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Potential Health Benefits of Pigment-containing Products on Creeping Bentgrass and Hybrid Bermudagrass

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Authors' contributions

This work was carried out in collaboration between all authors. Authors LBM, AWG, SBM and CEW designed the study and wrote the protocol. Author AWG conducted the research, data collection, performed the statistical analysis and managed the literature searches. Author PJB wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

The objective of this research was to analyze the use of pigmented products in the management of heat stress on creeping bentgrass [*Agrostis stolonifera* L. var *palustris* (Huds.)] and low temperature stress on bermudagrass [*Cynodon dactylon* (L.) *Pers.* × *C. transvaalensis* Burtt-Davy]. Studies utilized: zinc oxide (ZnO), green pigment + titanium dioxide (TiO₂) (Turf Screen); Cu-based pigment (PAR); fosetyl-AI (Fosetyl-AI); fosetyl-AI + Cu-based pigment (Signature); potassium phosphite (KH₂PO₄) (Title Phyte); Turf Screen + Title Phyte; and, PAR + Title Phyte. Products were applied bi-weekly for 12 wk. Bentgrass canopy temperatures increased ~0.5 to 3°C, photosynthesis reduced ~6 to 20 μ mol CO₂ cm⁻² s⁻¹, and relative chlorophyll content decreased ~8.5% by treatments, while bermudagrass was unaffected. Bermudagrass field studies indicated reduced photosynthesis (~8 to 21 μ mol CO₂ cm⁻² s⁻¹) for Title Phyte, stressed control, and Turf Screen. Root

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mass was unaffected by treatments. Turf Screen alone and + Title Phyte increased Zn plant (~820 mg kg⁻¹) and soil (4.75 kg ha⁻¹) levels. PAR alone and + Title Phyte; and Turf Screen + Title Phyte increased tissue Cu ~27 mg kg⁻¹. Overall, pigmented products promoted bentgrass heat stress and heavy metal accumulation but had minimum effect on bermudagrass turf performance.

Keywords: Turf pigments; carbon dioxide exchange rate; normalized difference vegetation index; photosynthetically active radiation; visual turfgrass quality.

1. INTRODUCTION

Creeping bentgrass is a cool season (C_3) turfgrass native to central Europe often grown in the cool and humid environments of the northern United States [1]. Due to its ability to tolerate low mowing heights (3 mm), soft texture, superb density, and narrow leaf blade (0.62 to 0.92 mm), it has become a popular putting green turf in warmer regions of the United States [2]. However, higher temperatures and greater humidity often negatively affect the quality of creeping bentgrass in summer months; a condition referred to as "summer bentgrass decline" [3,4].

Hybrid bermudagrass is a warm-season (C_4) turfgrass native to the hot, dry summers around the Indian Ocean [1]. Hybrid bermudagrass is favored over its common type form in warm subtropical and tropical climates due to its ability to tolerate very close mowing heights (3.2 to 4 mm) while sustaining a dense stand of turf and exhibiting good recovery potential [5]. Optimum soil and air temperatures for bermudagrass growth are 24 to 35°C and 29 to 37°C, respectively [6]; bermudagrass is used less frequently in cooler climates due to a dormancy (brown) state that occurs below 10°C [1]. Bermudagrass is also prone to low temperature kill, particularly in late winter and early spring; this most commonly occurs during periods of alternating freezing and thawing, and is aggravated by increased crown hydration [6], shade, traffic, and crown desiccation [1]. Chilling and freezing stress result in the reduction of photosynthetic assimilation of CO2 via the reduction of stomatal conductance, modification of thylakoid lipids, ice formation, and restriction of electron transport, as well as loss of chlorophyll and cessation of growth [7-11]. To prevent potential damage caused by freezing temperatures, plants can obtain chilling and freezing tolerance when exposed to low temperatures still greater than freezing [12].

The application of pigments onto playing areas to provide desirable turfgrass winter color is an

increasing trend in lieu of overseeding. Most of these products have a green coloration, thus, help create more aesthetically pleasing playing areas during periods of turf dormancy. However, reduced overall stand quality has been noted with repeated applications of pigmentcontaining products. This may be due, in part, to the altering of light intensity and spectral quality [13]. Pigments consist of dry powders with varying chemical compositions based on desired color. Common pigments in white, black, and red paint are TiO₂, C, and iron oxide (Fe₂O₃) [13].

