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Review

Indigenous African agriculture and plant associated microbes: Current practice and future transgenic prospects

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Reliance on non-sustainable inputs of fertilizers and pesticides does not hold the answer to obtaining higher yields from sustainable cash as well as food crops grown in Africa. Hence, the use of transgenic plants/crops and plant growth-promoting microbes with the aim of increasing productivity is attractive. In most parts of Africa, the farmers work at a subsistence level, the prevalent agricultural practice can have a significant effect on the relative abundance of the rhizosphere microbial populations. Presented here is illustrated view of plant associated microbes to soils especially in indigenous African agriculture focusing on current practice and future transgenic prospects.

Key words: Agricultural practice, crop, indigenous knowledge systems, microbial inoculants, transgenic.

INTRODUCTION

Given reliance on agrochemicals/fertilizers for maintenance of soil quality and addition of useful microbial inoculants (bacteria/fungi/VAM) to soil could provide reliable and sustainable means for maintaining soil quality and fertility. Plant growth promoting bacteria (PGPR) synergise with arbuscular mycorrhizal (AM) fungi to stimulate plant growth through nutrient acquisition and inhibition of fungal plant pathogens and therefore, contribute to the maintenance of tropical soil fertility**,** plant health and sustainable agriculture. For this and many other reasons PGPR are biofertilizers.

In parts of Africa, zinc deficiency in crop plants (Ajouri et al., 2004; Zuo and Zhang, 2009) is a well-known problem. Zn deficiency may have been worsened in recent years as a result of fertilizer over use. Fertilizers sometimes speed up eutrophication. Eutrophication is a process where chemical induced excessive plant growth and water bodies receive excess plant nutrients and thus stimulate undesirable pollution. It is better to use an environmentally friendly approach, which will not injure the microbial community in the rhizosphere soil, to solve the problems of infertile soil, biotic and abiotic stresses. Prevalent agricultural practices such as burning, tillage and pesticide use can reduce the diversity of soil microbes. Chemical, physical, and biological factors contribute to soil structure and soil factors may play a role in determining the composition of microbial rhizosphere communities.

In Africa, the use of transgenic microorganisms is not a mature industry. Some microbes are engineered for strain improvement and mutation-selection (Babalola, 2009), others are engineered to enhance microbial fertilizers and pesticides for example, *Pseudomonas putida* R20 (Staley and Brauer, 2006). Similarly, there is the notion that the planting of transgenic plants may not be financially within the reach of peasant farmers. South Africa is the only country in Africa that is currently growing transgenic crops commercially. Notwithstanding the positive results obtained in other countries, in the

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minds of some individuals, significant concerns remain in terms of having the transgene contained (Douville et al., 2007). A concern regarding the negative views of Genetically modified (GM) crops has been shown by the governments of Zambia, Angola, Namibia (Eicher et al., 2006) and this skepticism tends to limit in the minds of many decision makers the acceptability of GM crops in Africa. Currently, approval is required for confined trials, larger location trials, and for commercial use.

There are several limitations to the use of transgenic; farmer would like to know how predictable the results will be season after season and the financial implication of such technologies. No peasant farmer wants increase costs without new additional benefits to the farmer. Unless farming is heavily subsidized by the government, farmers may be hesitant to adopt a technology whose gains are more likely to appear only in the long term. This survey will describe the current practice in African agriculture, and briefly state future directions that might lead to the integration of indigenous knowledge systems into biotechnology revolution.

AFRICAN AGRICULTURE

A brief overview of current practice

The use of legume and non-legume multiple cropping systems, either rotation or intercropping, contribute to the restorative processes that operate in a sustainable system (Ferreira et al., 2000). Symbiotic nitrogen (N) fixation plays a key role in these cropping systems (Ferreira et al., 2000). For example, agriculture in Burkina Faso is largely dominated by 75% of rain fed crop production. Many Africans practice peasant farming, as they are resource poor farmers.

Major crops in various regions

There are cash crops, food crops and economically important crops. Cash crops are crops that are used in industry such as sugar for refining and grapes for the wine industry. Cash crops grown in different regions of Africa include pineapples, lemons, bananas, grapefruit, pears, peaches, tomatoes, sugarcane, sunflower seeds, apples, oranges, grapes, and soybean (Table 1). The main remaining cash crops are cinchona (a woody plant cultivated for its medicinal bark) and coffee followed by palm oil, cotton, cocoa, rubber and tobacco. Other major crops include tubers, plantains, maize, rice, groundnuts, beans, and sugar cane.

