



## Review

## Humic and fulvic acids as biostimulants in horticulture



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## ABSTRACT

Maintaining food production for a growing world population without compromising natural resources for future generations represents one of the greatest challenges for agricultural science, even compared with the green revolution in the 20th century. The intensification of agriculture has now reached a critical point whereby the negative impacts derived from this activity are now resulting in irreversible global climate change and loss in many ecosystem services. New approaches to help promote sustainable intensification are therefore required. One potential solution to help in this transition is the use of plant biostimulants based on humic substances. In this review we define humic substances in a horticultural context. Their effects on nutrient uptake and plant metabolism are then discussed and a general schematic model of plant-humic responses is presented. The review also highlights the relationship between the chemical properties of humified matter and its bioactivity with specific reference to the promotion of lateral root growth. Finally, we summarize and critically evaluate experimental data related to the overall effect of humic substances applied to horticultural crops. Current evidence suggests that the biostimulant effects of humic substances are characterized by both structural and physiological changes in roots and shoots related to nutrient uptake, assimilation and distribution (nutrient use efficiency traits). In addition, they can induce shifts in plant primary and secondary metabolism related to abiotic stress tolerance which collectively modulate plant growth as well as promoting fitness. In conclusion, the exogenous application of humic substances within agronomic systems can be used to aid the development of sustainable intensification. As most humic substances used in agriculture are currently derived from non-renewable resources like coal and peat, the promotion of this technology also requires the development of new sustainable sources of humic products (e.g. organic wastes).

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## 1. Introduction

The world is currently facing the combined challenges of feeding a growing population whilst also protecting the environment and producing renewable sources of energy. Demand for food is expected to increase 2–5 fold by 2030 and food production is predicted to increase by 60% in the coming decades to meet these demands (Clair and Lynch, 2010). In the last century, agricultural intensification was driven by inputs derived from non-renewable energy sources (i.e. synthetic fertilisers). Although this approach greatly enhanced crop yields, these practices have also resulted in a major decline in ecological heritage as a result of deforestation, soil erosion, industrial pollution, declines in surface- and groundwater quality and loss of biodiversity (including genetic erosion). These negative consequences of food production continue to proceed at an alarming rate and show no signs of reducing (Altieri, 2002). In addition, it is widely acknowledged that an increase in agricultural activities will further exacerbate the negative impacts of global climate change leading to greater uncertainty in food security (Tilman et al., 2011). Current unsustainable farming practices therefore need to be reviewed since current models of agricultural intensification are neither socially or environmentally sustainable.

The new challenge is to build systems of food production based on alternative intensification strategies (termed “ecological intensification”) which promote nutrient-use efficiency, reduce the need for disease and pest control, increase water-use efficiency and conservation, and which restore soil fertility (Tittonell, 2014). Ecological intensification aims to reduce the reliance on external inputs while maintaining high productivity levels (Tilman et al., 2011). Within this context, humic substance-based products may provide a potential technology to integrate different biotechnological approaches for ecological intensification related to both promoting plant growth and plant adaptation to new ways of food production.

Humic substances (HS) are formed by chemical and biological transformations of plant and animal matter and from microbial metabolism, and represent the major pool of organic carbon at the earth's surface. They contribute to the regulation of many crucial ecological and environmental processes. For example, HS sustain plant growth and terrestrial life in general, regulate both soil carbon and nitrogen cycling, the growth of plants and microorganisms, the fate and transport of anthropogenic-derived compounds and heavy metals, and the stabilization of soil structure (Piccolo, 1996). A major breakthrough in understanding humus chemistry in the last decade has come with the recognition that humus is a self-assembled supramolecular associations of small heterogeneous molecules held together mainly by weak hydrophobic linkages (Piccolo, 2002). In solution, HS are better depicted as a collection of diverse, relatively low molecular mass components forming dynamic associations stabilized by hydrophobic interactions and hydrogen bonds. The hydrophilic/hydrophobic ratio governs its environmental reactivity (Piccolo, 2012).

