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Response of Interaction between Aboveground and Belowground Herbivorous to Corn Development

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Key words
Aboveground herbivores, Belowground herbivores, Plant development, Community structure, Interactions

ABSTRACT

Plant and soil animal communities interact through plant-associated processes. How the interactions between the aboveground and belowground herbivores communities affect plant development remains unclear? In this study, *Holotrichia diomphalia* Bates, the belowground herbivore grub that feed on the roots, and *Rhopalosiphum maidis*, the aboveground herbivore aphid that feed on the leaf were chosen as research objects. Four groups were set based on field investigation, including aphid with grub (A & G), aphid (A), grub (G) and blank control (C) groups, and the effects on the host plant corn (*Zea mays* L.) was investigated. One-way ANOVA and Pearson analysis was performed to process the data. The results showed that, the interactions between above- and below-ground herbivores had a negative effect on corn. Belowground herbivore grubs inhibited the aboveground herbivores of aphids, and aboveground herbivores prevented the increase of grubs, whereas plants regulated population change through interaction with above- and below-ground herbivores, thus reducing the survival rate of above- and below-ground insect herbivores. In naturally occurring populations, behavior of leaf-feeding were influenced by root-feeders, causing multitude interactions at the same time. In conclusion, it is suggested that there may be a mutual inhibition between different aboveground and below-ground herbivores when the development of corn declined. These findings will contribute to a better understanding on the interaction mechanism between above-ground and below-ground herbivore on the host plants, and have guiding significance for agricultural pest control.

INTRODUCTION

Corn (*Zea mays* L.), also called maize, is widely cultivated worldwide and has become a staple food in many parts of the world. It is also one of the main crops in China, there are 420 million mu of spring corn in northern China alone. In recent years, the corn farmland has been affected by the traditional agricultural cultivation modes, and the diseases and pests have become increasingly serious. Aphids are the representatives of the main pests in the aboveground parts of corn, while grubs are the representatives of main pest of belowground parts of corn crop, which seriously affect the grain quality and yield of corn (Liu *et al*., 2018a, b). Aphids are kind of small sap-sucking insects which belong to the superfamily Aphidoidea, *Rhopalosiphum maidis* is the most representative of aphid in the agricultural production of northeast China. Grubs are the larval or immature form of northeastern beetles species, belong to the subfamily Melolonthinae, among which *Holotrichia diomphalia* Bates is the most representative (Liu *et al*., 2021).

In agroecological systems, aboveground and belowground herbivores are important components of harmful insect communities (Johnson *et al*., 2012; Johnson *et al*., 2013). More and more empirical studies in the past two decades have shown that herbivorous insects interact with each other and the population dynamics are formed by multiple indirect interactions between aboveground and belowground plants (Bardgett and Wardle, 2003; Rasmann *et al*., 2007; Kaplan *et al*., 2009; Wang *et al*., 2014). Furthermore, researches have shown that herbivores have important effects on soil organisms and their activities, and may have a great impact on plant growth and development, and herbivorous population

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dynamics (Bardgett and Wardle, 2003). It is also reported that arthropods may play role in maintaining soil fertility (Coulibaly *et al*., 2010). The above- and below-ground herbivores and their interactions can be mediated by plants, but it is difficult to clearly distinguish their roles, which may be due to differences of taxa on the spatial scale (Jing *et al*., 2015); however, few studies have further revealed their relationships (Bardgett and Wardle, 2010). Corn planting is affected by many associated environmental and ecological impacts, and corn cultivation has important effects on above- and below-ground insect communities. Studies have shown that corn can regulate the insect community structure and biodiversity, and changing the corn cropland patterns can increase the number of insect populations and the number of above- and below-ground taxa (Norris *et al*., 2016). However, at present it is unclear how herbivores induce changes in plant growth and development through aboveground and belowground herbivore interactions (Huang *et al*., 2013). Little is known about how individuals respond to the interactions of these populations at large spatial scales (Nadell *et al*., 2016).