Many pigments have a molecular structure similar to chlorophyll, however, pigment centered molecules are Cu ions instead of Mg ions as with chlorophyll. This similar molecular structure of pigment molecules to chlorophyll is one possible means of increasing a plant's photosynthetic efficiency.

Applications of pigmented products can enhance spring green-up when compared to traditional overseeding practices and dormant turf, due in part to the increased soil temperatures [14]. Summer applications of pigmented products to creeping bentgrass decreased CER as well as NDVI [15]. Reynolds et al. [16] determined that the long-term application of pigments, specifically those of darker colors such as green, black, and dark blue. reflected 87 to 95% of photosynthetically active radiation (PAR).

Lucas [3] observed that applications of aluminum tris + mancozeb or aluminum tris + chlorothalonil at 14 d intervals could reduce summer bentgrass decline. Building upon that, Lucas and Mudge [17] enhanced bentgrass quality using a monoester salt of phosphorous acid and an ethylene bisdithiocarbamate fungicide. The eventual conclusion was that the mixture of aluminum tris + mancozeb, which contains Pigment Blue 15, provided greater quality and color than any other fungicide combinations. Pigment Blue 15 enhances the activity of mancozeb and aluminum tris [17]. In previous research in cool-season turfgrasses, sequential applications of potassium phosphite (KH₂PO₃), either applied alone or in combination with iprodione, were found to reduce the occurrence of *Michrodochium nivale* (Fr.) Samuels and Hallett and other disease such dollar spot (*Sclerotinia homeocarpa* F.T. Benn.) and possibly pythium (*Pythium* spp.), as well as improve overall quality of turf canopy [18].

The presence of heavy metals in a soil environment may be beneficial or toxic to plants, depending on their concentrations [19]. A sufficient Zn level is suggested between 20 to 200 mg kg⁻¹ in various turf tissues with variation occurring between grass species [1,20]. Various Cu and Zn containing products as well as several fungicides are thought to help relieve environmental stresses on turfgrasses [3,14,17, 18].

Soil concentrations of Cu may increase over time due to the repeated application of Cu-containing fungicides, organic fertilizers, and effluent irrigation water [21,22]. Faust and Christians [23] reported soil Cu levels increasing from 0 to 600 mg kg⁻¹ caused a 16% decrease in bentgrass dry clipping weight as well as 52% less dry root mass than the untreated.

The objective of this study was to investigate if various pigments and fungicides could reduce high temperature heat stress on bentgrass and low temperature heat stress on bermudagrass grown in the transition zone. This research was undertaken to address the potential benefits of this common and growing practice in the turf industry.

2. MATERIALS AND METHODS

Field research was conducted in Clemson, SC at the Clemson University Research Facility on two 14 yr old putting greens, L-93 bentgrass and

TifEagle bermudagrass, both constructed to USGA specifications [24] from 24 June to 16 September, 2013 and 7 July to 29 September, 2014. Treatments consisted of: untreated control; zinc oxide (ZnO) + titanium dioxide (TiO₂) + green pigment (Turf Screen) (TurfMax LLC., Erdenheim. PA); Cu phytalocyanine pigment (PAR) (Harrell's LLC, Lakeland, FL); potassium phosphite (KH₂PO₄) (Title Phyte) (Harrell's LLC. Lakeland, FL); Turf Screen + Title Phyte; PAR + Title Phyte; fosetyl-Al + green pigment (Signature StressGard) (Baver CropScience + AG. Monheim Am Rhein, Germany); and, fosetyl-Al (Fosetyl-Al) (Quali-Pro, Pasadena, TX) (Table 1). Due to the potassium content of Title Phyte, soluble potash derived from potassium acetate (C₂H₃KO₂) (Stress Relefe, 0-0-25) (Harrell's LLC, Lakeland, FL) at 12.57 L ha⁻¹ (4 oz/1,000 ft²) was non-potassium added to treated plots. Treatments were applied every 14 d at the labeled rates using a CO₂ back pack sprayer delivering 187.3 L ha⁻¹ (20 gal a⁻¹). Plots were 2.5 x 1.5 m and replicated 4 times in each experiment. Both greens were maintained at mowing heights of 3.175 mm. Plots were arranged using a randomized complete block design and results analyzed using Analysis of Variance and Fisher's LSD (alpha=0.05).