Food crops are grown to feed people. The growth and sale of food crops such as yams, cassava, groundnuts, maize (corn), sorghum, millet, sesame, and plantains dominates the Central African Republic's economy (FAO, 2008).

Other economically important crops associated with Africa are apples, carrots, cotton, grapes, lettuce, onion, peas, plum and sweet corn (*Zea mays* L.). Sorghum and pearl millet are the most important coarse-grain cereals in the semi-arid tropical regions of both Asia and Africa.

Some food crops are sold and could be regarded as cash crops; however, some ambiguity exists in crop classifications. Different climatic and socioeconomic conditions prevail in Africa. Some countries are low in exportation of farm products. In general, Africa produces a wide range of food and cash crops but at low levels. Moreover, several of the countries are not self sufficient in food production.

Use of chemicals versus microbial inoculants

Zinc (Zn) deficiency in crop plants (Ajouri et al., 2004; Zuo and Zhang, 2009) is a well-known problem in parts of Africa; Zn deficiency may have been worsened in recent years as a result of fertilizer over use. Thus, it has been suggested that the large scale use of phosphate (P) fertilizer is detrimental to mycorrhizal functioning, leading to a lower uptake of Zn (Cardoso and Kuyper, 2006), and the addition of N fertilizers can lead to a lower diversity of bean-nodulating rhizobia (Ferreira et al., 2000). These are but two examples of the fact that reliance on non-sustainable inputs of chemical fertilizers and pesticides may not hold the answer to sustainable agriculture.

Fertilizer use has been reported to cause eutrophication, even beyond the farmer's field, and it has been shown that chemicals increase the number of resistant or tolerant weeds (Schooler et al., 2008, 2010). In addition, the use of fertilizers in conventional farming does not take into account the activity of mycorrhizal fungi. A higher level of fertilization may also negatively affect the rhizobacterial community (Marschner and Rumberger, 2004; Sturz et al., 2004). The fertilizers received by the soils as nutrient beyond the crop requirement can result in over-saturation of upper soil layers with phosphorus and a consequent negative effect on crop yield. This observation indicates that the bacterial community in the rhizosphere soil is likely to be more sensitive to modifications in the agricultural practices than in the rhizoplane (Roesti et al., 2006). Thus, it is better to use an environmentally friendly approach (that is, a paradigm that emphasizes the use of biological soil amendments in place of chemicals) to solve the problems of infertile soil.

Types of agricultural practices

Aside biotic and abiotic stresses agricultural practices (burning, tillage, pesticide use), inadequate management

Table 1. Cash and food crops grown in different regions in Africa.

Regions in Africa	Cash crops	Food crops
Northern Africa	Beniseed, cashew nuts, cocoa, cotton groundnuts/peanuts, gum Arabic, kolanut, palm kernels, rubber, soybean, yams and maize	Wheat, barley, and sorghum
Western Africa	Cotton, karite (shea nuts), sesame, coffee, cocoa, pineapple, banana, copra, sweet potatoes, citrus, groundnuts, palm oil, palm kernel, benniseed, rubber, millet, cowpea, and sorghum	Rice, cassava, maize/corn, sorghum , millet, yams, manioc, plantains, sesame, cowpea, groundnuts, sweet potatoes, tare, banana, sago palm, cocoyams, beans, bananas, and a variety of fruits and vegetables
Central Africa	Palm oil, sunflower oil, vegetables, fruits, coffee, tea, cotton, tobacco, sugarcane. palm kernels, pyrethrum, rubber, sugar, groundnuts, and cocoa	Bananas, sugarcane, coffee, maize, manioc, sweet potatoes, Irish potatoes, beans, peas, wheat, vegetables, plantains cassava, sorghum, taro, rice, millet, groundnuts, Yam, bananas, soybean, and wheat
Eastern Africa	Tobacco, sugar, cotton, cloves, coffee, oil palm trees, rubber, horticulture, tea, coffee, cloves, vanilla, sugarcane, cocoa and sisal	Cowpea, maize, pigeon pea, rice, cassava, sweet potatoes and groundnuts
Southern Africa	Millet, sorghum, corn, sunflower, and cotton	Beans, pumpkins, melons, gourds, bambaranuts, groundnuts, spinach, oriental tobacco, cotton, cowpea, maize, beans, pumpkins, potatoes, and sweet potatoes

