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RESEARCH

A post-emergence herbicide program for weedy sunflower (*Helianthus annuus* L.) control in maize

Milan Brankov · Milena Simić · Theresa Piskackova · Miloš Zarić · Miloš Rajković · Natalija Pavlović · Vesna Dragičević

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Abstract During the last 15 years in Serbia, there has been an invasion of *H. annuus* across the country. Plants were initially limited to non-cultivated areas near arable fields, while in recent years the species has started to occur and establish populations in crop fields, especially into wide-row crops. We tested eight herbicides in two greenhouse experiments: 1) a dose-response study; 2) an efficacy study with reduced herbicide rates adding an adjuvant. The tested herbicides showed satisfactory weed control, where all estimated effective doses 90 (ED_{90}) were lower than the recommended field rate for each herbicide, except for dicamba. The addition of non-ionic surfactants significantly increased the efficacy of glyphosate, mesotrione, rimsulfuron, and foramsulfuron. Whereas, there was no clear advantage to adding an adjuvant to bentazone and tembotrione, as the *H. annuus* population was already very sensitive (plants died in 1/8 of recommended rate in a dose-response study). All tested herbicides, except dicamba, can be used for satisfactory *H. annuus* control in maize, while glyphosate can be used for control of the species in non-agricultural lands.

Keywords Invasive species · Chemical control · Adjuvants · Effective doses

M. Brankov (✉) · M. Simić · N. Pavlović · V. Dragičević
Maize Research Institute "Zemun Polje", Belgrade, Serbia
e-mail: mbrankov@mrizp.rs

T. Piskackova
Food and Natural Resources, Czech University of Life Sciences Prague, Prague, Czech Republic

M. Zarić
West Central Research, Extension and Education Center, University of Nebraska–Lincoln, North Platte, NE, USA

M. Rajković
Institute for Medicinal Plant Research "Dr. Josif Pančić", Belgrade, Serbia

Introduction

Recent changes to the typical climate across Europe have resulted in an increased frequency of certain weed species (Krähmer et al. 2020). In Serbia, several of the most severe weeds are classified as invasive: common ragweed (*Ambrosia artemisiifolia* L.), common milkweed (*Asclepias syriaca* L.), field dodder (*Cuscuta camprestris* L.), and common lambsquarters (*Chenopodium album* L.). Weedy sunflower (*Helianthus annuus* L.) is an invasive species in southern Europe and Serbia and a primary contributor to yield losses in row crops (Stojićević and Vrbničanin 2022). The invasion of *H. annuus* started about 15 years ago, when weeds were found close to roads, field margins, or irrigation channels; however, their expansion has now extended into fields, where *H. annuus* is a highly competitive species with crops (Kanatas et al. 2021). Stojićević (2018) reported more than 200 populations across Serbia dominant on uncultivable areas, on roadsides and fields, with some having a density of more than 200 plants per square meter. While it is an economic problem only within the field, the spread along roadsides is the mainspring that needs to be targeted. It has been reported that competition from *H. annuus* has caused significant losses in row crops, especially in maize (*Zea mays* L.) (Deines et al. 2004) and soybean (*Glycine max* L. [Merit]) (Allen et al., 2000). Furthermore, according to Deines et al. (2004) *H. annuus* at a density of just four plants m^{-2} are enough to reduce maize yield up to 46%. Moreover, recent reports from Serbian fields (Ilić et al. 2022) have indicated high abundance in field and vegetable crops, causing loses from 5 to 33%. Taken together, a post program for controlling this weed is an imperative task for producers.

Such a task is not as straightforward as many other invasive weeds. Domesticated sunflower (also *Helianthus annuus* L.) is a major crop in Serbia and can be grown with two herbicide resistance (HR) traits, either to imazamox (IMI) or tribenuron (SU); both are herbicides that inhibit aceto-lactate synthase (ALS) (Presotto et al. 2012). Recent adoption of Clearfield® technology in sunflower resulted in heightened awareness of possible gene transfer from ALS tolerant sunflower to wild *H. annuus*, as natural gene flow can happen from herbicide-tolerant varieties to wild relatives (Presotto et al. 2012). This flow is possible depending on the overlap in flowering period, wind speed and direction, and distance between plants (Bozic et al. 2015). Božić et al. (2019) tested the successive generations of weedy *H. annuus* progeny which grew close to imazamox and tribenuron-methyl resistant hybrids and found evidence of some increased tolerance to tribenuron-methyl, but not to imazamox.