Plant mediated biotic interactions between above- and below-ground herbivores are the core of agricultural ecosystems. Previous studies have revealed the relationship between plant and soil animal communities through root-associated processes (Bednarek, 2012). It is reported that plant mediated process enables the root development to be estimated from the shoot development, which is easier to be measured. This also reflects the different effects between aboveground and belowground herbivores on plant organs (shoot and root) (Crispus *et al*., 2020) in response to changes in environmental conditions (Muller *et al*., 2000; Shipley and Meziane, 2002; Zhou *et al*., 2014; Peng and Yang, 2016). Lina (2017) studied the resistance of the stems, leaves and roots of maize to the aboveground insects *Rhopalosiphum maidis* and the underground insects *Diabrotica virgifera*, respectively, the results showed that corn was more resistant to belowground insects and more susceptible to aboveground insects. At present, there are few reports on the interaction between corn and grub. In different development stages of corn, different species of inserts have completely different effects on the growth and yield of corn. Therefore, it is particularly important to study the population changes and interactions of the two main above-ground and below-ground insects regulated by maize at different development stages (Kumar and Arivudainambi, 2019; Arifie *et al*., 2019). However, so far, few research has been done to explain how the aboveground and belowground herbivore interact to effect plant development at large field scale.

Based on the above scientific questions, this study selected grubs and aphids as research objects to compare the insect populations at different developmental stages of corn, and to study the interaction between aboveground and underground insects and their effects on the growth and development of corn. The experiment aims to help better understand the interaction mechanism of above- and under-ground insects and crop, and has guiding significance for further agricultural pest control.

MATERIALS AND METHODS

Experimental site

The experiment was carried out in a corn field in Dehui City (125°30’ E, 46°36’ N, Fig. 1), the central part of the Songliao Plain in Jilin Province, China from May to September 2015. According to Chinese Soil Classification System, the soil belongs to black soil, where corn (*Zea mays* L.) has been planted for over 30 years. It belongs to temperate monsoon semi humid climate, with an annual temperature of 4.4°C, and an annual average precipitation of 520 mm, of which 80% occurs in July and August.

Fig. 1. Location map of experimental site.

In the study area, grubs and aphids two of the most important pests that can affect the growth of corn were taken as objects. The sampling site was in the center of arable land to avoid the potential interference of surrounding shelter forests, and was set into 400 × 400 m plot with a total of 1000 corn plants. The site was further divided into 64 small quadrats, each quadrat was 50 m × 50 m. The planting method of all quadrats was the same as that of cultivation, no pesticides were used in all treatments.

Experimental design and measurements

Corn was planted on May 2, and the planting and cultivation methods were the same in all the quadrats. According to the corn growth stages that were classified as vegetative (V) or reproductive (R), there were three investigates conducted on July 6 (6-leaf stage, V6), August 1 (12-leaf stage, V12) and September 2 (filling stage, R2) respectively, to investigate the occurrence of aphids and grubs in each quadrat. The occurrence levels of corn aphids was classified into 1-10 levels. The number of aphids in 100 crops in level 1 was $n \leq 500$, 2 was $500 < n \leq 1500$, 3 was $1500 < n \leq 2500$, 4 was $2500 < n \leq 3500$ and 5 $n > 3500$. The following classification of damages at mild, moderate and severe levels caused by grub was used.

Mild damage: A small part of the fibrous roots of the corn plant were damaged, and the top leaves of the plant were slightly wilted.

Moderate damage: Part of the fibrous roots or a small part of the main root were damaged, and some leaves of the plant wilted.

Severe damage: Most fibrous roots or taproots were destroyed, and the whole plant wilted or died.

Based on the investigates, four groups were set according to the occurrence of aphids and grubs in quadrats: aphid and grub (A & G) group, aphid (A) group, grub (G) group and the blank control (C) group. For each treatment group, 16 plots were selected as parallel repeat. In A & G group, included the occurrence of both aphids and grubs in each quadrat, 10 corn plants with the occurrence of grubs below moderate damage level and the occurrence of aphids at level 1 were selected (labeled A & G No. 1-10) to investigate the number of aphids and grubs, repeated 3 times in each quadrat. In A group, 10 corn plants that the occurrence of aphids at level 1 and not damaged by grubs (labeled A No. 1-10) were selected, 3 replicates were set in each quadrat to investigate the number of aphids. In G group, aphids on corn were manually removed with a brush, 10 maize plants (labeled G No. 1-10) with grub damage at or below the moderate level were selected, and repeated 3 times in each quadrat to investigate the number of grubs. In C group, 10 corns plants that not attacked by grubs were selected (labeled C No. 1-10), and covered with 40 mesh gauze to avoid the damage of aphid, 3 replicates were set up in each quadrat. At the same time, the growth height of 10 cron plants was measured with 3 replicates in each quadrat.

