

Full Length Research Paper

Does benzyladenine application increase soybean productivity?

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Although soybean flowers are produced abundantly, a large number of flowers and young pods abort naturally. Abortion reduction may result in an increased number of pods, thus leading to a growth in grain yield. The objective of this study was to evaluate the effect of benzyladenine application on soybean pod abortion and, consequently, to increase the productivity of soybean cultivation. The soil of the experimental area is classified as oxisol. After soil analysis, fertilization and pH correction were performed according to technical recommendations for cultivation. Pioneer 98Y12 RR soybean was sown by mid-November, during the rainy season. Benzyladenine application at the end of flowering, with pods of about 1.5 cm length, provided a significant increase in productivity of the species for all used concentrations, with the treatment of 300 mg L⁻¹ corresponding to the highest increase, around 11%. The increase in productivity was determined by the higher number of total pods fixed to the plants by reason of abortion reduction in the three canopy positions. Other factors that contributed to the increased productivity were the higher number of seeds per plant, higher weight and seed diameter. Benzyladenine application is a promising practice for getting high productivity in the cultivation of soybean.

Key words: Growth regulator, abortion, grain yield.

INTRODUCTION

The growing global demand for food, especially in the light of the population growth, has intensified the search for a fair balance between increased food production and environmental, economic and social questions.

Fao (2009) estimates that, for the first half of the 21st century, the global demand for food will grow about 70%, a problem connected with intense competition for arable lands between food crops, energy crops and other industrial purposes. The most dynamic products of Brazilian agribusiness should be cotton, soybeans, chicken meat, sugar, maize and cellulose (Ojima, 2011).

Among agricultural products, soybean has a significant importance for supplying the growing world population with food.

Soybean (*Glycine max* (L.) Merr.) is one of the most widely grown and consumed oilseeds in the world. The large growth of soybean production can be attributed to various factors, with special mention of: High protein content (around 40%) of excellent quality, both for human and animal feeding; high oil content of the seeds (around 20%), which can be used for various purposes, especially for human feeding and biofuel production (Lazzarotto and

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Hirakuri, 2010).

Brazil has one of the world's largest areas of arable land, with capacity to expand the cultivation of this oilseed to meet the demand for food and biofuel (Yu et al., 2013). However, the expansion of the planted area has been facing challenges, as the deadlock of environmental questions involving deforestation and its impact on environment, like greenhouse gas emission and biodiversity loss (Lazzarotto and Hirakuri, 2010). The growth of soybean production will occur depending on higher grain yield of the crop, and researches will need to be developed to adopt new management practices that guarantee higher productivity.

Studies indicate that soybean grain yield is more decisively determined by the number of pods than by other components of production (Yashima et al., 2005). The amount of flowers that give rise to the pods until reaching maturity is a key factor for getting high yields. Although soybean flowers are produced abundantly, a large number of flowers and young pods abort naturally (Nonokawa et al., 2012). Some researches show that, in normal conditions, the abscission of the reproductive structures of soybean can vary between 20 and 82% of the total number of flowers produced (Crosby et al., 1981; Carlson et al., 1987; Yashima et al., 2005; Peterson et al., 2005).

The mechanisms responsible for flower and pod fixing are not completely established. According to Dario et al. (2005), the application of growth regulators could raise productivity above levels established until now. Researches point out the use of plant growth regulators to reduce pod abortion (Crosby et al., 1981; Nonokawa et al., 2012; Passos et al., 2011). In soybean cultivation, there seems to be a link between exogenous benzyladenine and reduction of flower and pod abortion (Crosby et al., 1981; Carlson et al., 1987; Nagel et al., 2001; Yashima et al., 2005; Nonokawa et al., 2012; Passos et al., 2011).

Abortion prevention may result in an increased number of pods and seeds, thus leading to a growth in grain productivity (Nonokawa et al., 2012). Therefore, studies to increase soybean productivity have deserved much attention of researchers in recent years, in order to meet the predictable growing world demand for the grain. Aiming at raising pod percentage through abortion reduction and, consequently, at increasing the per hectare productivity of soybean, this study's objective is to evaluate the morpho-physiological effect of benzyladenine application on soybean pod abortion and, consequently, to increase its productivity.

