Pest Management Science

Pest Manag Sci 64:319–325 (2008)

Mini-review

Glyphosate: a once-in-a-century herbicide

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Abstract: Since its commercial introduction in 1974, glyphosate [*N*-(phosphonomethyl)glycine] has become the dominant herbicide worldwide. There are several reasons for its success. Glyphosate is a highly effective broad-spectrum herbicide, yet it is very toxicologically and environmentally safe. Glyphosate translocates well, and its action is slow enough to take advantage of this. Glyphosate is the only herbicide that targets 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS), so there are no competing herbicide analogs or classes. Since glyphosate became a generic compound, its cost has dropped dramatically. Perhaps the most important aspect of the success of glyphosate has been the introduction of transgenic, glyphosate-resistant crops in 1996. Almost 90% of all transgenic crops grown worldwide are glyphosate resistant, and the adoption of these crops is increasing at a steady pace. Glyphosate/glyphosate-resistant crop weed management offers significant environmental and other benefits over the technologies that it replaces. The use of this virtually ideal herbicide is now being threatened by the evolution of glyphosate-resistant weeds. Adoption of resistance management practices will be required to maintain the benefits of glyphosate technologies for future generations.

Keywords: glyphosate; GMO; herbicide; herbicide-resistant crop

1 INTRODUCTION

The era of weed management with synthetic herbicides began in earnest after World War II with the introduction of 2,4-D. So the title of this paper is perhaps presumptuous, considering we are only 60 years into this era. Nevertheless, the simple molecule glyphosate [*N*-(phosphonomethyl)glycine] is the most important herbicide of this period. This review will discuss why glyphosate more closely approximates to a perfect herbicide than any other, and considers how the advent of transgenic crops has catapulted glyphosate to the dominant herbicide of this time. The review concludes by discussing how the combination of glyphosate overreliance and the evolutionary potential of weed species threatens glyphosate's efficacy and sustainability as a precious herbicide resource for world agriculture. There are other, more encyclopaedic reviews and books on glyphosate[1–4] and glyphosate-resistant (GR) crops,[5–7] but there is none that approaches this topic from the viewpoint that is taken in this short review.

2 THE HERBICIDE GLYPHOSATE

2.1 History

As reported by Franz *et al.*,[1] the glyphosate molecule was apparently first synthesized by Henri Martin of a small Swiss pharmaceuticial company (Cilag), but was not tested or at least patented for herbicidal use. John E Franz of Monsanto Co. first synthesized and tested glyphosate as a herbicide in 1970,[1] and it was soon after patented for herbicide use.[2] Glyphosate is anionic at physiological pH levels. It is active as a salt with various cations (e.g. the sodium or isopropylamine salts). The isopropylamine salt of glyphosate first reached the market in 1974 as a post-emergence, non-selective herbicide, and its popularity grew steadily for the many reasons outlined below.

2.2 Attributes that contribute to glyphosate success

2.2.1 *Mode of action*

Glyphosate mode of action is unique in that it is the only molecule that is highly effective at inhibiting the enzyme 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS) of the shikimate pathway (Fig. 1). Glyphosate is a transition state analog of phosphoenylpyruvate, one of the substrates for EPSPS. Inhibition of EPSPS leads to reduced feedback inhibition of the pathway, resulting in massive carbon flow to shikimate-3-phosphate, which is converted into high levels of shikimate.[3] The high levels of shikimate that rapidly accumulate in glyphosate-treated plant tissues was the clue that led N Amrhein and his coworkers to discover EPSPS as the molecular target site of glyphosate.[8]

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(*Received 13 June 2007; accepted 9 August 2007*)