of soil and crop rotation exclusively with grasses (Alvey et al., 2003; Ferreira et al., 2000) can reduce the diversity of soil bacteria; however, this reduction is typically more prominent in bulk soil than in the rhizosphere. Prevalent agricultural practices in Africa could have a magnified effect on soil microbes. By comparison with fallow fields, annual cropping stimulates soil microbial activity. In relatively undisturbed agro-ecosystems promoted by agro ecology, especially those include perennial plants and involve minimal tillage, the root biomass increases and the mycorrhizal mycelium network is kept intact. Minimum tillage, organic amendments, and crop rotation all contribute to promote soil quality. Expression of microbial activity is influenced by the interactions of direct or proximal factors in the soil biota. Proximal factors include temperature, water, oxygen content, substrate availability and substrate quality (Mosier and Parkin, 2007). The increase in biomass and activity of microorganisms may be attributed to higher input of carbon from the resident vegetation.

One of the reasons change arises in the presence of bacteria is because of the increased production of carbonic acid from the CO_2 liberated by the rhizobacteria. The production of CO_2 in the rhizosphere and the formation of organic and inorganic acids aids in the solubilization of inorganic plant nutrients. Rhizobacterial populations may be influenced by crop species or plant cultivars (Anandham et al., 2007; Babalola, 2007), plant type (Germida and Siciliano, 2001; Montealegre et al., 2002; Schwieger and Tebbe, 2000), developmental stage or plant age (Anandham et al., 2007; Marschner et al., 2004), distance from the soil to the root (Miethling et al., 2000; Montealegre et al., 2002), soil characteristics (Watt et al., 2006), irrigation history at the site (Dodd, 2009), root exudates and mycorrhizal infection (Marschner et al., 2001). These changes in the rhizosphere community might affect plant growth negatively (for example, root pathogen development) or positively (for example, increase in the proportion of plant growth promoting bacteria [PGPB] populations) (Roesti et al., 2006).

The PGPB are free-living bacteria of beneficial agricultural importance (Babalola, 2010a). In summary, agricultural practice can have a significant effect on the relative abundance of the rhizosphere bacterial populations. Potential benefits of agronomic practices such as the no-tillage system (sowing directly through the mulch) typically includes increases in soil biomass, a high level of active soil microbes, enhanced soil organic matter content and structure stability, and increased moisture content, as well as reduced soil temperature and soil erosion (Ferreira et al., 2000).

Soil structure and management

Chemical, physical, and biological factors contribute to soil structure. Apart from that, when plant root penetrates the soil, they help to improve the soil structure. Microorganisms also affect the physical and chemical soil properties (for example, aggregate stability and pH value), and also, soil fertility. The rhizobacteria may favour plant development by producing growth stimulating substances, contributing to the formation of a stable soil structure, releasing elements in organic forms through the mineralization of organic complexes, and by entering into symbiotic root associations. Observations like divergence from initial soil microbial community of the field by sown and naturally colonized soil (Hedlund, 2002) is well documented. Bacteria enhance aggregate soil structure and contribute to the maintenance of soil structure along with mycorrhizal fungi. As suggested from previous finding (Wertz et al., 2007), using substrate-induced respiration rates and ribosomal intergenic spacer analysis (RISA) technique, the colonization process in the sterilized soil clod led to the establishment of a bacterial community different from the surrounding soil aggregates and there is evidence of succession of bacteria during decolonisation. During investigation on the bacterial communities associated with the rhizosphere, it was established that soil factors played a minor role in affecting the microbial community in the rhizosphere (Miethling et al., 2000). Soil factors had a larger impact in determining the composition of microbial rhizosphere communities.