MATERIALS AND METHODS

Experimental design

The research was carried out in the Panorama farm, located in the municipality of Ipameri, State of Goiás (Lat. 17° 67' 90" S, Long. 48° 19' 59" W, Elevation 805 m). This region has an Aw climate,

according to Köppen classification, characterized as a tropical wet climate with rainy summers and dry winters. The soil of the experimental area is classified as Oxisol.

After soil analysis, pH correction and fertilization were performed according to technical re-commendations for cultivation (Prochnow et al., 2010). 120 kg ha⁻¹ broadcast potassium chloride (KCl) were used 10 days before sowing and fertilization was performed at the time of sowing with application of 350 kg ha⁻¹ of the 04-30-10 formula. Pioneer 98Y12 RR soybean was sown on November 23, 2012.

Initially, a benzyladenine stock solution was prepared by weighing 2000 mg benzyladenine and dissolved in distilled water with 8 ml NaOH 1 N solution, and then the volume was completed with 50 ml distilled water. From the dilution of the obtained solution, soybean plants received the following treatments: 0, 100, 200, 300 and 400 mg L⁻¹ benzyladenine. The experiment was conducted in a randomized block design, with application in R₃ phase, broth volume of 200 L ha⁻¹ and five replications. We attempted maximum uniformity in the application, by spraying benzyladenine on the leaves and flowers; to this end, we used a dosing valve coupled to a backpack sprayer. 90 plants were grown in an experimental plot of 3 × 2 m, with 0.5 spacing between the rows and 10 plants per linear meter.

The following variables were analyzed: Stomatal density, specific leaf area, length and width of leaves, number of pods in upper, middle and lower canopy and total leaf nitrogen concentrations were measured when the pods were fully developed and grains were perceptible to the touch with 10% grain fill, corresponding to R_{5.1} stage.

Pod abortion

To analyze pod abortion, we counted the number of pods in the three canopy positions (lower, middle and upper) of soybean plants in the reproductive R_{5.1} phase and at harvest maturation point, which corresponds to the reproductive R₉ phase. The counting difference in these two phases corresponded to the number of aborted pods in the lower, middle and upper third.

Stomatal density determination

Replicas of the adaxial and abaxial leaf surfaces were removed with colorless nail polish in the region of the middle third of previously dehydrated leaves. Stomata in the replicas were counted with the help of an optical microscope equipped with a camera lucida. Stomatal density was determined by counting the stomata located in an area of 1 mm², giving the number of stomata/area (Jadrná et al., 2009). Five replicas of the adaxial surface and five replicas of the abaxial surface of each replication were analyzed to determine stomatal density.

Specific leaf area (SLA)

To get the specific leaf area, we removed six leaf disks of 12 mm diameter from fully-expanded leaves which were dried in a greenhouse at 70°C for 72 h to determine dry weight. SLA was obtained through the equation proposed by Radford (2013).

Leaf area (LA)

Leaf area was determined following the equation proposed by Adami et al. (2008). For this purpose, we used an mm-graduated tape to obtain length and width of the leaves.

