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Lectin Histochemistry of *Agrotis segetum* Midgut Cells and Peritrophic Membrane

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Abstract

Midgut cell and peritrophic membranes of grain pest *Agrotis segetum* larvae were examined by light microscope. Six biotinylated lectins were used in the assays by applying histochemical methods (Avidin-Biotin-Peroxidase). The aim of this study was to find lectins binding on peritrophic membranes if they were available as insecticidal agents. Results of the study indicated that lectins BPA (*Bauhinia purpurea*) and GS-I (*Griffonia simplicifolia*) strongly stained whereas, PNA (Pea Nut Agglutinin) and UEA-1 (*Ulex europaeus*) moderately stained the tissues. However, WGA (Wheat Germ Agglutinin) and Con-A (*Canavalia ensiformis*) stained the tissues weakly. The common feature of two binding proteins, BPA and GS-I lectin was binding to D- galactose. Our examinations revealed that D-galactose mostly exists in *A. segetum* larvae midgut cell membranes and peritrophic membrane and this means BPA and GS-I lectins can be used as insecticidal agents against *A. segetum.*

1. INTRODUCTION

Agrotis segetum (Denis and Schiffermuller) (Lepidoptera: Noctuidae) is a harmfull black cutworm that feeds on roots of vegetable and cereal crops [1-3]. As a grain pest, it is of importance to combat with this agricultural pest. So, we have examined the histochemical properties of the peritrophic membrane and midgut cell membranes of *A. segetum* larvae to use the lectins binding on these membranes as insecticidal agents. In insects, alimentary canal consists of five layers; peritrophic membrane, epithelial surface, basal membrane, intima and muscle surface [4-6]. Peritrophic membrane is an acellular layer composed of a meshwork of chitin fibrils embedded in a matrix of proteins, proteoglycans, and mucopolysaccharides [7-9]. This layer protects the midgut epithelium from mechanical damage caused by food, pathogen and toxins [10, 11]. Four types of cells have been recognized in insect midgut epithelium; the columnar cells, goblet cells, endocrine cells and stem cells [12].

Lectins exist in plants, animals and prokaryotic cells [13]. The classification of lectins depends on their source [14-16]. Lectins specifically bind to carbohydrates or glycoconjugates of cell membranes. They are cytochemical probes in the search for changes in membrane organization [17]. There are several researches indicating altered glycosylation in cells and tissues by lectin histochemistry [18, 19]. Helliwell et al. (1989) studied lectin binding during necrosis, regeneration and neurogenic atrophy [20]. Welburn et al. (1994) suggested that the midgut lectin is normally responsible for the agglutination of trypanosomes in the fly midgut by binding to the procyclic surface coat, prior to establishment in the ecto-peritrophic space [21].

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Tinel et al. (2014) reported that *Dioclea violacea* lectin (DVL) interacts with midgut surface of *Lutzomyia migonei* [22]. Li-Byarlay et al. (2016) stated that they have identified two kinds of protease inhibitors and lectin to the fruit fly *Drosophila melanogaster* and alpha-amylase inhibitors and lectins to the cowpea bruchid *Callosobruchus maculatus*. Their study revealed that the midgut of parasitic insects was damaged by WGA inhibitor agent in the fed [23].

Lectin-carbohydrate recognition mechanism of *Plasmodium berghei* in the midgut of malaria vector *Anopheles stephensi* was studied using two lectins; wheat germ agglutinin (WGA) and Concanavalin A (ConA) [24]. Basseri et al. (2016) showed that Con A did not interrupted ookinete penetration into the midgut wall of *A. stephensi* but WGA inhibited ookinete invasion into the midgut cells [24]. The main aim of presented study was to use histochemical markers, lectins, to evaluate their specificity to the midgut and perithrophic membrane structure of *A. segetum* larvae.

2. MATERIAL and METHODS

2.1. Insect Culture

This study was performed in the Research Laboratories of Ankara University Faculty of Science, Department of Biology, Ankara, Turkey. Fifth instar larvae of *A. segetum* were used in this study. The larvae were raised with *Beta vulgaris var. rapa* and kept under 14 hour light cycle at 26 ± 1 C° and 60-65% relative humidity [25].