2.1 Turf Quality

To quantify treatment effects on turfgrass quality, two measurements were recorded. Normalized Difference Vegetation Index (NDVI) was measured twice weekly using a Field Scout Turf Color Meter (Spectrum Technologies, Plainfield, IL). The device estimates turf quality by measuring the red and near-infrared light reflected off of the plant's surface. A "greener" surface is indicated by a higher NDVI ratio ([near infrared light(NIR)-Red light)]/[NIR + Red]) measured on a 0-1 scale [25]. Three ratings were averaged per plot. Turfgrass quality was also measured visually (1-9, 9 = best) twice weekly.

Table 1. Treatments and rates applied bi-weekly to L-93 creeping bentgrass and TifEagle bermudagrass for field studies in 2013 and 2014 at Clemson University, Clemson, SC

Treatment [†]	Rate
	L ha ⁻¹ (oz 1,000 ft ⁻²)
Zinc oxide + titanium dioxide + pigment (Turf Screen)	7.97 (2.5)
Copper phytalocyanine pigment (PAR)	1.17 (0.37)
Potassium phosphite (Title Phyte)	12.57 (4)
Turf Screen + Title Phyte	7.97 (2.5) + 12.57 (4)
PAR + Title Phyte	1.17 (0.37) + 12.57 (4)
Fosetyl-AI + StressGard (Signature)	19.13 (6)
Fosetyl-Al	12.57 (4)

[†]Treatments not including Title Phyte received a K supplement using potassium acetate ($C_2H_3KO_2$) (Stress Relefe, 0-0-25) at 12.572 L ha⁻¹ (4 oz 1,000ft²)

2.2 Canopy Temperatures, Chlorophyll Content, and CER

Due to the presence of pigments in products tested, quality based on plant color or appearance could be misleading. Therefore, physiological measurements not dependent on plant color were also performed. Daily canopy (°C) temperatures were recorded at approximately 2 pm EST (1 h past solar noon) using a handheld infrared thermometer (Raytek Corporation, Santa Cruz, CA). Three readings per plot were averaged. Daily chlorophyll content was measured based on relative concentration of chlorophyll usina Field Scout а CM 1000 Chlorophyll Meter (Spectrum Technologies, Plainfield, IL) at the same time as canopy temperatures. This is a number count from 0 to 999 based off of light reflectance at 700 and 840 nm using the formula: Index = [(S840/A840) ÷ (S700/A700)] x 1,000, where: S=sensor; and, A=ambient light. Three readings per plot were averaged. In addition, CO₂ rates (CER) were measured exchange twice weekly using a CIRAS-2 Portable Photosynthesis System (PP Systems, Haverhill, MA USA) which included the differential CO₂/H₂O analyzer attachment. This gas system involved a clear polyethylene 150 mm diameter chamber placed around the plug for 75 s for each reading. Two readings per plot were averaged.

2.3 Volumetric Soil Moisture Content

To determine if turf quality decline was associated with soil moisture stress, volumetric soil moisture content (cm³ water cm⁻³ soil) in the top 12 cm was recorded from each plot twice weekly using a FieldScout TDR 100 (Spectrum Technologies, Plainfield, IL). Three readings per plot were averaged.

2.4 Root Weight

Root weights were collected at the beginning and conclusion of each field trial. Three cores (2.5 cm diameter x 15 cm deep) from were removed each plot. Green shoots and thatch layers were removed and the remaining vegetation used for root weight. To determine actual weights, root layers were dried at 80°C for 7 d, weighed, then ashed at 500°C for 3 h. Weight of ash was then subtracted from the dried weight to determine dried root mass.