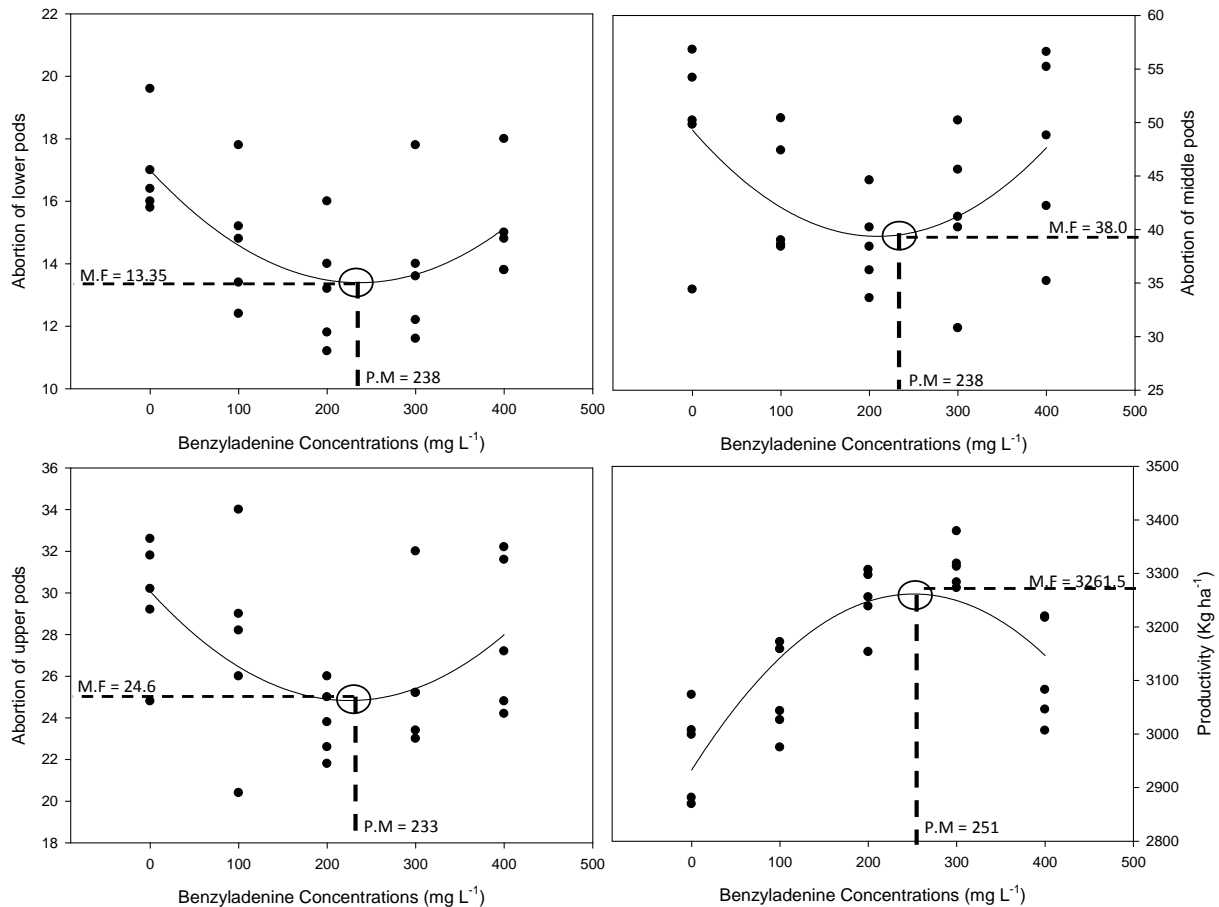


Figure 1. Regression equations for pod abortion of the lower third ($Y = 16.9874 - 0.0305x + 0.000064x^2$, $R^2 = 0.98^{**}$), pod abortion of the middle third ($Y = 49.2949 - 0.0950x + 0.0002x^2$, $R^2 = 0.97^*$), pod abortion of the upper third ($Y = 30.0766 - 0.0466x + 0.0001x^2$, $R^2 = 0.96^*$) and productivity per hectare ($Y = 2932.7643 + 2.6152x - 0.0052x^2$, $R^2 = 0.99^{**}$) of soybean plants treated with different doses of benzyladenine.

Nitrogen concentration

Samples of fully expanded leaves were collected and total N concentration was determined following Cataldo et al. (1975).

Productive variables

Number of seeds per plant, 100 seed weight, seed diameter and productivity were measured in the reproductive R_9 phase. 100 seed weight and productivity were adjusted to 13% moisture.

Experimental design and statistical procedures

Analyses of variance were processed following the randomized block design with five treatments, five replications and plot with 90 plants. Data were submitted to regression analysis using SISVAR 5.3 software (Ferreira, 2011).

RESULTS

The data obtained were adjusted using the quadratic

regression model (Figure 1). Results show that benzyladenine application at the end of flowering, with pods of up to 1.5 cm length, provided a significant increase in the productivity of the species for all concentrations used, with a peak for the concentration of 251 mg L^{-1} , which corresponds to the highest gain in productivity around 11%. Abortion reduction was proportionally higher in the lower and middle third of the plants. The lower third featured a reduction of 21.3% of pod abortion at a concentration of 238 mg L^{-1} compared to control, resulting in an increase of 3.6 pods per plant. In the middle and upper third of the canopy, the reduction of pod abortion provided an increase of 22.8% (11.3 pods per plant) and 18.0% (5.4 pods per plant) respectively. The peak corresponding to the benzyladenine concentrations in the middle and upper third were 238 and 233 mg L^{-1} , respectively.