2.2. Tissue Preparation

Midgut tissue samples were dissected from *A. segetum* fifth instar larvae. Tissues were washed in distilled water and fixed overnight in 10% formaldehyde at 4 C°. After fixation tissues were dehydrated in ethanol series (70-100%) and embedded in paraffin wax. Sections of 4µm thickness were cut and after dewaxing with xylol for 30 min they were hydrated in ethanol series for 30 min of descending concentration [20, 26].

2.3. Lectin Histochemistry

Biotinylated lectins used in this study were shown in Table 1. Lectins were Con A, WGA, PNA, UEA-1, GS-I and BPA (Sigma, USA).

Table 1. *List of lectins, binding specifity to carbohydrates and used concentrations*

Biotinylated Lectins	Carbohyrate specifity	Lectin concentrations (µg/ml)
Canavalia ensiformis (Con-A)	α-D- Mannose > α-D-Glucose	2.5
Triticum vulgare (WGA)	N-acetylglucosamine>sialic acid	1.0
Arachis hypogaea (PNA)	β-D-galactose-(1→3)-D-N-acetyl-galactosamine	5.0
Ulex europaeus (UEA-1)	α-L-fucose	5.0
Griffonia simplici folia (GS-I)	α- D- galactose	5.0
Bauhinia purpurea (BPA)	N-acetyl-D-galactosamine, D-galactose	5.0

After deparaffination, tissue sections were incubated in H_2O_2 for 10 min at room temperature to block endogenous peroxidase activity then sections were washed in PBS (Phosphate-Buffer Saline, pH 7.4) and incubated with biotinylated lectins for 60 min at room temperature [20,27]. Sections incubated with Avidin-Biotin-Peroxidase complex for 60 min at room temperature and washed with PBS buffer two times. Air dried sections were incubated with diaminobenzidine (DAB) (0.6 mg/ml with 3µl of H_2O_2) for 5 min. Finally, they were stained with Harris Haematoxylin for 5 seconds, washed with PBS buffer and examined by light microscope (Vanox, Olympus, Japan).

3. RESULTS and DISCUSSION

Lectins used in the study showed a particular binding to cell surfaces and peritrophic membrane. The BPA and GS-I conjugates strongly stained the columnar and goblet cell membrane and peritrophic membrane. N-acetyl-D- galactosamine, D-galactose and α-D-galactose specificity may be related to their presence in columnar cell membranes, goblet cell membranes and peritrophic membrane. Light microscope observations revealed that these two lectins were more heavily stained apical surfaces of midgut cells than basal surfaces. These carbohydrate residues of receptors were found in apical cell membrane more than basal cell membrane (Figures 1-4).

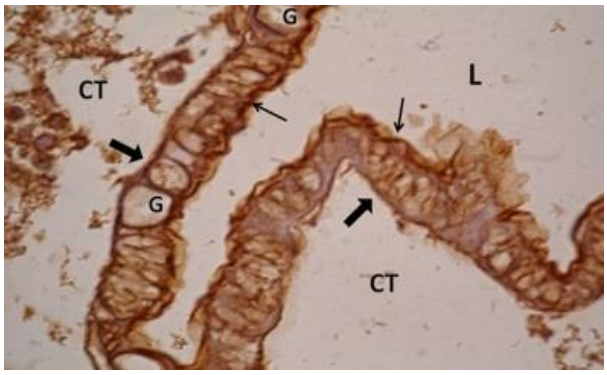

Figure 1. *Staining of midgut columnar cells and goblet cells of A. Segetum 5th larvae with BPA. Apical cell membrane (→), Basal cell membrane (bold arrow), Connective Tissue (CT), Goblet cell (G), Lumen (L). ×670*

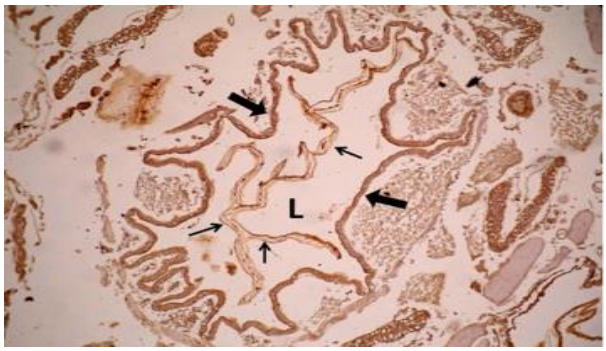

Figure 2. *Strongly staining of cells membranes and peritrophic membrane of A.segetum 5th larvae with BPA. Epithelial cells layer (bold arrow), Lumen (L), Peritrophic membrane (→). ×67*

