IS MINERAL NUTRITION OF GLYPHOSATE-RESISTANT CROPS ALTERED BY GLYPHOSATE TREATMENT?

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Summary
Claims have been made that glyphosate application to glyphosate-resistant (GR) crops can result in deficiencies in certain mineral elements in those crops and that this is a cause of increased plant diseases. Strong evidence from multiyear and multisite studies has not verified these claims. Furthermore, these studies and others have found that glyphosate has no effect or a slight stimulation of yield of GR crops.

Introduction
Crops made resistant to the herbicide glyphosate represent about 80% of the acreage of transgenic (GM) crops grown worldwide (Duke, 2018a). Their continued phenomenal success over the past 23 years has resulted from the ability to use perhaps the best herbicide yet devised (Duke & Powles, 2008) with high-yielding varieties of soybean, maize, canola, sugarbeet, alfalfa, and cotton. Yet, there has been controversy over whether glyphosate adversely affects mineral nutrition of glyphosate-resistant (GR) crops (*e.g.*, Bott *et al.,* 2008; Yamada *et al*., 2009; Zobiole *et al*., 2010a, 2011). These claims have been linked to claims that GR crops are more susceptible to some plant diseases due to manganese deficiencies and other causes (Yamada *et al*., 2009) and to yield decreases (*e.g.*, Bott *et al*., 2008; Zobiole *et al*., 2010b). Two proposed mechanisms of purported glyphosate effects on plant mineral nutrition have been proposed: 1) direct effects by chelation of mineral cations, especially divalent cations such as Mn^{++} and 2) toxic effects on rhizoshere microbes involved in plant mineral assimilation. Although, an analysis of all the literature on this topic concluded that most of the literature did not support the view that glyphosate use in GR crops caused these problems (Duke *et al*., 2012), these claims of adverse effects have received considerable attention from farmers and the general public, and they persist in reviews (*e.g.*, Martinez *et al*., 2018) and on websites (*e.g.*, https://fluidfertilizer.org/wp-content/uploads/2016/05/58P20-22.pdf). More recently, even stronger evidence refuting claims of altered mineral nutrition in glyphosate-treated GR crops has been published, while virtually no findings to the contrary have been reported. This short review discusses these new papers and the strong case for a lack of an effect.

Direct evidence
The tour de force study was that of Kandel *et al.* (2015), who examined the effect of glyphosate on plant mineral content, disease, and yield in GR soybean over three years at sites in five US states and one Canadian province with a wide variety of growing conditions. There was no effect of glyphosate on Mn content. Although there were a few inconsistent significant effects of glyphosate treatment on some elements, the effects appeared random among years, sites, and treatments, sometimes with higher levels in the glyphosate treatments (*e.g.*, Zn in Ontario in one of two years). This level of random effects is consistent with the rate of false positive and false negatives that one might expect at the 95% level of confidence. The authors stated that no nutrient deficiency symptoms were apparent, and yield was unaffected or slightly increased by glyphosate.

In a two-year study with GR soybean and at two sites near each other in the state of Mississippi (one with a previous multiyear continuous glyphosate use and another with no history of glyphosate use), no short term or long-term effects of glyphosate use were found on mineral content of leaves or harvested seed (Duke *et al.,* 2018) (Table 1). Similarly, in a two year study with GR maize in Mississippi and Illinois, with a long-term use and a no previous glyphosate use site in both states, no effect of glyphosate was found on the content of any element in leaves or harvested grain (Reddy *et al*., 2018) (Table 1). In both the soybean and maize studies, a non-GR variety was used as a control to see if the GR genes influence mineral nutrition. It did not.

The three studies discussed above are the most rigorous studies to examine the effect of glyphosate on mineral content of GR crops. They were done over a wide geographic area over multiple years, and the results consistently indicate no effect of glyphosate on GR crop mineral nutrition. Another recent study from Brazil reported no effect of glyphosate on mineral nutrition or yield of GR maize (Costa *et al*., 2018).