Data relating to 100 seed weight, seed diameter and number of seeds per plant were adjusted using the quadratic regression model (Figure 2). The results represented by the 100 seed weight show that there was

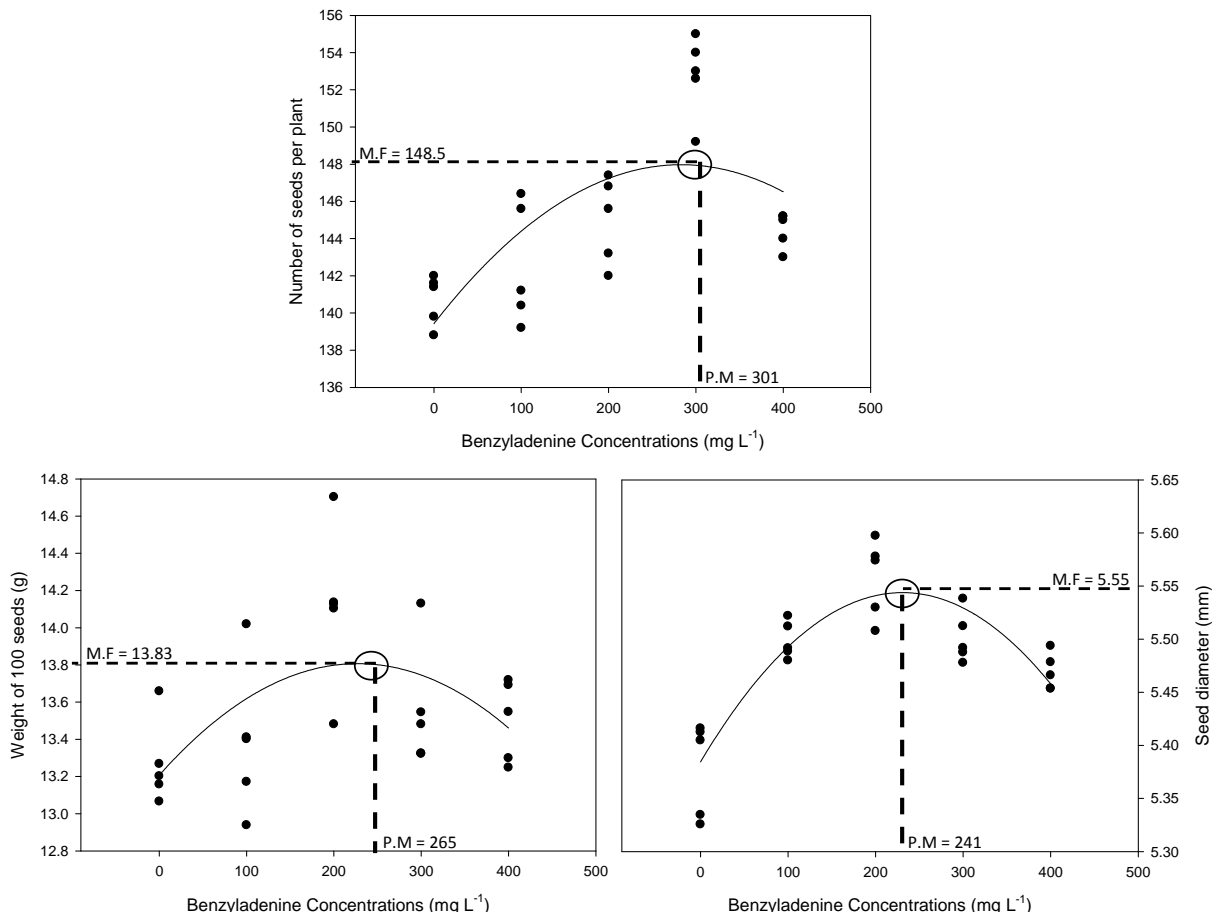


Figure 2. Regression equations for 100 seed weight ($Y = 13.2073 + 0.0053x - 0.000011x^2$, $R^2 = 0.99^*$), seed diameter ($Y = 5.3845 + 0.0014x - 0.0000029x^2$, $R^2 = 0.99^{**}$), and number of seeds per plant ($Y = 139.4286 + 0.0603x - 0.0001x^2$, $R^2 = 0.99^{***}$) of soybean plants treated with different doses of benzyladenine.

a significant increase at all concentrations used in relation to control, presenting a peak corresponding to a concentration of 265 mg L^{-1} with a 5% increase in the seed weight. As for the seed diameter and number of seeds per plant, peaks were verified with concentrations of 241 and 301 mg L^{-1} , with contributions of 3 and 7% respectively, decreasing at higher doses.