Still, little research has been focused on possible solutions for *H. annuus* in row crops other than sunflower. The research in Serbia has been mostly focused on evaluating the possible gene flow from sunflower to weedy relatives, rather than finding practical solutions for weedy sunflower control. There are only a few investigations regarding possible control of weedy sunflower. Ilić et al. (2022) reported nicosulfuron efficacy of two *H. annuus* populations and found lower susceptibility of the tested population. Furthermore, Vrbničanin et al. (2017) reported weedy sunflower fitness followed by nicosulfuron applications. As maize is the most extensively planted crop in Serbia with an area of 0.9–1.1 million of hectares per year (Anonymous, 2021), and post-emergence (POST) herbicides are most commonly used as correction treatments following pre-emergence herbicides for weed control, it was of particular importance to assess the possible herbicides for POST herbicide treatments to control *H. annuus* in maize and adjoining areas, such as field margins and irrigation channels, as the most likely source of new field infestations.

With the ambition to reduce herbicide applications across the European Union (Tataridas et al. 2022), the EU Green Deal aims to reduce the herbicide use up to 50% by 2030. As herbicides are registered for application in certain rates, any deviation from application rates might result in herbicide resistance evolution, bearing in mind that weeds could survive those rates, as they might be sub-lethal (Gressel, 2011). Nevertheless, there are still available options to apply reduced rates and maintain high level of efficacy. Adding adjuvants into the tank together with herbicides may lead to increased weed control, hence adjuvants change physico-chemical characteristics of the solution, enabling higher uptake of herbicides (Hazen 2000). Therefore, our research sought to evaluate the response of *H. annuus* to seven POST applied herbicides for weed control in maize analysing dose-response of each herbicide. The research also included the total, non-selective herbicide glyphosate, given that many populations still grow on field margins and irrigation channels. The second study aimed to evaluate possible reduction of herbicide rates by adding a NIS adjuvant into the tank.

Material and methods

Even near the research station of the MRIZP, there are many instances of *H. annuus* populations expanding from field margins into fields, therefore many local populations were available for sampling. *H. annuus* seeds were collected across 10 localities near the MRIZP (Fig. 1) in the October 2021 and combined into one composite collection of sunflower seeds. These locations were selected because MRIZP grows maize on more than 1000 ha and recently, weedy sunflower has started invasion on maize fields, reducing yields significantly. Seeds were cleaned and stored in the refrigerator at 5 °C until sowing.

Fig. 1 The locations where *H. annuus* seeds were collected (the area of 8 km^2), Google Maps, accessed 16. Sep 2023

Two greenhouse experiments were conducted at the Maize Research Institute "Zemun Polje" (MRIZP), Belgrade, Serbia, during 2022 (a dose-response study) and 2023 year (an efficacy study). Eight herbicides were used in the experiment (seven herbicides labelled for weed control in maize, and a total herbicide glyphosate) (Table 1).

For both experiments, *H. annuus* seeds were planted and grown in D40H cone-tainer cells plastic cones (6.9 cm in diameter, 35.6 cm depth, the volume of 983 mL) (Stuewe and Sons, Inc., Corvallis, OR 97389, USA) filled with growing medium (Floragard, Oldenburg, Germany). Plants were watered and fertilized as needed. The greenhouse was maintained at 30/20 °C day/night and 16/8 h photoperiod (850 µmol m^{-2} s^{-1} photosynthetic photon flux). Initially, 5–10 seeds were planted per cone, and later thinned to one plant per cone, representing one replication. When reached 10–15 cm height (4–6 true leaves) plants were moved to a research spray chamber (Avico Praha, Prague, Czech Republic), and following the application were returned to the greenhouse, and grown for another 21 days. For applications, an AI95015EVS nozzle was used calibrated to deliver 93.5 L ha^{-1} at 414 kPa. After 21 days, plants were harvested (cut at soil surface) and dried at 60 °C to constant mass. All data were converted into a percentage (%) of reduction compared to untreated control (4.5 g ± 0.31).