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Table 1. Effect of glyphosate on mineral content of leaves and seed of GR soybean, GR maize, and GR sweet corn.

Ca	Cu	Fe	Mg	Mn	Ni	Zn
			Soybean leaf[a]			
NE[b]	NE	NE	NE	NE	NE	NE
			Soybean seed			
NE	NE	NE	NE	NE	NE	NE
			Maize leaf[c]			
NE	NE	NE	NE	NE	NE	NE
			Maize seed			
NE	NE	NE	NE	NE	NE	NE

[a]combined results from two sites over two years in Mississippi (from Duke *et al.*, 2018)
[b]NE = no effect of glyphosate compared to non-treated control
[c]combined results from two sites over two years each in two U S states (Mississippi and Illinois) (from Reddy *et al.*, 2018)

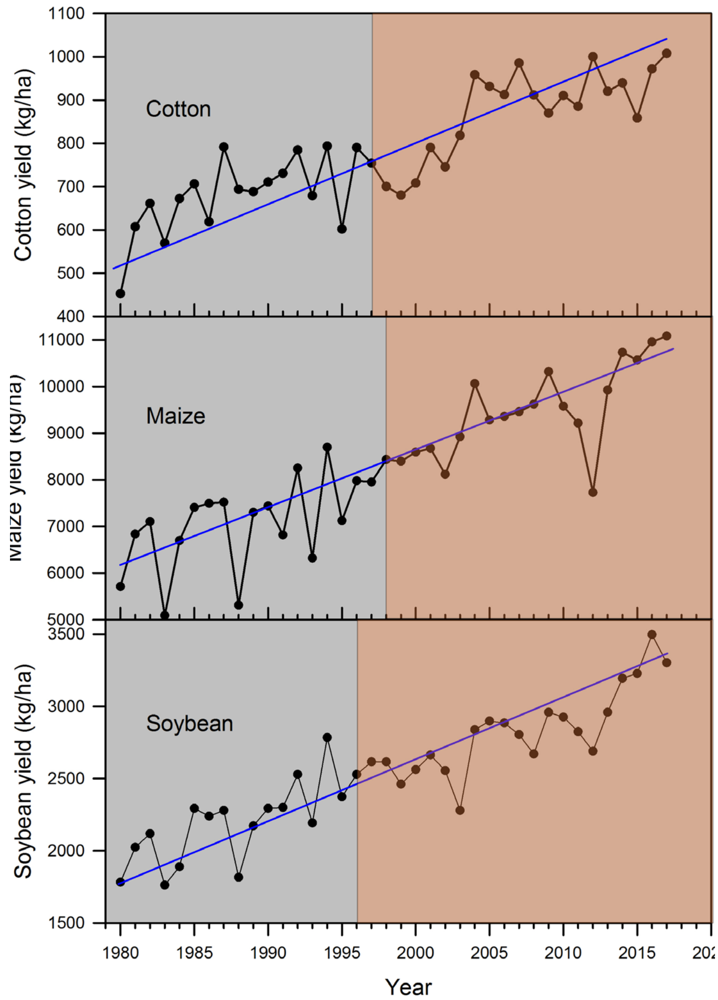

Figure 1. U.S. yields of the three crops over the past 38 years that are now grown mostly as GR cultivars. The shaded areas represent the years since the introduction of each GR crop. Data are from the USDA, National Agricultural Statistics Service Data and Statistics Web site: https://www.nass.usda.gov/Publications/Todays_Reports/reports/croptr18.pdf. This is an updated version of a previous figure from Duke *et al.* (2012).