DOI: 10.1002/ps.1518

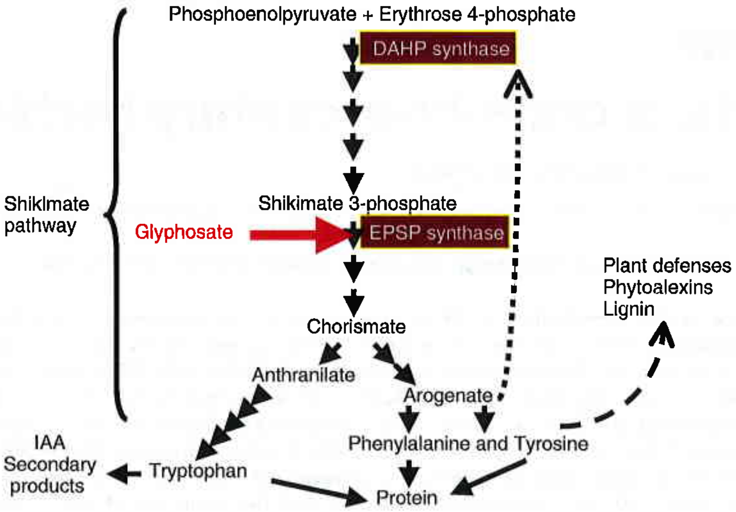

Figure 1. The shikimate pathway and the site of its inhibition by glyphosate. Products of the pathway and regulatory feedback inhibition (dotted arrow) are shown.

How glyphosate-induced inhibition of the shikimate pathway actually kills plants is not entirely clear. Many assume that insufficient aromatic amino acid production to maintain necessary protein synthesis is the primary effect, and this is consistent with the slow development of symptoms. Yet others have produced evidence to support the view that the increased carbon flow to the shikimate pathway by deregulation of the pathway by inhibiting EPSPS results in shortages of carbon for other essential pathways.[9] The rapid cessation of carbon fixation in glyphosate-treated sugarbeet[10] is better explained by this mechanism than by reductions in aromatic amino acid pools.

The EPSPS of all higher plants appears to be inhibited by glyphosate, making it a non-selective herbicide, active on a very wide range of plant species. Only glyphosate has been found to be an excellent EPSPS inhibitor, with no analogs or alternative chemical classes targeting this enzyme having been commercialized. This, coupled with the many other desirable properties of glyphosate, makes it a unique, ideal herbicide.

2.2.2 *Uptake and translocation*

Glyphosate is taken up relatively rapidly through plant surfaces.[11,12] Leaf uptake rates vary considerably between species, accounting for at least some of the differences in glyphosate susceptibility between species. Diffusion is the most likely mode of transport across the plant cuticle. The physicochemical properties of glyphosate enable it to be translocated from the leaf via the phloem to the same tissues that are metabolic sinks for sucrose.[9] Thus, phytotoxic levels of glyphosate reach meristems, young roots and leaves, storage organs and any other actively growing tissue or organ. Good uptake, excellent translocation to growing sites, nil or limited degradation and a slow mode of action are the primary reasons for the excellent efficacy of glyphosate. In species in which it acts faster, such as sugarbeet, glyphosate can limit its own translocation.[13]

2.2.3 *Toxicology*

Glyphosate is one of the least toxic pesticides to animals.[1,4,7] Accordingly, it is used for weed control throughout the world in urban and recreational areas, as well as on industrial and agricultural land. Glyphosate is less acutely toxic than common chemicals such as sodium chloride or aspirin, with an LD_{50} for rats greater than 5 g kg^{-1}. Some formulation materials and cationic salt ions used with glyphosate are more toxic than the glyphosate anion. Glyphosate is not a carcinogen or a reproductive toxin, nor does it have any subacute chronic toxicity. In a lengthy review, Williams *et al.*[14] conclude that, when used according to instructions, there should be no human health safety issues with glyphosate.

2.2.4 *Environmental profile*

In general, glyphosate is an environmentally benign herbicide.[1,14–16] Glyphosate binds tightly to soil constituents, with very little movement to soil and groundwater. In soils with macropores and pronounced preferential flow, glyphosate can move readily to groundwater,[17] but cases of this occurring in the field have not been well documented.[7] The major glyphosate degradation product, aminophosphonic acid (AMPA), is significantly more mobile than glyphosate in soil.[17] Glyphosate has a relatively short environmental half-life owing to microbial degradation in the soil. Glyphosate is not volatile, so there is no atmospheric contamination.

Because glyphosate is tightly bound by soil, it has essentially no soil activity (hence its use only as a post-emergence, foliar-applied herbicide). At commercial use rates, the glyphosate molecule itself has little or no effect on non-target organisms, other than some fungi.[1,7,16] At exposures to glyphosate likely to be found in the environment after application, there is no evidence of adverse effects. Studies with very low levels of glyphosate have found stimulation of growth of some plant species,[18] although this phenomenon has not been investigated in the field. Some studies with formulated glyphosate have found effects on amphibians, the study by Relyea,[19] for example, but the studies did not differentiate between the effects of formulation materials versus the glyphosate molecule.