The unit and specific leaf area and the number of stomata of the leaf adaxial and abaxial surfaces were described by quadratic models show in Figure 3. Variations in leaf expansion were also found with the increase in benzyladenine concentrations up to the dose of 220 mg L^{-1} , with maximum gain of 4%, decreasing at higher concentrations. Specific leaf area showed significant differences with the various benzyladenine concentrations, the highest result being found for the dose of 250 mg L^{-1} , with average variation of 9% in relation to control. The number of stomata of the adaxial and abaxial surfaces presented significant variation with benzyladenine application. On average, this variable showed an increase of 16 and 32%, when the control was compared with the corresponding peak at 325 and

227 mg L^{-1} , respectively.

DISCUSSION

Responses to benzyladenine application strongly show the importance of cytokinins to soybean, for influencing pod fixing and seed development and, consequently, raising the crop yield.

Levels of endogenous cytokinins in the xylem of soybean are high at the beginning of anthesis and decrease with the progress of flowering (Carlson et al., 1987). Low availability of cytokinin associated with intense competition for nutrients and assimilates between developing fruits and vegetative organs limits the production potential of seeds in the cultivation of soybean and promotes an intense abortion of the reproductive structures. We suggest that benzyladenine application raises the endogenous hormone levels in the plant, increasing the drain strength. Strengthening the drain intensifies the unloading of assimilates, influencing directly the photosynthesis balance, which results in a