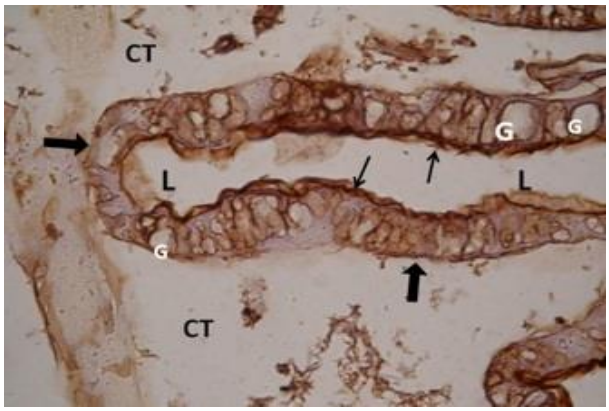

Figure 3. *GS-I staining of midgut cells of A. segetum larvae. Apical cell membrane (→), Basal cell membrane (bold arrow), Connective Tissue (CT), Lumen (L), Goblet cell (G). ×670*

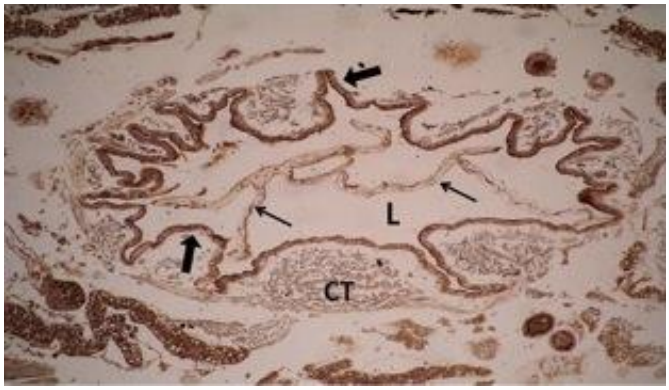

Figure 4. *GS-I staining of midgut cells and peritrophic membranes of A. segetum. Connective Tissue (CT), Epithelial cells layer (bold arrow), Lumen (L), Peritrophic membrane (→).× 67*

Arachis hypogaea (PNA) lectin normally binds to β-D-galactose- (1→3) and N-acetyl galactosamine residues. The apical surface of columnar cells were moderately stained with PNA. This lectin has strongly stained goblet cell membrane but weakly the peritrophic membrane. However, apical surfaces of midgut columnar cells were stained much more than the basal cell membranes (Figures 5-6).

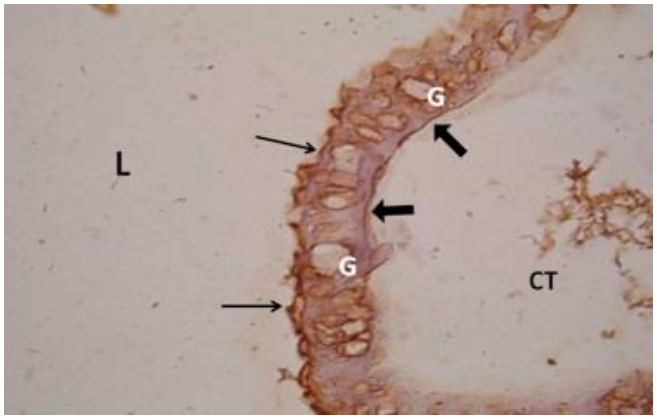

Figure 5. *Staining of midgut goblet cells and columnar cells of A.segetum with PNA. Apical cell membrane (→), Basal cell membranes (bold arrow), Connective Tissue (CT), Goblet cell (G), Lumen (L). × 670*