According to Hayes (2006), the operationally-defined fractionation of humic substances is based on their solubility and was first introduced by Sprengel in 1837. Soil scientists define humic acids (HA) as humus materials that are soluble in aqueous alkaline solutions but precipitate when the pH is adjusted to 1–2. In contrast, fulvic acids (FA) remain in solution after the aqueous alkaline extracts are acidified. This classical definition persists in the older scientific literature but chemically HS are nothing more than a product of a saponification reaction by alkaline extraction from soils and sediments. Piccolo (2002) redefined FA as associations of small hydrophilic molecules in which there are enough acid functional groups to keep the fulvic clusters dispersed in solution at any pH, while humic acids are made of associations of predominantly hydrophobic compounds (polymethylenic chains, fatty acids, steroids compounds) which are stabilized at neutral pH

by hydrophobic dispersive forces (van der Walls,  $\pi$ – $\pi$ , and CH– $\pi$  bonds). Their conformations grow progressively in size when intermolecular hydrogen bonds are increasingly formed at lower pH until humic matter flocculates.

New formation of intermolecular hydrogen bonding and alteration of pre-existing hydrophobic interactions accounts for the disruption of original supramolecular associations of humic matter. This interpretation implies that water soluble humic associations are mainly stabilized by weak forces and that root-excreted organic acids (typically present in soil solution) may affect the stability of humic conformations and, hence, their effects on plant processes (Piccolo, 2002). This concept suggests that humic molecular complexity may be reduced by the progressive breakage of inter- and intra-molecular interactions that stabilize the complex suprastructures, thus, releasing single humic molecules that can be isolated and identified by combined advanced analytical techniques. This field of analytical chemistry has been termed humeomics (Nebbioso and Piccolo, 2011, 2012; Nebbioso et al., 2015) and allows a holistic evaluation of the chemical constituents of humic assemblies, thus, providing the basis for identifying HS that influence plant performance. For example, the effects of HS on specific plant metabolic processes can now be better understood through humeomics paving the way for the targeted development of HS biostimulant products for use in agriculture.

The aim of this review is to firstly present the main effects of soluble humic matter on plant growth and metabolism and to describe the relationship between its chemical properties and biological action in a structural-activity model. Secondly, we will report on the mechanistic effects of biostimulant humic-based products on horticultural crop production.

## 2. Direct effects of humic substances on plant growth and development

The promotion of plant growth by HS, defined here as biostimulation, is well documented in the literature (Piccolo et al., 1992; Nardi et al., 2002; Chen et al., 2004; Nardi et al., 2009; Canellas and Olivares, 2014). In support of this, Rose et al. (2014) used a random-effects meta-analysis to show that shoot and root dry weights of different plant species increased about 22% in response to the exogenous application of HS. In view of this, it remains important to understand how HS act on plant metabolism in order to support the future development and successful development and deployment of humic-based technologies. In addition, it is also important to highlight that plant responses to HS also appear to be highly dependent on plant species and ontological state, mode and rate of application, source of HS, and finally the prevailing management and environmental conditions (Trevisan et al., 2010a,b).

Overall, the growth response of monocotyledonous to exogenously applied HS appears to be greater than for dicotyledonous plants although the molecular and physiological basis for this difference remains unclear. In addition, plant physiological responses to HS isolated from brown coal (e.g. lignite, leonardite, subbituminous coals) are less than those observed in response to the addition of HS isolated from peat, composts or vermicomposts (Canellas and Olivares, 2014). Although this information is important for maximising the impact of HS under field application, it is also proving essential to understanding the indirect and direct effects of HS on plant growth.

Humic substances comprise more than 60% of the soil organic matter and are the major component of organic fertilizers and are known to contain significant amounts of nutrients (e.g. N and S; Stevenson, 1994). Due to the stabilization of HS in humic–clay aggregates and their intrinsically slow rate of mineralization in soil,

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