2.5 Heavy Metal Analysis

Soil heavy metal (Cu and Zn) analysis was performed by extracting 5 cores (2 cm diameter x 15 cm deep) per plot, blending each plot's respective cores, and then placing the mixed soil into individual paper bags. Tissue analysis was performed by obtaining clippings from each plot using a standard walk-behind greens mower with bucket attachment. Three passes were made on each plot before removing the clippings and placing them in paper bags. Due to insufficient growth of the bermudagrass plots, tissue analysis was not performed. Both tissue and soil samples were sent to Clemson University Agricultural Service Laboratory for heavy metal analysis.

3. RESULTS AND DISCUSSION

In bentgrass and bermudagrass field studies, results varied between yearly studies, therefore data from each was separated and analyzed individually.

3.1 Bentgrass

3.1.1 Canopy temperatures

The reduction of summer canopy temperatures has been shown to positively affect overall bentgrass health and is a major claim for several health promoting products, including several in this study. None of the studied products, however, produced lower canopy temperatures. All treatments in study 1 showed similar average summer temperatures to untreated control with temperatures between 37 and 37.8°C (98.6 and 100°F) (Fig. 1). In study 2, the untreated control (37.6°C) exhibited significantly lower temperatures than Turf Screen, PAR, and PAR + Title Phyte with an average difference of ~1.39°C (2.8°F) suggesting these treatments may actually be heat sinks.

3.1.2 Chlorophyll content

Reduced chlorophyll content is indicative of possible stress. In both studies the untreated control exhibited significantly higher levels (~9.2%) of chlorophyll than all treatments in study 1 and ~7.6% in study 2 (Fig. 2). In study 1, Signature exhibited higher chlorophyll content averaging 297 compared to 282 of PAR but less than the untreated (327 relative concentration). No differences were observed between Fosetyl-

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AI, PAR + Title Phyte, Title Phyte, Turf Screen, and Turf Screen + Title Phyte in study 1. In study 2, differences were observed between Title Phyte (290); Signature (283); Fosetyl-AI (276); Turf Screen (273); Turf Screen + Title Phyte (268); PAR (267); and PAR + Title Phyte (262) with all treatments less than the untreated (315).

3.1.3 Visual turf quality

Visual quality for areas treated with Title Phyte and Fosetyl-Al averaged 5.8 and 5.5 in study 1, significantly less than all other treatments which averaged ~6.8 (Fig. 3). In study 2, turf quality for Fosetyl-Al was less than Signature, PAR, untreated, and PAR + Title Phyte averaging 5.4 compared to ~6.3 for treatments tested. However, Fosetyl-Al showed similar visual quality to that of Turf Screen, Turf Screen + Title Phyte, and Title Phyte averaging ~5.9.

Dernoeden and Fu [26] concluded that applications of aluminum tris + chlorothalonil and potassium salts + mancozeb improved the overall quality of creeping bentgrass during the summer in addition to reducing scalping injury. Though, not proven, it was suggested the fungicides modified the plant morphology, growth habit, and/or rate.

3.1.4. Normalized difference vegetation index

In both studies, the NDVI average of the untreated control was similar to Fosetyl-AI (~0.74 in study 1, ~0.71 in study 2) (Fig. 4). Differences were not observed between Turf Screen, Turf Screen + Title Phyte, PAR + Title Phyte, and PAR in either study (~0.72 in study 1, ~0.68 in study 2). Similar NDVI averages were observed between Title Phyte and Signature in both studies (~0.73 in study 1, ~0.70 in study 2).

3.1.5 Soil moisture

No significant differences were measured in volumetric soil water content for all treatments in both studies (data not shown).

3.1.6 Carbon dioxide exchange rate

Carbon dioxide exchange rate (CER) measures the net CO_2 change from the surface of the turfgrass. A positive measurement is indicative of respiration exceeding photosynthesis and negative if photosynthesis exceeds respiration. Differences were not observed between treatments in study 1 with CER levels ranging between 2.4 and 21.5 μ mol CO₂ cm⁻² s⁻¹ (Fig. 5). In study 2, significantly lower CER (10.67 μ mol CO₂ cm⁻² s⁻¹) was measured in the untreated compared to PAR + Title Phyte (30.35 μ mol CO₂ cm⁻² s⁻¹); Turf Screen + Title Phyte (29.42 μ mol CO₂ cm⁻² s⁻¹); Fosetyl-Al (27.52 μ mol CO₂ cm⁻² s⁻¹); PAR (27.02 μ mol CO₂ cm⁻² s⁻¹); and Turf Screen (22.27 μ mol CO₂ cm⁻² s⁻¹). Similar levels of CER were observed between Title Phyte (18.78 μ mol CO₂ cm⁻² s⁻¹); Signature (20.99 μ mol CO₂ cm⁻² s⁻¹); Turf Screen + Title Phyte; Fosetyl-Al; PAR; and Turf Screen in study 2. All levels indicate failure to reduce net CO₂ exchange rate suggesting products do not alleviate summer photosynthetic stress on creeping bentgrass.