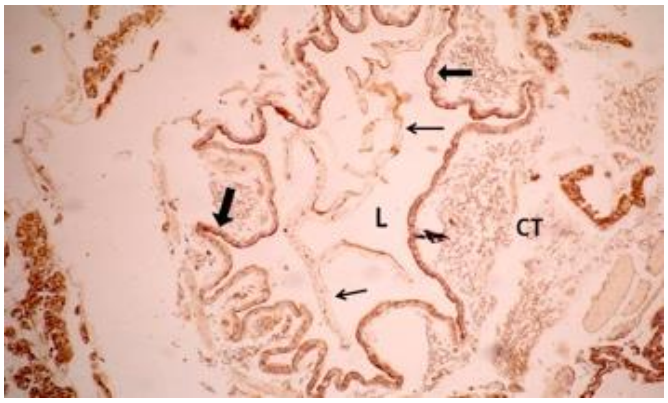

Figure 6. *Moderately staining of cells membranes and peritrophic membrane of A.segetum with PNA. Epithelial cells layer (bold arrow), Connective Tissue (CT), Lumen (L), Peritrophic membrane (→). × 67*

Lectin *Ulex europaes* (UEA-1) is known to react with α-L-fucose residues. Our studies represented that UEA-1 was weakly bounded to the goblet cell membrane and peritrophic membrane, but it was moderately observed to bind to the apical surfaces of columnar cells. Apical surfaces of these cells were seen to be stained better than the basal cells membranes (Figures 7-8).

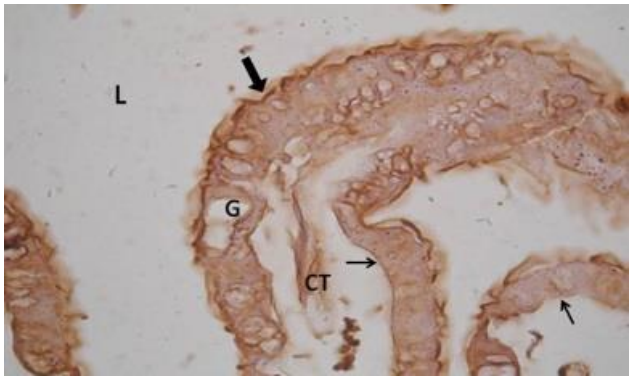

Figure 7. *Staining of columnar cells and goblet cells of A. segetum with UEA-1. Apical cells membranes (bold arrow), Basal cells membranes (→), Connective Tissue (CT), Goblet cell (G), Lumen (L). ×670*

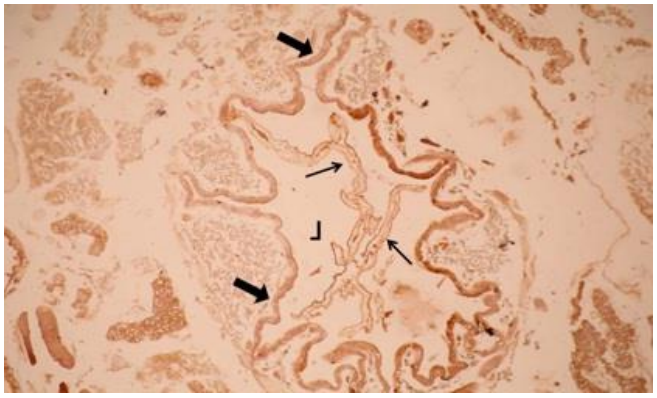

Figure 8. *UEA-1 staining of midgut columnar cells and peritrophic membrane of A. segetum. Epithelial cells layer (bold arrow), Lumen (L), Peritrophic membrane (→).×67*

Lectin Wheat Germ Agglutinin (WGA) specifically binds to N-acetyl-glucosamine and sialic acid residues. The apical surfaces of midgut cells and goblet cell membrane were weakly labelled by WGA also, WGA was observed to bind poorly to the peritrophic membrane. WGA reactivity was weaker in the basal surface of midgut lumen than in the apical surface. At the same time, N-acetyl-glucosamine and sialic acid residues were a few in the apical surface, goblet cell membrane and peritrophic membrane (Figures 9-10).