USE OF TRANSGENIC MICROBES

Microbes have the ability to act as defense systems for infection and weed control. The interrelationship between plant root and the rhizosphere bacteria is appreciably more thorough than that found in the rhizoplane. Unlike some beneficial root-associated *Bacillus* spp, *Pseudomonas* spp. are typical examples of bacteria with specific plant-microbe interrelationships where a single strain can act as a biological control agent for several fungi. Although many plant pathogens are not sufficiently pathogenic for weed control when used alone as a bio-herbicide, genetically engineered microorganisms (GEMs), altered for hyper-virulence, have potential (Babalola, 2009) for such uses. It should be noted that the aim of biological weed control is not to eradicate weeds but to reduce them to a non-economic level. For this purpose, the microbial agent needs to be specific in action and directed to a targeted weed (Gentry et al., 2004).

Transgenic microorganisms may also find use in bioremediation although it is important to avoid horizontal gene flow to wild or cultivated relatives. Some microbes are engineered for strain improvement and mutation-selection (Babalola, 2009), others are engineered to enhance microbial fertilizers and pesticides for example, *P. putida* R20 (Staley and Brauer, 2006). Some examples of transgenic microbes and the reasons for their uses have been demonstrated (Table 2).

USE OF TRANSGENIC PLANTS

Transgenic plants are plants/crops with exogenous gene(s) introduced, usually by recombinant DNA technology, from other genera, species or strains, with the aim of producing a superior plant which provides some benefits to mankind (Table 3). Regarding the African continent, there is the notion that the planting of GM crops may not be financially within the reach of peasant farmers. For example, particular reference has been made to Ethiopia where the likelihood of subsidizing the provision of GM seeds to smallholder farmers is very slim (Azadi et al., 2011). In most parts of Africa, the farmers work at a subsistence level, and it is logical to think that the introduction of GM crops will widen the differences between socio-economic classes, thus raising the issue of equitability, among other factors such as productivity and sustainability. There is also a concern that the genes from engineered plants may integrate and become established in other organisms following environmental release of the transgenic plants (Gentry et al., 2004). However, although transgenic plant DNA persists in soil, there is no documented example of transgenic plant DNA being transferred into indigenous soil microorganisms (Azevedo and Araujo, 2003; Dunfield and Germida, 2004). Thus, a recent finding suggests that residues of insect resistant Bt-corn (that is, corn engineered to express an insecticidal protoxin gene from the bacterium *Bacillus thuringiensis*) is degraded just like other corn cultivars, and that the persistence of the insecticidal Cry1Ab protein in soil is negligible (Daudu et al., 2009). Interestingly, South Africa is the only country in Africa that is currently growing transgenic crops commercially. Notwithstanding the positive results obtained in other countries, in the minds of some individuals, significant concerns remain in terms of having the transgene contained (Douville et al., 2007).

With particular reference to Egypt (Eicher et al., 2006)

Table 2. Examples of the use of transgenic microbes with potential for use in agriculture.

Transgenic microbe	Crop inoculated	Reason	Reference
Pseudomonas putida Biovar B. Strain HS-2	Non-transformed and transgenic canola plants	Phytoremediation and plant growth promotion	Rodriguez et al. (2008)
Pseudomonas fluoresences Z30-97	Wheat	To monitor possible alterations of bacterial community structure	Bankhead et al. (2004)
Pseudomonas asplenii AC	Common reed (*Phragmites australis*) [cav. Trin. Ex. Steudel]	Significant increases in shoot and root size and partial protection of the plant from inhibition by either copper or creosote	Reed et al. (2005)
P. asplenii AC	Canola (*Brassica napus*)	Effective growth promotion and partial protection of the plant from inhibition by either copper or creosote	Reed and Glick (2005)

Table 3. Examples of transgenic plants.

Transgenic plant	Purpose of transgene	Gene expressed	Reference
Lycopersicon esculentum (Solanaceae) cv. Heinz 902	Less subject to deleterious effects of metals (Cd, Co, Cu, Mg, Ni, Pb or Zn)	ACC deaminase	Grichko et al. (2000)
Arabidopsis thaliana	Protection against fungal pathogen (*Fusarium oxysporum* f. sp. Matthiolae)	Fusion proteins (*Fusarium*-specific antibody and antifungal peptide)	Peschen et al. (2004)
A. thaliana	Production of arachidonic and eichosapentaenoic acids	*IgASE1*, the gene encoding activity from *Isochrysis galbana*	Qi et al. (2004)
Canola (*Brassica napus*)	Increase plant Ni uptake. Enhanced plant biomass accumulation	ACC deaminase	Farwell et al. (2006)
Canola (*B. napus* cv. Westar)	Improve tolerance to inhibitory effect of salt stress	ACC deaminase	Sergeeva et al. (2006)
Canola (*B. napus*)	Phytoremediation of arsenate contaminated soil	ACC deaminase	Nie et al. (2002)
Canola (*B. napus* cv. Westar)	Tolerance to (flooding stress and Ni stress) multiple stressors protection against growth inhibition by flooding	ACC deaminase	Farwell et al. (2007)

Table 3. Contd.