2.2.5 *Resistance*

As mentioned above, there is no evidence of any higher-plant EPSPS being naturally resistant to glyphosate, although some plant species and biotypes of species are less susceptible than others owing to other physiological and/or biochemical mechanisms. For example, some bermudagrass [*Cynodon dactylon* (L.) Pers.] and field bindweed (*Convolvulus arvensis* L.) biotypes are more naturally resistant to glyphosate than others.[20,21] For 20 years after the introduction to world usage of glyphosate there was no evidence of evolved glyphosate-resistant weed populations. Scientists from the manufacturer of glyphosate stated that evolution of glyphosate-resistant weeds would be very slow, and the levels of resistance would be very low.[22] However, at about the same time, the first studies of evolved glyphosate resistance were published.[23,24] Since then, reports of evolved glyphosate-resistant weed populations have appeared at a brisk pace, especially associated with the advent and high adoption of transgenic glyphosate-resistant (GR) crops (Fig. 2) (see Section 3.5). The current state of evolved glyphosate resistance in weed species worldwide has been recently reviewed,[25,26] and, considering the current intense selection pressure from the massive glyphosate usage, the appearance of resistant weed populations should not be a surprise.

2.2.6 *Degradation in plants*

Until recently, the metabolic degradation of glyphosate by plants was neither well documented nor accepted.[3] In at least some species, glyphosate is degraded slowly to aminophosphonic acid (AMPA) and glyoxylate by a glyphosate oxidoreductase (GOX).[27-29] This is best evident in transgenic GR soybeans, because the plant is protected from the glyphosate toxicity by a resistant form of EPSPS, so that the healthy plant can metabolically degrade glyphosate. In GR soybeans treated with commercial doses of glyphosate at late developmental stages, glyphosate metabolism is evident as substantial AMPA in harvested seed.[27] Similar studies have not been done with GR maize or cotton. This experiment cannot be done with GR canola because it contains a transgene that encodes a bacterial GOX and therefore very little glyphosate is found after treatment, compared with GR soybean.[29] Large amounts of AMPA would be expected in these tissues, but the levels were no higher than in GR soybeans, suggesting that canola readily degrades AMPA.

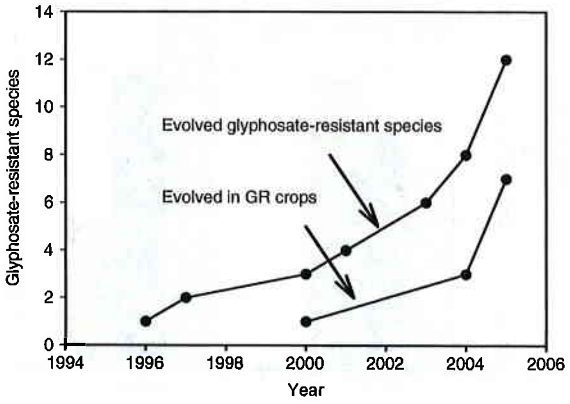

Figure 2. Evolved glyphosate-resistant species worldwide. Data plotted from the website of Ian Heap: http://www.weedscience.org/in.asp.

2.3 Approaches to overcoming glyphosate's non-selectivity

Because glyphosate is non-selective, for the first 20 years after commercialization its use was restricted to removing weeds before crop planting and to situations where glyphosate could be directed to avoid contact with foliage of crops or other desired vegetation. Even small amounts of glyphosate reaching plant foliage can cause some phytotoxicity owing to the excellent translocation of glyphosate from any exporting leaf to growing points. Thus, glyphosate is widely used in a range of well-established tree, nut and vine crops if it is directed at basal weeds within and between the crop rows, ensuring that the foliage is not contacted. Some of the first weeds to evolve glyphosate resistance occurred in such tree, nut and vine crop areas where glyphosate could be applied several times in a season.[25,26]

Until the transgenic crop era (from 1995 onwards), glyphosate use in the major annual world grain crops was restricted to preplanting weed control. For example, glyphosate is the herbicide of choice for early-season weed control before planting wheat crops, worldwide. Mechanical innovations enabled some glyphosate use within crops. These included shielded sprayers and devices to wipe the herbicide onto weeds that were taller than the crop.[30] These approaches in annual crops have not been widely utilized. Therefore, the value of crop varieties resistant to glyphosate had been long recognized, although attempts to find naturally resistant crop varieties were without success. The advent of methods to move genes from one organism to another offered the possibility of breaking this impasse.