Dose-response study The experiment was conducted as a complete block design with four replications in two experimental runs (the 1st run April-Jun 2022; the 2nd run July–September 2022). One *H. annuus* plant was considered as one replication. All herbicides were applied in the following doses: 0.125X, 0.25X, 0.5X, 1X, 2X, 4X, 8X, where X matches to the field use rate of each herbicide (Table 1). The experiment contained the untreated check, where plants were grown under the same

Table 1 The list of tested herbicides for *H. annuus* control in maize

Herbicide	HRAC group	Product	Producer	1X rate (g a.i. / ha)
Bentazone	6	Bentamark 480 SC	Ningbo Sunjoy Agroscience	1440
Dicamba	4	Plamen SC	Galenika-Fitofarmacija	288.9
Foramsulfuron	2	Equip	BASF	45
Glyphosate	9	Bingo 480	Agroarm	960
Mesotrione	27	Intermezzo	Agrosava	120
Nicosulfuron	2	Motivell Extra 6 OD	Belchim	45
Rimsulfuron	2	Rimex	Agrosava	60
Tembotrione	27	Laudis	BASF	88

Hrac Herbicide resistance action committee, *Glyphosate* a total herbicide, not registered for use in conventional maize production, *1X rate* recommended rate of product, *a.i.* active ingredient conditions. The model selection function *mselect* tool in R software (R Foundation for Statistical Computing, Vienna, Austria) was used to compare models, and Weibull (type 1) was selected as the best-fit model based on Akaike's information criterion (data not shown) for *H. annuus* biomass reduction, which was analyzed using the *drc* package in R software (Ritz et al. 2015) following the Eq. (1):

$$Y = c + (d - c)\exp(-\exp(b(\log(x) - \log(e)))) \quad (1)$$

where y represents biomass reduction (%), b is the slope at the inflection point, c is the lower limit of the model, d is the upper limit, and e is the inflection point (distance to 50, 90, and 95 biomass reduction (%)). Data from the two experimental runs were combined, with replications and experimental runs considered random effects.

Efficacy study The experiment was conducted as a randomized complete block design with four replications in two experimental runs (the 1st run Feb-Apr 2023; the 2nd run May-Jun 2023). Again, one *H. annuus* plant was considered as one replication. The same herbicides were applied as in the previous study (Table 1), while using reduced doses 0.25X, and 0.5, as well as 1X alone and including an adjuvant - non-ionic surfactant (NIS, 1 L ha^{-1}) (Dash, BASF, Germany). Justification for only including this adjuvant for these trials was confirmed by previous research under field conditions (Brankov et al., 2023a). The experiment also included untreated control plants. The data obtained were processed using the statistical package STATISTICA 8.0 for Windows (TIBCO Software Inc., Palo Alto, CA, USA). The differences between the treatments were determined by two-way analysis of variance (ANOVA), with mean separations made at $\alpha = 0.05$ level using Tukey's post hoc test. Since the effects of herbicides and adjuvants were significant, comparisons were made for each herbicide within rate and adjuvant used.

Results

Dose-response study

According to the obtained data, sunflower was the most sensitive to bentazone and tembotrione, where plants initially died 7 days after treatment, at all rates. Under such conditions, the model could not estimate the following values: ED_{50}, ED_{90}, or ED_{95} (Table 2). Also, the model could not estimate values for foramsulfuron and rimsulfuron, indicating high susceptibility of *H. annuus* to those herbicides. Less than half of the recommended field rate of glyphosate was needed for 90% biomass reduction. ED_{95} for nicosulfuron and mesotrione were 25 and 28 g, respectively. *H. annuus* showed tolerance only to dicamba, where ED_{50} was close to recommended field rate, while ED_{95} was 2.5-fold higher than the field recommended rate.