Nicotiana benthamiana	To combat plant viruses, e.g. tomato bushy stunt virus and cucumber necrosis virus	Single-chain Fv fragments	Boonrod et al. (2004)
Soybean	Herbicide resistance	Phosphinothricin N-acetyltransferase	Kita et al. (2009)
A. thaliana	To regulate ethylene production	ACC deaminase	McDonnell et al. (2009)
Tomato	Increase tolerance to flooding stress, partial protection from root hypoxia	ACC deaminase	Grichko and Glick (2001)
Golden Rice 2	Increase production of β-carotene (pro-vitamin A)	*psy* – daffodil gene encoding phytoene synthase	Paine et al. (2005)
Wheat (*Triticum aestivum* cv. Bobwhite)	High resistance to virus infection	RNase 111 – Bacterial ribonuclease	Zhang et al. (2001)
Rice	Folate (Vitamin B9) biofortification	*Arabidopsis thaliana* genes of pterin and PABA branches of the folate biosynthesis pathway from a single locus	Storozhenko et al. (2007)
Alfalfa	Increase sugar yield from cell wall	Downregulated lignin biosynthetic enzymes	Chen and Dixon (2007)
Rice	Enhanced grain yield irrespective of dense planting	Brassinosteroid deficiency	Sakamoto et al. (2006)
A. thaliana	Remediation of lead and cadmium contamination	*Saccharomyces cerevisiae* protein YCF1	Song et al. (2003)

and South Africa are varieties of GM sweet potato that are resistant to potato weevils and viral diseases, for example, sweet potatoes feathery mottle virus, and potato tuber moth (*Phthorimaea operculella* Zeller). The Bt sweet potato is not yet commercialized because Egypt does not want to risk the possible loss of its future export market to Europe (Eicher et al., 2006), while South Africa is hindered by liability issues with regards to cross-boundary movement of Bt sweet potatoes to neighbouring countries where Bt technology is not registered. Similarly, insect resistant GM cowpeavarieties that have been developed are far from being commercialized (Eicher et al., 2006). While Europe does not accept GM crops, some African countries anxiously await GM crops because of their potential to boost agricultural productivity and alleviate poverty, reduce the need for fertilizer and minimize irrigation. USA, Canada and Australia accept the technology; developing countries like Argentina, Brazil, China and India are in favour of the use of GM crops (Economist, 2010). South Africa is the leading light for transgenic research and transgenic crop in Africa (James, 2005). In Africa, Burkina Faso, Egypt and South Africa are in support of the technology. Since Africa is the poorest and the most food insecure region in the world, it has been argued that GM crops are one of the solutions to Africa's food crisis (Wambugu, 2003). There exist many startling legal and political barriers by so-called anti-GM advocates to hinder the more widespread adoption and use of GM crops. The concerns of African governments are about potential health, environmental and trade effects of importing food in this era of no biosafety regulations in any of her states with the exception of South Africa. A concern regarding the negative views of GM crops has been shown by the governments of Zambia, Angola, Namibia (Eicher et al., 2006), and this skepticism tends to limit in the minds of many decision makers the acceptability of GM crops in Africa. Currently, approval is required for confined trials, larger location trials, and for commercial use. An exorbitant cost is incurred for the regulatory approval of every transformation event. It is obvious that Africa needs help in erecting the biosafety and regulatory capacity, and in expanding its human capacity in biotechnology without which GM technology will long remain in its infancy in most African countries. Unfortunately, in 2002, six African nations afflicted by drought, refused U.S. food aid (corn) because of concerns that the European Union would boycott their local exports, after the corn was found to be genetically modified. Those governments preferred to subject millions of their people to possible starvation rather than anger the European Union (Anon, 2002).