3 TRANSGENIC, GLYPHOSATE-RESISTANT CROPS

3.1 Approaches

Various genetic engineering and biotechnology approaches to producing GR crops were tried with limited success until the *CP4* gene of *Agrobacterium* sp. was found to encode a GR form of EPSPS.[5] When this *CP4* gene plus a promoter was placed into the genome of certain crops, high levels of glyphosate resistance were expressed. In addition to the *CP4* gene, a gene from *Ochrobactrum anthropi* Holmes *et al.* encoding GOX was employed to contribute to resistance in canola.[5] The resistance factors for GR *CP4* soybean and *CP4* plus GOX canola are each about 50-fold.[29] For maize, the EPSPS has been altered by site-directed mutagenesis of a maize gene to provide a form of GR EPSPS that is used in some GR maize varieties. Genes that encode other forms of GR-EPSPS[31] and glyphosate detoxification enzymes[32] are being proposed for future GR crops, but, at present, the *CP4* gene is responsible for glyphosate resistance in most commercial GR crops.

3.2 Commercialization and adoption

Six GR agronomic crops have been deregulated (approved for growing by farmers) in the USA (Table 1). Only four of these crops (soybeans, cotton, maize and canola) are being grown at this time. The adoption rate of GR soybean, cotton, maize and canola in the USA has been spectacular, with over 90% of all US soybeans and almost 70% of cotton GR by 2006. Similarly, about 75% of the canola planted in Canada and the USA in 2005 was GR.[33] Almost 100% of the soybeans in Argentina are GR, and the adoption rate of GR soybeans in Brazil has been very rapid since it became legal to grow these crops in 2004.[34] More than 80% of all transgenic crops planted worldwide are GR crops, the planted area approaching almost 100 million hectares in 2006.[35] Therefore, the adoption of this technology, where it has been approved, has been sweeping. A more detailed discussion of the spectacular rates of adoption of GR crop technology is available in Dill *et al.*[36] GR crops have catapulted glyphosate to the most used herbicide in the USA, accounting for more than 60% by volume of herbicides used in 2001.[37] The use rate has gone up significantly since then owing to the increased adoption of GR crops. The significant decline in cost of glyphosate after the patent expired in 2000 has also contributed to this dominance.

The driving force for this rapid adoption is that the combination of glyphosate and a GR crop generally provides better, simpler, cheaper and more flexible weed management than the conventional alternatives.[33,38] Clearly, the economic benefits of GR crops are evident to farmers. Consequently, glyphosate has largely replaced the selective herbicides previously used in soybean, cotton and other GR crops, so reducing their value.[39] This has caused economic difficulties for competitor international herbicide manufacturers without this technology, and has contributed to substantial rationalization in this industry.

Table 1. Transgenic GR crops that have been deregulated in the USA

Crop	Year deregulated
Soybean	1996
Canola	1996
Cotton	1997
Maize	1998
Sugarbeet[a]	1999
Alfalfa[b]	2005

[a] Removed from market, but to be reintroduced in 2007.

[b] Returned to regulated status in 2007 by court.

2.3 Environmental benefits and risks

Overall, GR crop technology has been found to be more environmentally benign than the weed management technology that it replaces.[6,7,39–41] This is because, as mentioned above, glyphosate is more environmentally benign than the destructive soil tillage and/or herbicides that it has replaced. Glyphosate is less likely to move or persist in ground and surface water than the herbicides it has replaced.[7,42]

Tillage has been reduced where GR crops have been adopted. Tillage is an environmentally harmful practice that causes loss of top soil and consequent pollution of surface waters and air. Utilization of tillage results in significant fossil fuel use with associated negative impacts. Brookes and Barfoot[41] estimated that GR crop use worldwide in 2005 resulted in a reduction of carbon dioxide emissions and potential additional soil carbon sequestration equivalent to the removal of about 4 million family cars from the road in terms of effects on global carbon balance. Tillage has been significantly reduced in GR crops (Fig. 3),[36] although the evolution of GR weeds and the movement of naturally GR weed species into GR crop fields is making returning to occasional tillage more desirable as an additional weed management tool in GR crops.