Efficacy study

Efficacy for bentazone, mesotrione, and tembotrione was high in all treatments (93.6% - 96.8%), and the influence of added adjuvants was not clearly seen (Table 3) Efficacy for bentazone, mesotrione,

Table 2 Percentage of biomass reduction of *H. annuus* influenced by herbicides and adjuvants

Herbicide	Treatments					
	0.25X	0.25X + adj	0.5X	0.5X + adj	1X	1X + adj
	% of biomass reduction (±SD)					
Bentazone	94.9 ± 1.9 a	95.3 ± 1.7 a	95.7 ± 1.9 a	94.8 ± 1.6 a	95.8 ± 1.7 a	94.7 ± 2.7 a
Dicamba	60.5 ± 6.5 b	63.4 ± 7.2 b	71.0 ± 6.5 b	72.3 ± 8.4 b	79.9 ± 3.0 a	82.0 ± 7.4 a
Foramsulfuron	88.7 ± 3.4 b	98.2 ± 1.6 a	92.4 ± 2.5 b	98.0 ± 1.7 a	92.2 ± 1.6 b	98.0 ± 1.3 a
Glyphosate	48.1 ± 9.6 c	96.2 ± 1.6 a	88.9 ± 2.5 b	98.0 ± 1.7 a	91.7 ± 2.7 b	97.0 ± 1.1 a
Mesotrione	94.7 ± 1.2 a	93.6 ± 1.7 a	95.8 ± 1.3a	93.7 ± 3.2a	95.3 ± 1.0a	94.3 ± 3.2 a
Nicosulfuron	40.1 ± 9.7 c	94.8 ± 2.8 a	81.0 ± 6.5 b	95.4 ± 3.3 a	92.3 ± 6.2 b	97.0 ± 1.0 a
Rimsulfuron	83.4 ± 7.2 b	90.7 ± 2.7 a	85.7 ± 5.8 b	91.4 ± 3.6 a	94.2 ± 2.0 a	96.9 ± 2.4 a
Tembotrione	96.1 ± 1.8 a	96.8 ± 0.8 a	95.7 ± 0.9 a	95.2 ± 1.5 a	94.4 ± 2.9a	95.2 ± 1.1 a

Means followed by the same letter within the same herbicide do not differ using Tukey's test at $\alpha = 0.05$. X: herbicide rate; adj: NIS adjuvant

Table 3 Influence of applied herbicides on 50 (ED_{50}), 90% (ED_{90}), and 95% (ED_{95}) of *H. annuus* biomass reduction at 21 DAT

Herbicide	ED_{50}		ED_{90}		ED_{95}		Field recommended rate
	g a.i. or a.e. / ha ± SE						
Bentazone	N/A		N/A		N/A		1440
Dicamba	274.1	± 35.7	551.8	± 215.8	720.9	± 400.9	288.9
Foramsulfuron	N/A		N/A		N/A		45
Glyphosate	301.1	± 19.9	459.3	± 70.5	539.8	± 103.1	960
Mesotrione	14.8	± 11.1	21.9	± 14.0	25.4	± 15.5	120
Nicosulfuron	13.7	± 0.61	23.2	± 2.9	28.4	± 4.6	45
Rimsulfuron	N/A		N/A		N/A		60
Tembotrione	N/A		N/A		N/A		88

ED effective dose needed to achieve a certain percentage of the biomass reduction, ED_{50} 50% of biomass reduction, ED_{90} 90% of biomass reduction, ED_{95} 95% of biomass reduction, *N/A* not applicable (the model could not estimate ED values due to very high susceptibility of *H. annuus*)

and tembotrione was high in all treatments (93.6% - 96.8%), and the influence of added adjuvants was not clearly seen (Table 3). However, all plants died 3–7 days after bentazone and tembotrione indicated very high susceptibility to those herbicides. Sunflower survived longer after application of mesotrione, 17–21 days following applications. Foramsulfuron and rimsulfuron efficacy was increased when NIS adjuvants were added (up to 9.5% at 0.25X of foramsulfuron and 7.3% using the same rate for rimsulfuron), in all treatments. Among all sulfonylureas, nicosulfuron showed the least efficacy, especially applied at 0.25X (40.1% of biomass reduction). The 0.5 rate of nicosulfuron did not provide satisfactory control (81.0%), while adding the adjuvant improved efficacy in all treatments. Satisfactory control of *H. annuus* was not obtained using reduced rates or the field recommended rate of dicamba. Furthermore, adding the adjuvant did not influence efficacy.