WHAT CURRENTLY LIMITS THE MORE EXTENSIVE USE OF MICROBIAL INOCULANT TECHNOLOGY

Farmers would like to know how predictable the results of microbial inoculants will be season after season. It is difficult to convince an African peasant farmer that only long-term benefits will result from the use of microbial inoculation technology. The coverage of many specific inoculants is for small niche markets and may not be wide enough to generate registration costs in a fairly short time (Babalola, 2010a, b, c). The registration costs of these inoculants may not be offset early enough as the bio-herbicide market is still in the infancy.

The data suggest that plants, engineered genes, environmental stresses and agricultural practices influence the microbial community structure more than any microbial inoculant, even when it is introduced at a high level (Castro-Sowinski et al., 2007). The increased costs of microbial inoculants could be a disincentive for some farmers. Microbial inoculant effects are often not as obvious as chemical herbicides or fertilizers. In addition, certain microbial experiments must be done under containment and there are different levels of containment because, for example, of issues regarding potential ecological effects of transgenic microbe releases. Besides, post-regulation monitoring must be followed and in many instances patent protection applies. Approval must be obtained from appropriate authorities before testing and release of new microbial inoculants. All of these requirements for approval cause delays and increase costs with no additional benefits to the farmer. The adoption of this technology comes with a variety of constraints for formulation and delivery of the inoculants. Environmental constraints include climatological factors. For example, the need for sub-optimal temperature, adequate moisture, and appropriate dew period is critical in weed biocontrol for efficacy of foliar herbicides (Babalola, 2007). Other environmental constraints include the level of humidity required, UV radiation and the occurrence of natural disasters. As well, there are numerous biological constraints including host variability and host range, synergistic interactions, facilitated infection (for example, plus enzyme), and genetic manipulation.

Inoculation results are often not very predictable, for example, in the findings of Schwieger and Tebbe (2000) inoculation of *S. meliloti* L33 had an effect on the structure of the rhizosphere bacteria from *Medicago sativa* but not on the structure of the rhizosphere bacteria from *Chenopodium album*. Moreover, inoculant density increases only temporarily, and then gradually decreases to a stable population. Thus, soils and rhizospheres appear to have a certain microbial threshold population size (Normander and Hendriksen, 2002). Another possible limitation is the overlapping of nutritional requirements of inoculated bacteria and indigenous microbes potentially resulting in competition for niche colonization between the inoculant bacteria and the indigenous microorganisms (Schwieger and Tebbe, 2000). Unless farming is heavily subsidized by the government, farmers may be hesitant to adopt a technology whose gains are more likely to appear in the long term.

COOPERATION BETWEEN AFRICAN COUNTRIES AND THOSE OUTSIDE OF AFRICA

Good infrastructural facilities are not common in many African countries. Subsistence farming is labor-intensive with relatively low inputs of new technology. National efforts to make more effective use of this technology are confronted with poor research funding, a problem in the majority of the nations on the continent of Africa. Public debate on the release of genetically modified crops has led to questions regarding their ecological compatibility. Regulations for the production and use of microbial inoculants have only been established for specific purposes. Support from local and international governments and agencies may not always be accessible or it may not be available where it exists. Industrial-scale production equipment including fermenters, dryers and formulation equipment are required for the technologies to thrive and subsistence farmers may not find such affordable facilities within reach, especially in remote communities in rural areas in Africa. The present restrictions on mass release of transgenic microbes, the paper work including efficacy, safety, composition, toxicity, and degradation, the painstaking risk assessment and such warrant that each country must isolate and develop its own microbial inoculants because current regulations do not encourage transfer of these inoculants between countries. Moreover, the EU scrutinizes chemicals that are endorsed for use, under directive 91/414/EEC, thereby discouraging the development of new microbial inoculants.

CONCLUSIONS

This report has provided useful information relevant to African agriculture stakeholders. Some promising line of investigation that could be suggested entails: the need for field trials, and the development of management strategies to foster indigenous knowledge system. It is still a challenge to use transgenic approach to tackle the problems of sustainable agriculture in Africa. However, it is hope that indigenous technological knowledge in agriculture would be harmonized with transgenic technologies without sending indigenous systems to extinction.

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