The only environmental risk of GR crops of which the authors are aware is that of transgene flow to

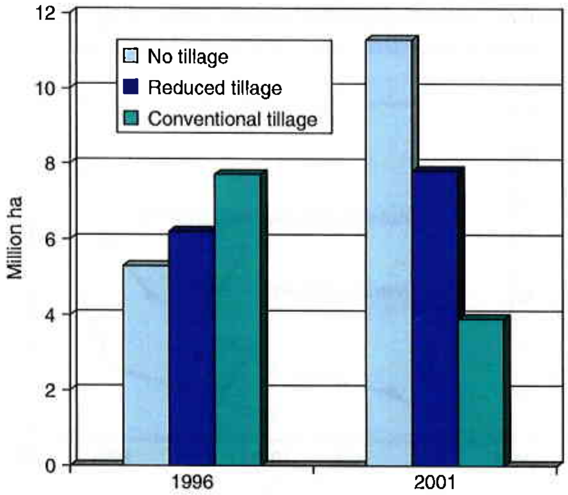

Figure 3. Soybean tillage methods by hectares farmed in the USA in 1996 and 2001. In 1996 and 2001 there were respectively 19.2 and 23 million ha of soybeans grown.[43].

wild or weedy relatives. Gene flow of GR transgenes to non-transgenic crops is known in canola, but has not been either a production or an environmental problem.[44] Glyphosate-resistant creeping bentgrass (*Agrostis stolonifera* L.), a wind-pollinated perennial, is being tested for use as a turf grass, and the *CP4* EPSPS gene has been readily transmitted to non-transgenic bentgrass.[45] The recent banning of GR alfalfa by a court in California was ostensibly because of gene flow to organic alfalfa. These cases of gene flow to the same species are more economic than environmental threats. Gene flow can also occur between closely related weed species.[46]

Introgression (full incorporation into another genome) of transgenes into wild plants does pose a potential environmental problem, in that the genes cannot be recalled. Hybrids between species or between crops and weedy variants of the crop are often unfit, making full movement of a gene or genes into another population, with the backcrosses required, unlikely. Before transgenic crops, herbicides were introduced for crops to which the crops were naturally resistant. There are no proven cases of complete introgression of herbicide resistance gene(s) from a naturally resistant crop to an associated weed in the field. That weeds closely related to crops are sometimes naturally resistant to the same herbicides as the related crop may partially account for this. Nevertheless, full introgression of traits from crops to weeds appears to be rare, even with extreme selection pressure.

Canola is the only GR crop commercially grown in North America that has a weedy relative with which it can readily interbreed.[47] Herbicide-resistant transgenes have been found in *B. rapa* × canola hybrids in the field, but complete introgression into *B. rapa* has not been confirmed. Maize genes could theoretically introgress into teosinte (*Euchlaena mexicana* Schrad.), the species from which maize originated, since the two species can interbreed.[48] The transfer of transgenes from soybean to weedy relatives is not considered a risk in the Western Hemisphere (which accounts for about 83% of the total soybean area worldwide), because there are no sexually compatible relatives of soybean growing wild in the Americas. There are tetraploid *Gossypium* species in South and Central America that potentially could cross with *G. hirsutum.* Where *G. hirsutum* L. and *G. barbadense* L. overlap in distribution, natural hybrids theoretically could occur, although there have been no reports of such hybrids occurring (Meredith W, private communication, 2007).

In spite of the lack of any significant problems with transgene introgression from a GR crop so far, introgression of transgenes to wild relatives is considered to be the largest potential risk of any transgenic crop, in that recalling the errant genes would be essentially impossible, once fully introgressed. Herbicide resistance transgenes should pose no threat to natural ecosystems, but, when stacked with transgenes imparting traits that would increase fitness (e.g. insect or disease resistance), the herbicide resistance trait could assist in introgression in the agroecosytem, eventually resulting in wild species with new traits that could alter species interactions in a natural ecosystem. Development of fail-safe technologies to prevent introgression of transgenes should be a high-priority area of future research for those interested in a secure future for transgenic crops.