3.1.7 Root weights

No significant differences were observed in root weight between treatments in either study (data not shown).

3.1.8 Heavy metals

Due to the presence of metal molecules in the pigmented products, heavy metal concentrations of plant tissue and soil was analyzed. Turf Screen and Turf Screen + Title Phyte exhibited significantly higher net change of Zn concentrations in bentgrass plant tissue in both studies (~825 mg kg⁻¹ in study 1, ~920 mg kg⁻¹ in study 2) (Fig. 6). Additionally, plots treated with both products were significantly higher than all other products in net soil concentration of Zn (~4.5 kg ha⁻¹ greater in both studies) (Fig. 7).

The concentration of Cu in bentgrass plant tissue and soil was also of interest due to the presence of Cu-based pigments in several of the other products. In study 1, PAR + Title Phyte (50 mg kg⁻¹); PAR (45 mg kg⁻¹); Turf Screen + Title Phyte (41 mg kg⁻¹); and Signature (37 mg kg⁻¹) exhibited significantly higher increases in Cu concentration of tissue than the untreated control (1.25 mg kg⁻¹) (Fig. 8). In study 2, PAR + Title Phyte (56 mg kg⁻¹), Turf Screen + Title Phyte (43 mg kg⁻¹), PAR (4 mg kg⁻¹), and Turf Screen (34 mg kg⁻¹) exhibited significantly great net increases in soil Cu concentration than the control (11 mg kg⁻¹) and Title Phyte (11 mg kg⁻¹).

No observed differences occurred in net soil Cu concentration in either year with study one net ranging between 0.03 and 0.4 kg Cu ha⁻¹ in both studies (data not shown).

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3.2 Bermudagrass

3.2.1 Canopy temperature

High temperature stress is not a major concern on hybrid bermudagrass due to its C_4 physiology. All treatments in study one showed similar canopy temperatures to that of the untreated with temperatures between 37 to 39°C (98.9 to 100.4°F) (Fig. 9). Temperatures for all treatments except Title Phyte in study 2, however, exhibited greater values (~2 to 3.5°C) to that of the untreated (36.6°C). These results are similar to those seen in the Bentgrass study, indicating these treatments are probable heat sinks.

3.2.2 Chlorophyll content

Chlorophyll content differences were not observed in either study year (data not shown).

3.2.3 Turf quality

In study 1, visual turf quality of PAR, Signature, Title Phyte, and Fosetyl-Al were similar to the untreated control with ratings between 5.7 and 6.4 (Fig. 10). PAR + Title Phyte, TurfScreen + Title Phyte, and Turf Screen yielded greater visual ratings of ~6.8. None of these ratings, however, meet an average acceptable turf quality level of 7. No differences in visual quality were observed in study 2, with all treatments ranging between 6.6 and 7.1.

3.2.4 Normalized Difference Vegetation Index

Differences in NDVI ratings were not observed in either study between untreated control and treated plots. Study 1 yielded reflectance averages between 0.65 and 0.69 and between 0.64 and 0.68 in study 2 (data not shown). This indicates plant leaves maintained a similar level of green, regardless if a pigment was applied.

3.2.5 Soil moisture

Similar levels of volumetric soil moisture were observed throughout both studies (data not shown).

3.2.6 Carbon dioxide exchange rate

Study 1 CERs were similar between treated areas and untreated with rates between -14.3 and -24.4 mg L⁻¹ CO₂ (Fig. 11). In study 2, Signature (-28.295 mg L⁻¹) was the only treatment different from the untreated control of -8.972 mg L⁻¹, indicating greater photosynthetic efficiency with it.