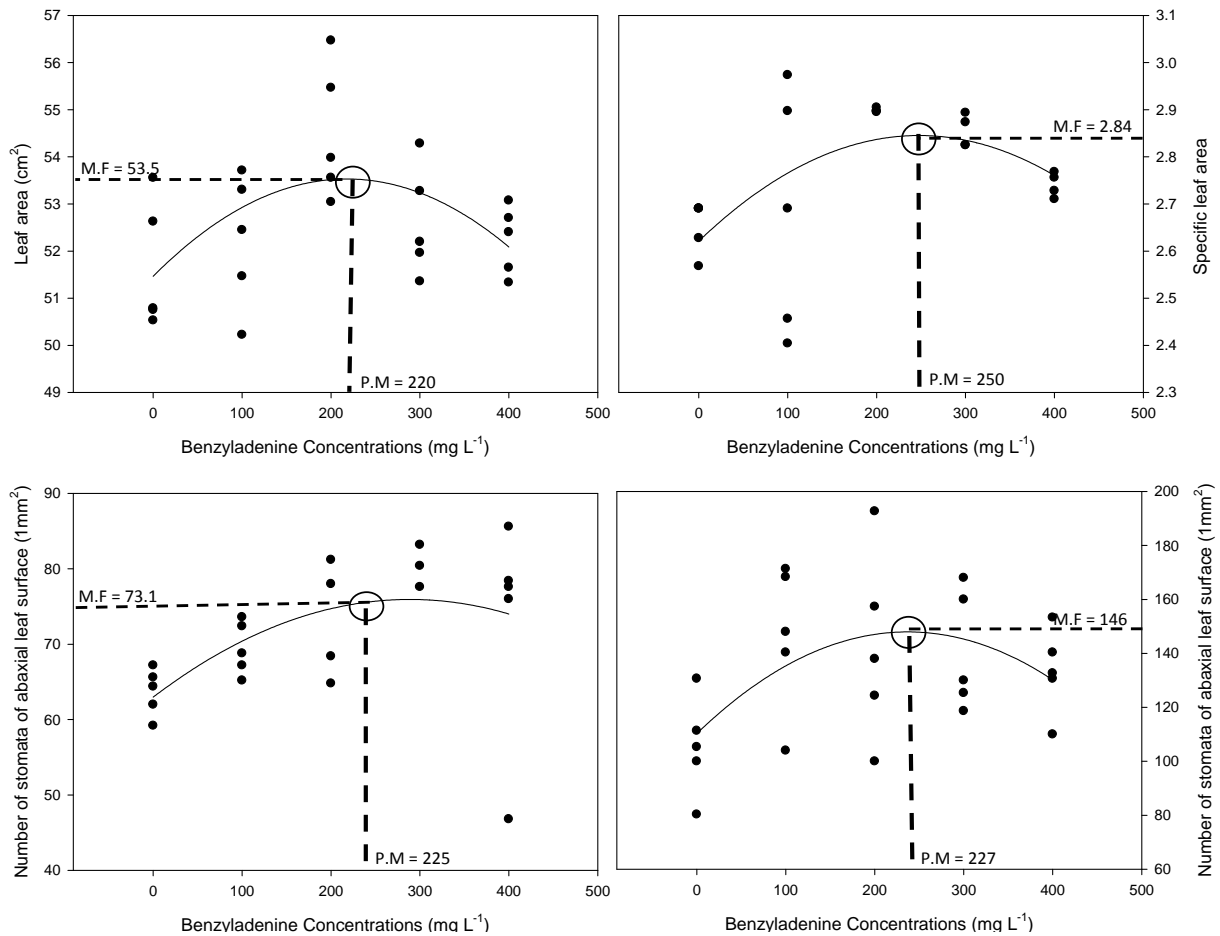


Figure 3. Regression equations for leaf area ($Y = 51.4672 + 0.0189x - 0.000043x^2$, $R^2 = 0.99^*$), specific leaf area ($Y = 2.6212 + 0.0018x - 0.0000036x^2$, $R^2 = 0.99^*$), number of stomata of the adaxial leaf surface ($Y = 62.9821 + 0.0898x - 0.0002x^2$, $R^2 = 0.98^*$) and number of stomata of the abaxial leaf surface ($Y = 110.2838 + 0.3179x - 0.0007x^2$, $R^2 = 0.95^{**}$) of soybean plants treated with different doses of benzyladenine.

higher production of assimilates.

The increased drain strength of the reproductive organs and the larger translocation of assimilates to these organs explain, at least in part, the higher 100 seed weight in plants treated with benzyladenine in relation to control. Similar results have been found by other authors using synthetic cytokinins in soybean plants (Carlson et al., 1987; Passos et al., 2011).

Benzyladenine resulted in significant gains in the number of pods fixed to the plants, with increases mainly in the pods located in the lower and middle third of the canopy. During the vegetative growth of soybean plants, stem tips and roots are normally the main drains; seeds and fruits become the dominant drains during the reproductive development, in particular for adjacent or close leaves. We may induce that the ability of benzyladenine to regulate the balance of power between sources and drains may have provided a higher mobilization of assimilates for the lower pods, reducing the amount of assimilates in direction of the roots,

resulting in a higher fixing of pods in the lower and middle third of soybean plants. An experiment carried out by Nagel et al. (2001) showed that plants treated with benzyladenine use to have a less developed root system, for he noted a more visible withering in the heat of the day when compared with control plants.

The increase of the productivity of soybean plants treated with benzyladenine is in part explained by morpho-anatomical changes in the leaf, like expansion of leaf area; increase of the specific leaf area and of the number of stomata per leaf. The larger leaf area and consequent lower leaf thickness may have contributed to optimizing the interception of the light that reaches and crosses the interior of the canopy, increasing the amount of photosynthetically active radiation able to reach the lower strata of the soybean plant. The higher transmittance allowed the realization of more photosynthesis at canopy level, with direct effect on the production of photoassimilates. Of course, all these factors contributed to the optimization of photosynthesis,

resulting in a significant increase in the number of pods and seeds, and consequently in productivity.

Benzyladenine application at the end of the flowering of soybean plants is a promising production technology, since it significantly increased the productivity.

Conclusion

1. Benzyladenine application reduced pod abortion in the lower, middle and upper third of the canopy of soybean plants.
2. Soybean plants treated with benzyladenine showed higher yields than control plants.
3. The highest productivity was obtained in soybean plants treated with a concentration of 300 mg L⁻¹.
4. Benzyladenine application at the end of the flowering phase is a promising management practice for soybean cultivation.

Conflict of Interest

The authors have not declared any conflict of interests.

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