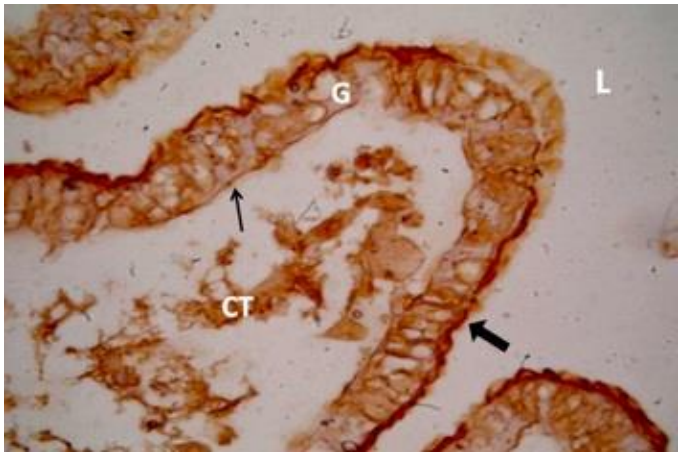

Figure 9. *WGA staining of midgut cells of A. segetum. Goblet cell (G), Apical cells membranes (bold arrow), Lumen (L), Basal cells membranes (→). ×670*

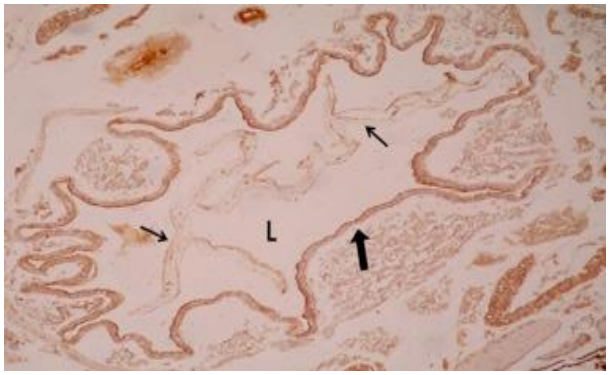

Figure 10.*Weakly staining cell membranes and peritrophic membranes of A. segetum with WGA. Lumen (L), Peritrophic membrane (→), Epithelial layer (bold arrow). × 67*

Canavalia ensiformis (Con-A) is specific for α-D-mannose and α-D-glucose residues. Our observations revealed that Con-A was bounded weakly to the goblet cell membrane and peritrophic membrane but, moderately to the apical surface of midgut columnar cells. Whereas, basal surface of cells was stained weaker than the apical surfaces. As a result, α-D-mannose and α-D-glucose residues were seen to be a few in the goblet and peritrophic membrane (Figures 11-12).

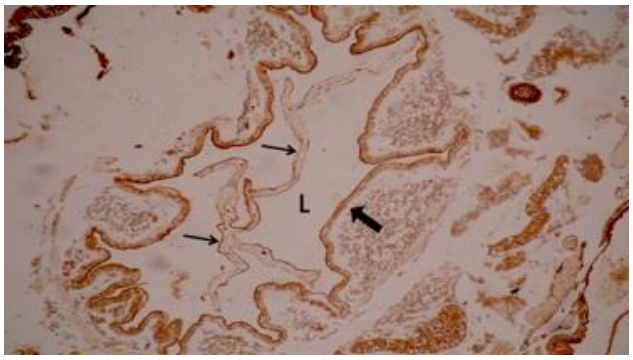

Figure 11. *Staining of columnar cells and goblet cells of A. segetum with Con-A. Goblet cell (G), Apical membrane (→), Lumen (L), Basal membrane (bold arrow).× 670*

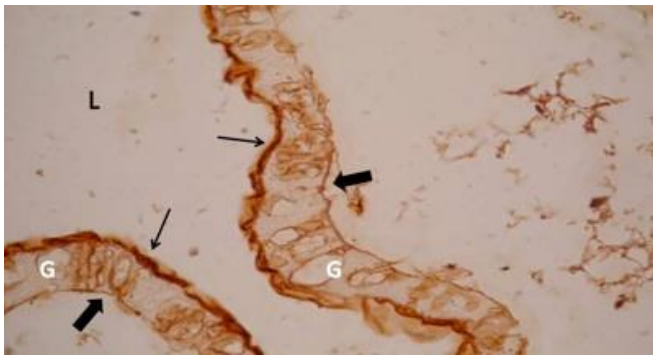

Figure 12. *Con-A staining of midgut cells and peritrophic membranes of A. segetum. Epithelial layer (bold arrows), Lumen (L), Peritrophic membrane (→).×67*

Eventually, we identified that while the lectins of BPA and GS-1 were intensively stained the cell membranes and peritrophic membrane of *A. segetum*, Con-Aand WGA were weakly stained that of *A. segetum* (Table 2).