To investigate the number of aphids, the aphids on corn plants were collected by suction traps method (Gao *et al*., 2014). In brief, suction trap was covered on the full plant of each cron, lasted for 5s to collect aphids. The extracted aphids were preserved in an alcohol solution, identified and counted (Mónica *et al*., 2010; Cai and Li, 2015).

Grubs were collected by hand sorting from soil samples located to the right of each test plant (0.5 m × 0.5 m × 0.25 m at length, width, depth). The grubs were fixed in a solution of 1:1 10% formalin and 96% ethanol for several minutes. Then the grubs were stored in a glass tube in 10% formalin for classification and identification (Gutiérrez-López *et al*., 2010).

Data analysis

All data were processed using SPSS 19.0 (IBM Corp., Chicago, IL, USA). The one-way ANOVA was used to analyze the statistical significance between the population coefficients of aphid and grub, and the interaction effect on corn growth. Pearson analysis was performed for estimating the correlation between the aphid and corps height, grub and plant height of average 30 plants. Niche analysis was performed to investigate the relationship between aphid, grub and plant height. For aphids and grubs, the significance of the effects of distance and the presence of grubs was assessed using a repeated-measures analysis of variance with plants (1, 2, 3, … 10) containing aphids and plants containing grubs as the variables.

RESULTS

Interactions of above- and below-ground herbivore insects

The survival of herbivore insects varied with different months. The population of aphids generally increased from July to September, while the population of grubs decreased gradually (Fig. 2) The presence of above-ground insects significantly inhibited below-ground insects ($p_{JUL} = 0.001$, $p_{AUG} = 0.004$, $p_{SEP} = 2.9 \times 10^{-5}$, Fig. 2A). In contrast, below-ground herbivores survival decreased when above-ground herbivores were present ($p_{JUL} = 0.623$, $p_{AUG} = 0.352$, $p_{SEP} = 0.015$, Fig. 2B). The aphid and grub greatly inhibited the population of aphids, and had little impact on the underground pest grub. The impact on the population of the both insects in September was significantly different, especially on aphids. This may be related to the temporal and spatial pattern of the population distribution of each insect.

Effects of above- and below-ground herbivore insects on corn

Herbivores significantly decreased plant development through the interaction above- and below-ground herbivores insects, resulting in the most highest performance with below-ground herbivores that were present during the different months (G > A & G >A group, $p < 0.01$, Fig. 3).