Discussion

While maize and soybean (*Glycine max* [L.] Merr.) present large crop area worldwide and *H. annuus* is an increasingly problematic weed in these crops, no previous research has directly examined the efficacy of POST herbicides of interest for possible *H. annuus* control in Serbia. This study demonstrates high sensitivity of *H. annuus* to bentazone, tembotrione, rimsulfuron, and mesotrione, even at 1/8 the recommended rate (Fig. 2). The susceptibility at partial rates should imply that full rate applications should not promote Non-Target Site (NTS) resistance in surviving plants (Suzukawa et al., 2021). This evidence may advise use of these herbicides at the full rates as part of an IPM strategy and herbicide rotation plan (Norsworthy et al., 2012). Furthermore, bentazone can be used in soybean as well.

Glyphosate is a non-selective herbicide, approved for 10 years more by the EC,and it can be used for weed control on non-agriculture lands in Europe and in certain HR crops in other parts of the world. In our research, we found that less than ½ of the field recommended rate was needed for 90% of *H. annuus* biomass reduction. While recent literature has reported the potential of glyphosate resistance across *H. annuus* populations (Singh et al., 2020), our results of increased efficacy by adding an adjuvant should recommend its use and safeguard the potential for NTS. At this time in Europe, glyphosate could efficiently control *H. annuus* growing adjacent to fields, directly reducing spreading potential of the species.

Dicamba is a useful mode of action against a variety of broadleaf weeds in maize and has become a popular herbicide addition in HR soybean in North and South America. However, our results indicate that dicamba is not an effective mode of action for this population of *H. annuus*, even when an adjuvant is added. While other adjuvants may also improve dicamba efficacy, NIS has been shown as an adequate partner for dicamba (Creech et al., 2016); although,

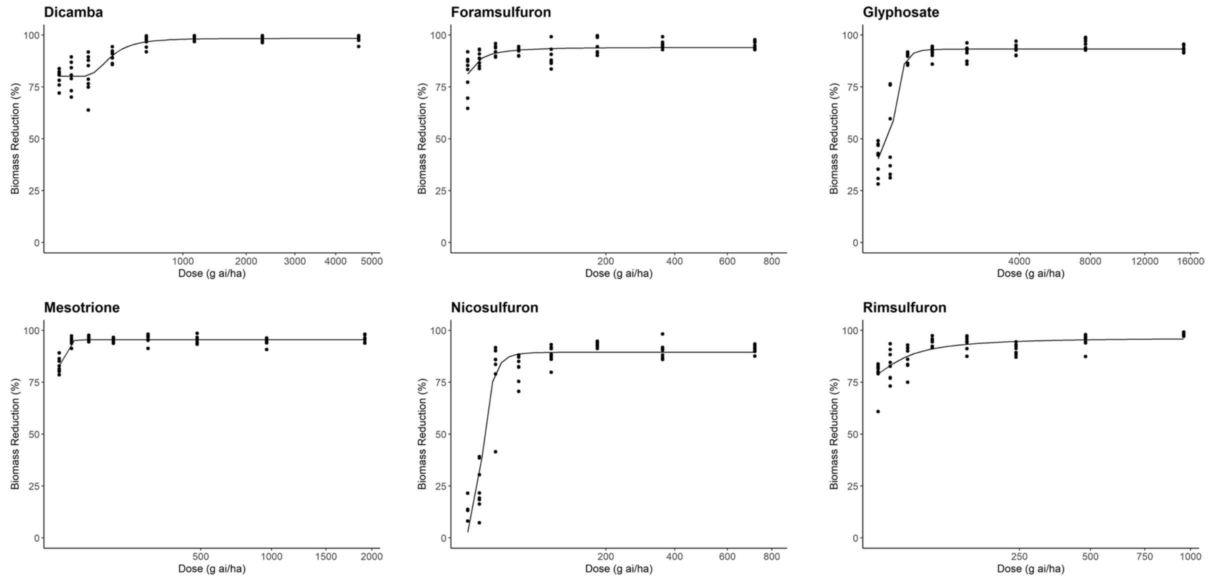

Fig. 2 Biomass reduction curves of *H. annuus* exposed to dicamba, foramsulfuron, glyphosate, mesotrione, nicosulfuron, and rimsulfuron (the graphs containing bentazone and tembotrione are not shown due to high efficacy and unsuitability for curve fitting)

coarser droplet size may improve herbicide uptake necessary for a systemic herbicide like dicamba (Creech et al., 2016).