Table 2. *Lectin binding patterns in Agrotis segetum midgut*

List of Lectins	Apical membrane	Basal membrane	Peritrophic membrane	Goblet cell membrane
Con-A	++	+	+	+
WGA	+	+	+	+
PNA	++	+	+	++
UEA-I	++	+	++	++
GS-I	+++	++	++	+++
BPA	+++	++	++	+++

It was found that WGA and PNA lectins bind strongly to epithelium cell membrane of midgut in several Lepidoptera species therefore, they play a role as insecticide materials [28]. We observed that WGA weakly but, PNA moderately were bounded to the apical surface of epithelium cell of *A. segetum* midgut. According to Czapla and Lang (1990) midgut epithelial cells of *Coleopter* species stained strongly with WGA and PNA [28]. However, *A. segetum* strongly stained by GS-I and BPA lectins (see above Table 2) in our study. The difference between our study and Czapla and Lang (1990) may arise from the orders of insects. The midgut of female sand fly *Lutzomyia longipalpis* from four sources Con-A, HPA (*Helix pomatia* agglutinin), PNA and WGA was conjugated with colloidal gold in as study of Evangelista and Leite (2002). Only HPA was found to be specific for N- acetyl-galactose amine and was observed to bind to the midgut cells and cytoplasmic secretory granules and microvilli of midgut epithelium [29]. In *A. segetum* larvae, Con A and WGA were observed to bind weakly to the apical surface of midgut cells and PNA was moderately bound to these cells. In a study of Sauvion et al. (2004) with *Lutzomyia longipalpis* lectins WGA, Con A and PNA did not bind to the peritrophic membrane but bound weakly to the peritrophic membrane of midgut of *A. segetum*. ConA acts as a feeding inhibitor for pea aphid (*Acyrthosiphon pisum* Harris). ConA, specific to α- D- mannose, was bound strongly to midgut epithelial cell surfaces of *A. pisum* and therefore inhibited feeding process in *A. pisum* [30]. Our observations showed weakly staining with Con A lectin that of *A. segetum*. This has shown that α-D-mannose residues were found to be few in this zone of *A. segetum*. It was reported that the sugar specificity of the purified *Aedes aegypti* midgut lectin (*Aelec*) was strongly inhibited by D (+)-mannose and raffinose, followed by D (+) glucose. N-acetyl-D-mannosamine and N-acetyl-D-glucosamine were moderate inhibitors [31]. Whereas our data were different from *Aedes aegypti* midgut lectin (Aelec). The midgut cell membranes of *A. segetum* were moderately inhibited by D (+)-mannose and glucose and weakly inhibited by N-acetyl-D-glucosamine.

Martin et al. (2006) studied morphology and permeability of peritrophic membrane of penaeid shrimp (*Sicyonia ingentis*) with WGA lectin staining [32]. As WGA was represented to bind strongly to peritrophic membrane of *S. ingentis* they had the opportunity to detect the structure of peritrophic membrane of midgut. Peritrophic membrane of *A. segetum* was labeled weakly with WGA lectin. This result indicates that N-acetyl-D-glucosamine was presented in the peritrophic membrane of *A. segetum* midgut. Our examinations demonstrated that the apical surfaces of midgut cells of *A. segetum* were stained better than basement surfaces. Zaccone et al. (1987), studied on the surface epidermis of *Ambystoma tigrinum* larvae by using five different lectins to detect sugar residues on cell membranes [33]. Con A, WGA, RCA-I and SBA were clearly labeled the cell surfaces especially the apical surfaces but, lateral and basement surfaces were only labeled with *Ricinus communis* Agglutinin (RCA-1) lectin. We obtained similar findings in *A. segetum* midgut cell membranes.

As a result, lectins were observed to bind better to the apical cell surface than basement cell surface of *A. segetum*. In our study, among the used lectins, GS-I and BPA were observed to bind strongly to the apical membranes of midgut columnar and Goblet cells. We believe that in the light of the data we have obtained from our work, GS-I and BPA lectins are available as insecticidal agents against *A. segetum.*

CONFLICTS OF INTEREST

No conflict of interest was declared by the authors.

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