Indirect evidence

Perhaps the best indirect evidence of the lack of an adverse effect on mineral health of GR crops is their extremely high level of resistance to glyphosate. It is surprising that there is only one paper that reports properly conducted dose/response studies to determine the resistance level. Nandula *et al.* (2007) found a 50-fold level of resistance to glyphosate in both GR soybean and canola. If glyphosate significantly impaired plant mineral nutrition, this level of resistance would be impossible because many fundamental biochemical and physiological processes depend on adequate levels of minerals such as iron, manganese, magnesium, etc. With GR soybeans, under some conditions, applied glyphosate can be metabolized to aminomethylphosphonic acid (AMPA) so rapidly that the AMPA can cause slight phytotoxicity in the form of a transient chorosis that has little or no effect on yield (Reddy *et al.*, 2004). This uncommon phenomenon is not associated with mineral deficiencies and is not seen in GR maize, which produces much less AMPA in glyphosate-treated tissues.

Likewise, lack of a glyphosate treatment effect on yield supports the view that plant mineral nutrition is not affected. Duke *et al.* (2012) plotted US yield data for soybean, maize, and cotton, before and after adoption of GR varieties of these crops. An updated version of this plot is provided in Fig. 1. In the US, about 90% of the acreage of these crops is planted with GR varieties (Duke, 2018). The general increase in yield with time is about the same before and after adoption of GR versions of these crops. Indeed, the recent multiyear, multisite studies of GR soybean and maize have reported either no effect of glyphosate on yield, or a small increase at some sites in the upper Midwest of the US and in Brazil (Kandel *et al.*, 2015: Duke *et al.*, 2018; Reddy *et al.*, 2018; Silva *et al.*, 2018: Williams *et al.*, 2014).

As mentioned earlier, claims have been made that the reduction of some minerals, especially Mn, leads to greater susceptibility to plant disease (Yamada *et al.*, 2009). Glyphosate treatment does increase non-GR plants' susceptibility to plant diseases through inhibition of synthesis of shikimate pathway-based plant disease defenses. Hammerschmidt (2018) reviewed this phenomenon, and the literature that shows that glyphosate reduction in plant disease defenses does not happen in GR crops. The large study of Kandel *et al.* (2015) found no influence of glyphosate treatments on sudden death syndrome (*Fusarium virguliforme*) in GR soybean at any of their many sites for either year of the study, and Williams *et al.* (2015) found no effect of glyphosate on incidence of Goss's wilt (*Clavibacter michiganensis* ssp. *Nebraskensis*) in GR maize at either site over two years. Indeed, glyphosate is fungitoxic to some plant pathogens (especially rusts) and can act as a fungicide, albeit a generally weak one compared to commercial fungicides, for these microbes in GR crops (Duke *et al.*, 2007; Duke, 2018b).

There are two arguments against the chelation theory of effects on mineral content that are discussed in detail in Duke *et al.* (2012). First, when one calculates the relationship between the number of divalent metal cations in the plant and the number of glyphosate molecules taken up by a treated plant at recommended glyphosate rates for weed management, the ratio is huge. So, glyphosate would chelate an insignificant proportion of the metal cations. Second, even better chelators than glyphosate are used to get metal cations, especially Fe, into plants, and they do not cause mineral deficiencies or phytotoxicity.

Lastly, successful farmers are intelligent. They would become aware of any significant adverse effects of glyphosate use on GR crops rather quickly. Adoption of GR crops has been very rapid, saving them billions of dollars in weed management costs worldwide (Brookes & Barfoot, 2015). Yields have not been compromised, and profits have increased in most places with GR crops. The only significant downside has been the evolution of GR weeds due to the overuse of glyphosate (Heap & Duke, 2018), a result of farmers being happy with GR crops. Clearly, if there were significant adverse effects of glyphosate on crop mineral nutrition and disease, farmers would use other means of weed management.

The papers finding no effects of glyphosate on mineral nutrition of GR crops may represent a small proportion of the existing data with this result, as journals are reticent to publish results indicating no treatment effect (no difference). Nevertheless, the majority of the existing literature related to this question supports the view that mineral nutrition in GR crops is not a problem.

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