3.2.7 Root weight

Differences between treatments were not observed in net root weight in either study (data not shown).



Fig. 1. Canopy temperature averages for two summer studies following bi-weekly applications to creeping bentgrass of various nonpigmented and pigment-containing products. Vertical bars represent standard errors. Different letters indicate significant differences between treatments within each study by LSD test (p ≤ 0.05)

3.2.8 Soil Zn

Plots treated with Turf Screen and Turf Screen + Title Phyte had higher soil concentrations of Zn than other treatments in both studies (Fig. 12). In study 1, soil Zn levels averaged \sim 5.4 kg ha⁻¹ and \sim 4.0 kg ha⁻¹ in study 2 for TurfScreen and TurfScreen + Title Phyte compared to <1.8 kg ha⁻¹ for all other treatments.



Fig. 2. Chlorophyll content averages for two summer studies following bi-weekly applications to creeping bentgrass of various nonpigmented and pigment-containing products. Vertical bars represent standard errors. Different letters indicate significant differences between treatments within each study by LSD test ($p \le 0.05$)







Normalized Difference Vegetation Index





Bentgrass CO₂ Exchange Rate





Net Bentgrass Tissue Zinc Concentration





Fig. 7. Net soil zinc concentration for two summer studies following bi-weekly applications to creeping bentgrass of various nonpigmented and pigment-containing products. Vertical bars represent standard errors. Different letters indicate significant differences between treatments within each study by LSD test ($p \le 0.05$)



Net Bentgrass Tissue Copper Concentration

Fig. 8. Net plant tissue copper concentration for two summer studies following bi-weekly applications to creeping bentgrass of various nonpigmented and pigment-containing products. Vertical bars represent standard errors. Different letters indicate significant differences between treatments within each study by LSD test ($p \le 0.05$)



Bermudagrass Canopy Temperature

Fig. 9. Canopy temperature averages for two summer studies following bi-weekly applications to hybrid bermudagrass of various nonpigmented and pigment-containing products. Vertical bars represent standard errors. Different letters indicate significant differences between treatments within each study by LSD test ($p \le 0.05$)



Fig. 10. Average visual turfgrass quality for two summer studies following bi-weekly applications to hybrid bermudagrass of various nonpigmented and pigment-containing products. Vertical bars represent standard errors. Different letters indicate significant differences between treatments within each study by LSD test ($p \le 0.05$)



Bermudagrass CO2 Exchange Rate

Fig. 11. Carbon dioxide exchange rates for two summer studies following bi-weekly applications to hybrid bermudagrass of various nonpigmented and pigment-containing products. A more negative numbers indicates a greater photosynthetic efficiency. Vertical bars represent standard errors. Different letters indicate significant differences between treatments within each study by LSD test ($p \le 0.05$)



Net Bermudagrass Soil Zinc Concentration

Fig. 12. Net soil zinc concentration for two summer studies following bi-weekly applications to hybrid bermudagrass of various nonpigmented and pigment-containing products. Vertical bars represent standard errors. Different letters indicate significant differences between treatments within each study by LSD test ($p \le 0.05$)

4. CONCLUSION

Pigment and sunscreen-containing products are often marketed as capable of relieving summer stress by way of reducing canopy temperatures while increasing a plant's basic photosynthetic efficiency. However, results of this study largely do not support these claims. Repeated applications of some of the products on creeping bentgrass, a turfgrass that historically suffers from heat and high light stress, resulted in an increase in canopy temperatures in addition to a reduction in overall efficiency as indicated by greater CO₂ exchange rates when compared to the untreated. The increase in temperatures may be linked to the covering and/or entering ("clogging") of stomata by ZnO and TiO₂ containing products, thus reducing plant transpiration.

Similar to the bentgrass study, in the bermudagrass study most products caused an increase in canopy temperature, however, this may be beneficial to bermudagrass, as could be a more negative CER rate with Signature. The cause of this justifies additional research as no major difference in relative chlorophyll content was observed between treatments.

Due to customer pressure for turfgrass managers to provide exceptional turf conditions with limited

financial resources, research should continue to investigate potential stress relieving strategies as well as possible methods to improving and increasing spring green-up timing with these and additional products.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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