3.4 The changing weed spectrum

It is a maxim in weed control that nature abhors a vacuum. Where GR crops are grown intensively with high reliance on glyphosate for weed control, the agroecological niches resulting when weeds are well controlled by glyphosate will eventually be filled by species that can naturally resist or avoid glyphosate. From 1996 onwards in the USA, where GR crops have been grown, this process has been observed and documented. At least some of the biotypes of the following species are not well controlled by recommended rates of glyphosate: *Amaranthus rudis* JD Sauer, *Amaranthus tuberculatus* (Moq.) JD Sauer, *Chamaesyce hirta* (L.) Millsp., *Chenopodium album* L., *Chloris polydactyla* (L.) Sw., *Commelina benghalensis* L., *Commelina communis* L., *Cyperus* spp., *Dicliptera chinensis* (L.) Jussieu, *Ipomoea* spp., *Lotus corniculatus* L., *Richardia brasiliensis* (Moq.) Gomez, *Sesbania exaltata* (Raf.) Cory, *Spermacoce latifolia* Aubl., *Synedrellopsis grisebachii* Heiron & Kuntze and *Tridax procumbens* L.[49–51] Some of these species have become problematic in GR crops. This is not an exhaustive list.

3.5 Evolved glyphosate resistance

In addition to weed species shifts, overreliance on glyphosate in GR crops has led to the evolution of GR weeds (Fig. 2). Most of the documented cases of evolved GR weeds in the past 6 years have been in GR crops. The evolution of GR weeds in various parts of the world has been reviewed recently[25,26] and therefore will not be elaborated upon here. It is clear that in the USA, Argentina and Brazil (to a lesser but significant extent) the massive levels of adoption of GR crops means an overreliance on glyphosate for weed control across massive areas with insufficient diversity. Thus, there is a high selection pressure for resistance, and, consequently, glyphosate-resistant weeds are evolving in these areas. Given the high popularity of GR crops, this process is likely to accelerate through the foreseeable future.

4 SUSTAINING GLYPHOSATE AND THE GLYPHOSATE-RESISTANT CROP WEED MANAGEMENT SYSTEM

By any criteria, the technological innovation of GR crops has been an outstanding success. Soybean, maize, canola and cotton producers, particularly in Argentina, Brazil, Canada and the USA, have overwhelmingly adopted this technology. As GR crops become approved by governments in other parts of the world, farmers in those countries are likely rapidly to adopt them because of the same benefits evident to North and South American producers. Currently, glyphosate dominates crop weed control in soybean, maize, canola and cotton in North and South America. Consequently, throughout large areas, glyphosate reliance without diversity in weed control practices is a strong selection pressure favoring the evolution and eventual domination of glyphosate-resistant weed populations. Given the tangible benefits of GR crops and glyphosate, it is unlikely that producers (and many that advise them) will readily diversify away from high reliance on glyphosate. Inevitably, then, glyphosate-resistant weeds will emerge, threatening the long-term efficacy of the world's most important herbicide resource. This lamentable scenario can only be minimized and managed through the reintroduction and maintenance of diversity in weed control tools acting to reduce the evolutionary selection pressure favoring glyphosate-resistant weeds. Put simply, glyphosate can only be sustainable in the long term if there is sufficient diversity in weed management practices. The challenge is to develop and implement this needed diversity given prevailing economic and other constraints. Diversity can be achieved in many different ways, and to achieve this there are essential roles for many sectors of the agricultural industry. Gene-driven and/or new herbicide innovations can increase herbicide diversity by providing novel full-dose herbicide mixtures and alternatives to glyphosate.[52] Equally, mechanical and precision application technologies offer the potential to reduce reliance on glyphosate. At the farmer level, better agronomic management to enable crops to suppress weeds and wise crop husbandry/rotations can enable producers to reduce glyphosate reliance. It is only through such diversity that the world's greatest herbicide will continue to control crop weeds and help ensure future harvests. Finally, individuals in those countries yet to introduce GR crops can learn much from the experience evident in North and South America and should act to sustain the longevity of the precious glyphosate herbicide resource.

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