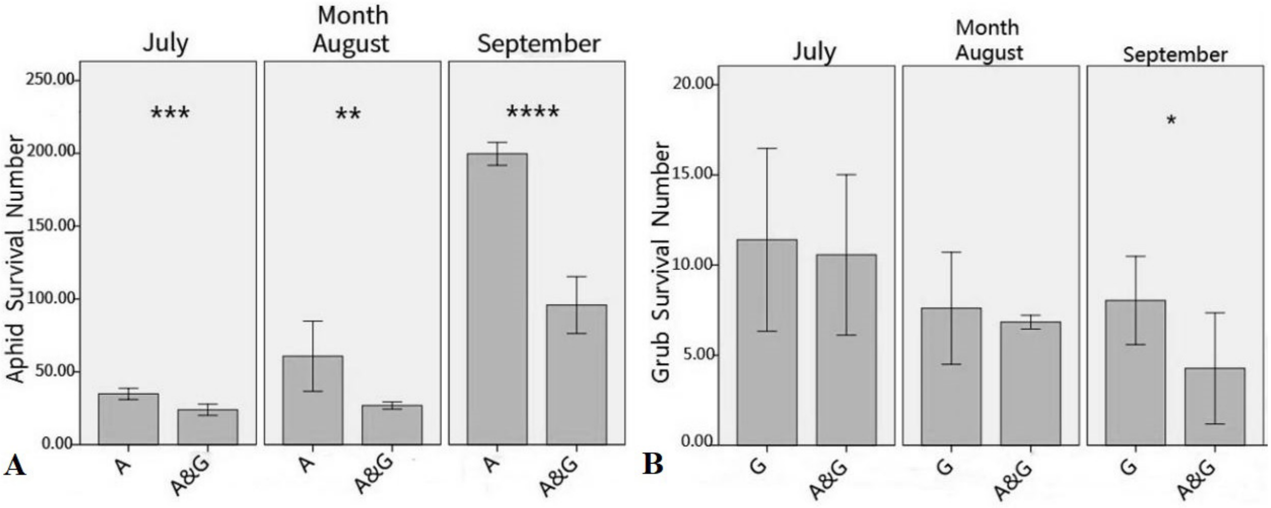

Fig. 2. Interactions between aphid (above-ground) (AG) and grub (below-ground) (BG) via mediated plant responses. (A) The densities of aphid survival with or without below-ground herbivore, both are AG and BG herbivore, and (B) The densities of grub survival with or without above-ground herbivore.
Notes: A & G, A, G groups are aphid and grub, aphid, grub, respectively. Values are means ± S.E. * $p< 0.05$, ** $p <0.01$, *** $p <0.001$, **** $p <0.0001$.

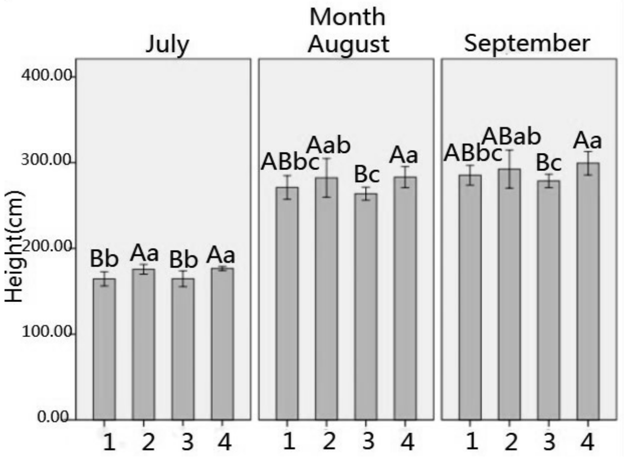

Fig. 3. The effects of above-ground herbivores (aphids) and below-ground herbivores (grubs) on corn.
Treatments 1, 2, 3, 4 in abscissa indicated: aphid and grub, aphid, grub, blank control, respectively; Lower case letters represented significant level of difference $p <0.05$; Upper case letters represented extremely significant levels of difference $p <0.01$.

Compared with C group, the plant height of G group decreased by 11.9 cm, 19.8 cm and 21.7 cm respectively in different months. The G group was slightly higher than A & G group. But there were no differences with A group (Fig. 3). Therefore, it was a significant negative effect between above- and below-ground insects by plant, and the plant development was related negatively and significantly to above-ground herbivores ($p <0.01$, $R_{AUG} = -0.701$, $R_{SEP} = -0.518$, Fig. 4A), BG herbivores ($p <0.01$, $R_{JUL} = -0.541$, $R_{AUG} = -0.479$, Fig. 4B) and their interaction.

Table I. The degree of linear correlation of among aphid (above-ground), grub (below-ground) and plant height.

Correlations		Aphid	Grub	High
Aphid	Pearson correlation	1	–0.555**	0.448**
	Sig. (2-tailed)		0.000	0.000
	N	99	99	99
Grub	Pearson correlation	–0.555**	1	–0.526**
	Sig. (2-tailed)	0.000		0.000
	N	99	99	99
Plant height	Pearson correlation	0.448**	–0.526**	1
	Sig. (2-tailed)	0.000	0.000	
	N	99	99	99

** $p<0.01$.

Correlation analysis of aphids, grubs and corn

Overall, all observations (experimental treatments) were significantly negative correlation between the above- and below-ground herbivores, below-ground herbivores and plant ($p <0.01$, successively r = –0.555, –0.526, Table I). However, the harms of both the above- and below-ground herbivores to the plant differed significantly.

