature of the juice obtained from untreated mango would be explained by the fact that this mango is more vulnerable to contamination by natural microflora. Thus, during their metabolism, the microorganisms will use the sugars and transform them into acid, promoting a better acidity of the environment. Unlike treated mangoes, the microorganisms do not have easy access to the mango to cause its alteration because of the bacterial biopesticide.

The titratable acidity values of mango juices range from 0.81% to 1.17%. These acidity levels are close to those of Belem et al. (2017) who after a physico-chemical analysis of Brooks mango obtained an acidity of 1.03 ± 0.99%. Total acidity contributes to the sanitary quality of food by limiting the development of spoilage and/or pathogenic microorganisms. The work of Kameni et al. (2003) revealed a titratable acidity content of 17.9% for the Amelie mango variety. The difference observed between the contents of these two mango varieties could reflect a variation in acidity according to the varieties, the stage of maturity of the fruit and the elimination of certain volatile acids during storage.

The mango juices analyzed have low ash contents. The presence of ash in a fruit reflects its richness in mineral elements, particularly micronutrients. These ash contents are significantly higher than those obtained in the work of Koffi (2018). Indeed, this author after measuring the ash contents in pineapple samples obtained contents between 0.2% and 0.28%. However, our results are different from those obtained by Millogo Dè Pierre (2011). His studies revealed an ash content between 2.04 and 2.54% on oven-dried mangoes. This difference could be explained by the fact that drying causes a condensation of minerals in the food when leaving from a fresh to a dry state. The mango juice samples analyzed have protein contents higher than those found in the literature (0.2 to 0.4%) (CNUCED, 2016). These results could be due to the nature of the fruit (maturity, variety …). Indeed, according to Hassan and Othman (2011), fruits are very low in protein. Mango, like most fruits is very low in protein but contains bromelain, a glycoprotein with protease activity commonly used in the food industry. These high protein levels are believed to be the result of the activity of microorganisms. Indeed, according to Onifade and Agboola (2003), the increase in protein content in foods could be related to the activity of microorganisms that synthesize several enzymatic proteins; thus increasing the protein content of these foods. Also, Musa et al. (2010) and Eshun et al. (2013) reported that the protein richness of foods could be due to varietal differences.

Juices from unpreserved mangoes had higher levels of reducing and total sugars than juices from preserved mangoes. This could be attributed to the storage temperature of the mangoes. Indeed, according to Koffi et al., (2017a), the storage temperature would favour the reduction of sugar content in the fruit when the storage time is extended. This difference could also be explained by varietal selection and the ripening stage of the fruit. Our results are close to those obtained by Koffi (2018) on pineapple analysis. The latter had found a reducing sugar rate from 20.70% to 26.79% and a total sugar rate from 59.42% to 62.32%. Microbiological analysis of these fruit juices showed lower loads of coliforms, yeasts and moulds and aerobic mesophilic germs. The microbial load values obtained in this study were contrary to those obtained by several other authors who have worked on fruit juices. Indeed, Kaddumukasa et al. (2016) obtained loads of more than 104 CFU/mL in coliforms, (5.95.102–6.3.108 CFU/mL) in presumed pathogenic staphylococci in pineapple, mango and passion fruit juices in Kampala (Uganda). In addition, work by Bello et al. (2014) on locally produced orange, pineapple, grape and papaya
juices sold on the streets in Nigeria showed loads ranging from $1.5\times10^4$–$4\times10^4$ CFU/mL, $1\times10^4$–$3.5\times10^7$ CFU/mL and $2\times10^4$–$4\times10^4$ CFU/mL in total coliforms, staphylococcus and yeast moulds respectively. According to the Food Safety Division of Luxembourg (2015), the yeast and mold load in pasteurized fruit juices must not exceed $m = 10^2$ CFU/g or mL and $M = 10^3$ CFU/g or mL (Quebec Standard, 2009). These results can be explained by the fact that these fruit juices have been pasteurized. Indeed, pasteurization makes it possible to destroy a certain number of microorganisms including the vegetative forms. The work of Baba-Moussa et al. (2006) has indeed shown that foods that do not require cooking during manufacturing such as fruit juices had high microbial loads.

The presence of these moulds in the fruit juices analyzed thus indicates a potential hazard for consumers. Indeed, these molds are capable of secreting toxins including aflatoxin (Aspergillus) and patulin (Penicillium) harmful to humans (Carballo-Pacheco et al., 2018). The mango juices analyzed have a low coliform load. Such a value could be explained by the action of pasteurization. N’Zi (2018) in his work on the microbiological quality of pineapple and orange juices indicated that the presence of coliforms in the analyzed juices is indicative of fecal contamination or the water supply probably contaminated (well water, rainwater . . .) for cleaning utensils. Also, the bottles used for packaging, the method of production and non-compliance with hygiene rules are all factors that would lead to contamination and increase of microorganisms in these products (Tambekar et al., 2009).

The high load of mesophilic aerobic germs in mango juice samples compared to coliforms, moulds and yeasts could be explained by the fact that these germs are present throughout the immediate handling laboratory environment with ease of growth when the environment is favourable ($T = 25$–$30 \degree C$).

5. Conclusion
The present study was carried out with the aim of highlighting the influence of Bacillus strains used as biopesticides on some organoleptic and biochemical characteristics of mango juices. These fruits were well preserved in the presence of biopesticides over a period of 15 days. The physicochemical analyses revealed that the juices produced had an acid pH, low dry matter content and titratable acidity. These juices had more than 2% protein and also contained a high content of total sugars (more than 56%). The mineral richness of these juices was characterized by ash levels between 0.24% and 0.32%. The presence of mesophilic aerobic germs, coliforms and yeasts and moulds was reported in each juice but at lower loads. It could be said that the use of bacterial biopesticides in mango preservation has little influence on the biochemical and organoleptic characteristics and preserves the sanitary quality of mangoes.

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Competing Interests
The authors declare no competing interest.

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