Acknowledging the concern for ALS resistance in *H. annuus,* either inherent or acquired through cross-pollination with domesticated sunflower (Bozic et al. 2015), we found the ALS herbicide results most interesting. The *H. annuus* population tested was very susceptible to foramsulfuron and rimsulfuron, where plants died at 1/8 of the field rate. Consequently the model could not estimate the ED values of interest (Table 3). While Ilić et al. (2022) reported lower susceptibility of some *H. annuus* population in Serbia to nicosulfuron, our results demonstrated 95% biomass reduction at less than ½ of the field rate, even without an adjuvant. Best management practices would still recommend using these herbicides with caution and probably in combination with other effective modes of action.

In our research we tested all herbicides in reduced rates, combining them with non-ionic surfactant. As adjuvants are known to increase herbicide efficacy, adjuvant inclusion could allow for effective control with lower rates of herbicides (Delvin et al., 1991; Bunting et al., 2004). Indeed, previous research in Serbia has also confirmed adjuvants as potential tools for increasing herbicide activity and efficacy (Brankov et al., 2023b). This study also supports the addition of NIS adjuvant as a key factor for increased efficacy in the SU herbicides and glyphosate (Table 3). The effect of the NIS adjuvant could be seen with ALS inhibitors clearly, supporting previous research where non-ionic surfactants increased efficacy when NIS adjuvants were added into the tank (Idziak et al. 2023; Sobiech et al. 2020). NIS adjuvants are water soluble chemical and lipid compounds that are not molecularly charged (positive or negative). They reduce the surface tension of the water molecule, which enable the water droplet to cover a greater leaf surface area. While bentazone, mesotrione, and tembotrione all performed well enough alone that no adjuvant advantage was apparent. Glyphosate applied with an adjuvant provided the best control improvement: from 48% applied alone to 96% applied with NIS.

Dicamba was the only tested herbicide which did not benefit from the inclusion of NIS. Those results are not in the line with the previous published results (Polli et al. 2021). Weedy sunflower plants had the characteristics symptoms of the auxin herbicides although biomass reduction was about 80% of the control (Table 2). As dicamba is an auxin herbicide, its might prolong growth of weedy sunflower up to certain time, indicating that biomass reduction was not on satisfactory level. On the other hand, visual observation of injury (data not shown) indicated that those plants on 21st day were highly damaged. Further tests will be needed using other adjuvants in order to increase dicamba efficacy.

While yield protection in maize is a major focus of this research, limiting *H. annuus* management to the boundaries of a field where maize is grown will not be enough to contain invasion. As already mentioned, *H. annuus* began in field margins and non-crop areas and has gradually spread into row crop fields. The most recent literature reported that weeds close to fields may receive sub-lethal herbicide doses, and surviving then may evolve metabolic resistance. Gressel (2011) reported that low pesticide rates may hasten the evolution of resistance. Furthermore, Vieira et al. (2020) reported *Amaranth* species increased tolerance to glyphosate, dicamba, and 2,4-D following exposure to sub-lethal rates via spray particle drift. Neglected and unmanaged, *H. annuus* found in the border areas and grassy corridors can be a seed source, reservoir for plant pathogens and pests, and harbour genetic variability for herbicide resistance. Therefore, it is of particular importance to manage the vegetative communities in field margins and areas adjacent to fields as well as promote beneficial species. A holistic strategy should include coordinated management between neighbours and land managers of shared borders.

Conclusions

Based on our research, selected herbicides present several options for treatment against *H. annuus*. The tested population was highly sensitive to tembotrione and bentazone, which can be recommended, especially for their contribution to diversifying herbicide mode of action. Nevertheless, mesotrione, and other ALS inhibiting herbicides tested in the study (rimsulfuron, foramsulfuron, and nicosulfuron) also indicated successful species control. Dicamba did not show satisfactory weed control with or without NIS, but further testing could be done with other adjuvant partners. At the present time, glyphosate applied with NIS could be a good option in for *H. annuus* control on non-agricultural areas. Furthermore, the recommendation to add adjuvants into the tank with herbicides might enable using lower herbicide rates for *H. annuus* control, if used in conjunction with other diverse tactics to reduce the risk of developing herbicide resistance.

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Data availability All data and materials are available on the reasonable request.

Declarations

Ethical approval Not Applicable.

Consent to participate All authors have read and approved the submission.

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