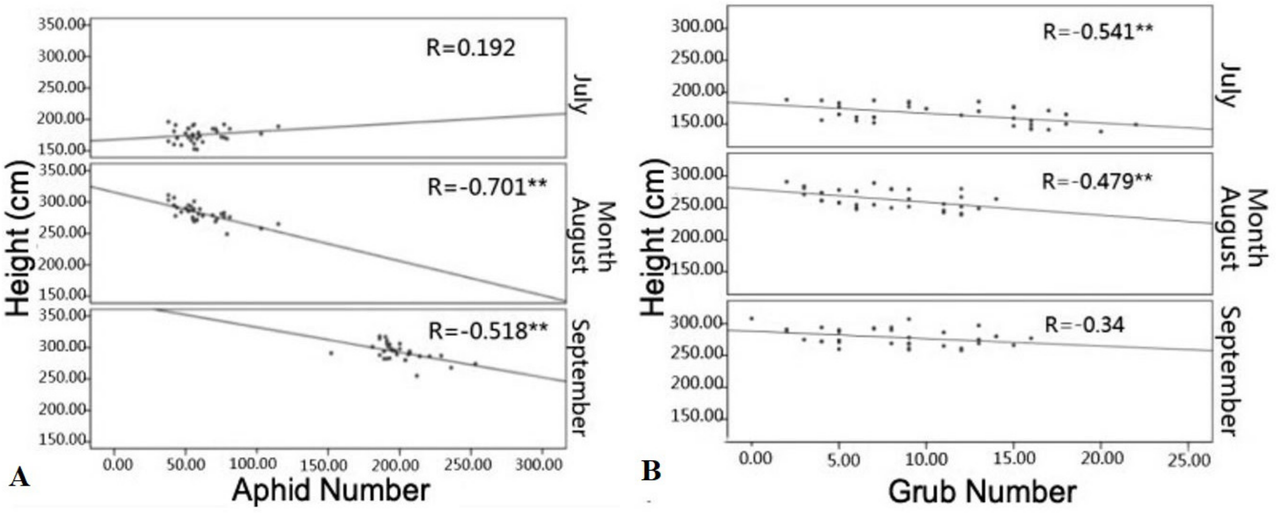

Fig. 4. The correlation analysis of (A) aphids and (B) grubs on corn plant.
Notes: ** $p<0.01$; R: Pearson correlation coefficient. Lines: the relationship between the two variables aphid or grub and plant (linear correlation).

The destruction of the plant roots by the BG herbivore influences the growth and development of the plant considerably. Therefore, it was significant negative relationship with plant development and BG or both herbivores. In the leaves, the plant was affected by above-ground herbivore, but its factor was significant as a main effect (*p* <0.01, r = 0.448, Table I), which showed that above- and below-ground herbivores were inhibited each other. Plant exposure to above- and below-ground herbivores resulted in high effect, when herbivores were on.

In terms of the relationship between aphids and maize niches, the discrete points were relatively clustered in July, indicating that the spatial niche occupied by aphids was narrow, and aphids had little impact on the growth of maize. The discrete points gradually dispersed in August and September, indicating that aphids occupied the spatial niche gradually increase, the impact of aphids on the growth of corn also gradually increases. In terms of the relationship between grubs and maize niches, the discrete points were extremely scattered in July, indicating that grubs occupied a wider spatial niche, and grubs had a greater impact on maize growth. The discrete points gradually gathered in August and September, indicating that grubs occupied the ecological space position gradually decreased, and the effect of grub on maize growth was weakened appropriately. The reason for this phenomenon may be related to the increase of grub's age and the enhancement of feeding function. In terms of the relationship between aphids and grub niches, the discrete points were most concentrated in August and the most dispersed in September, indicating that the interaction between the two insects was strongest in September, followed by July and the weakest in August (Fig. 5).

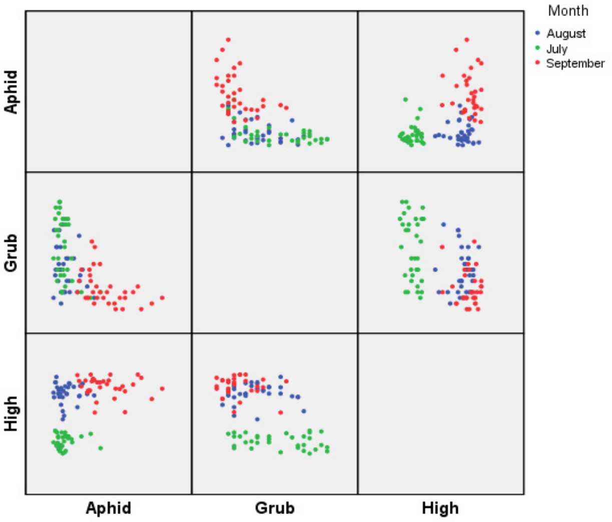

Fig. 5. The niche relationship among aphid (above-ground), grub (below-ground) and corn height.

DISCUSSION

The interaction between aboveground and underground insects was studied in this work. The results showed that the interactions of aboveground and underground insects was negative correlation with plants. As corn was consumed by leaf- and root-herbivores simultaneously, its development level decreased significantly, resulting in intense a significant reduction in the above- and below-ground insect herbivore populations. The interaction of two insects decisions in naturally occurring populations of leaf-feeding insect herbivores can be influenced by root-feeders, where a multitude of interactions occur simultaneously. Poveda *et al.* (2005) found that, the aboveground plant-insect interaction patterns was affected not only by aboveground factors, but also by a large number of different belowground processes mediated by wild mustard (*Sinapis arvensis*). Moreover, it was also found that plant-mediated interactions between above- and below-ground herbivores may vary according to the herbivorous species, herbivores and plant types (Johnson *et al*., 2013; Huang *et al*., 2013).

Studies have shown that plant-insect interactions are important for herbivore insect population change, as well as for their impact on population dynamics (van Geem *et al*., 2013). This is considered as a potential mechanism of compromise between plant growth and insects survival (Strauss and Irwin, 2004). Furthermore, since plants can fine-tune the relevant consumers of herbivorous insects through various interactions, it is worth investigating how they play a role through the relationship between above- and below-ground insects. Studies on the relationship between above- and below-ground interactions in food webs among large-scale crops are often overlooked. This may be a major problem in explaining why individual populations of insect are dominant pests. Thus, the study of multiple interactions between above- and below-ground insects and plants must be undertaken in order to understand the competitive pressures of coevolution of biological interactions (Masters and Brown, 1992; van Geem *et al*., 2013; Karban and Baldwin, 1997).

In different development stages of maize, A & G group had extremely significant inhibition on the individual number of aphid, while grub had a significant inhibitory effect only in the late growth stage of maize, indicating that the two insects inhibited each other and affected the population growth. Previous studies have shown that due to the simultaneous attack of host plants by two types of insects, the development level is significantly decreased and the population of both insects is suppressed and decreased in number (Brown and Lugo, 1992; Hatfield and Prueger, 2010). Poveda *et al.* (2005) suggested that there may be reciprocal inhibition between below-aboveground insects when host plants are fed. This interaction is important for both phytophagous insect population change and development trends and its impact on population dynamics (van Geem *et al*., 2013). Our findings are consistent with the conclusion of Pereira *et al*. (2020) that the belowground pest *Diabrotica speciose* and the aboveground pest *R. maidis* inhibit each other in cornfields. Due to the inhibitory effect of aboveground and underground herbivorous insects and host plants, in the farmland, the diversity of insect populations can be maintained. Through the interspecific competition relationship, the rapid increase of the population of individual dominant pests can be effectively suppressed, the outbreak of individual pests can be prevented, and the control of farmland pests can be realized.

During the experiment, only the plant height was measured, but the dry weight and yield of corn plant was not measured. The reason is that to measure the dry weight of corn, a large number of corn plants will be cut, which will disturb the sample plot, affect the change of farmland ecosystem habitat, thus affecting the population dynamic distribution pattern of phytophagous insects. The impact on yield will be considered in future relevant studies.

CONCLUSION

Overall, in A & G group significantly inhibited the number of above-ground individuals, while grubs only had a significant inhibitory effect in the late growth stage of maize. Grubs significantly affected the growth and development of maize, followed by the combined action of grubs and aphids. Only aphids had little effect on the plant height of maize. Through the correlation analysis, the interaction between the above-ground insects and the underground insects and the host was discussed, and the results showed that there was a very significant negative correlation between the three.

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Ethical statement

There are no research conducted on animals or humans.

Statement of conflict of interest

The authors have declared no conflict of interest.

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