

Review

Revisiting the concept of stress in forest trees at the time of global change and issues for stress monitoring

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ARTICLE INFO

Keywords:

Acclimatization
Adaptation
Chlorophyll fluorescence
Fitness
Non-structural carbohydrates
Optimal and sub-optimal conditions
Resilience

ABSTRACT

The definition of stress comes from the physical sciences and refers to the ability of a force applied to a material to induce a deformation (strain). Unlike materials, living organisms and biological systems react to stress with responses to prevent or repair the damage. Plants exposed to stressful conditions move from an optimal (non-stressed) to sub-optimal state, until they reach a new equilibrium with the changed environmental conditions, through physiological, biochemical, and genetic pathways to cope with the new environmental conditions. Multiple fluctuating environmental pressures often act simultaneously, then the effects of individual stresses cannot be isolated. In natural ecosystems, plants, and plant communities, naturally adapted to harsh environments, are in equilibrium with their environment. This equilibrium can be considered as “optimal” albeit in relative terms (i.e., for a specific genotype at a specific site). The mechanisms of acclimatization and adaptation are important especially in trees, which are sessile long-living organisms, unable to escape from the worsening of the environmental conditions. Rapid climate change, with severe drought and heat waves, can have different effects in relation to their level of equilibrium before the impact (starting point). The new equilibrium can be defined in terms of fitness (capacity of individuals to grow and reproduce) and resilience (capacity to cope to stress and restore the condition prior to the disturbing factor). Field studies suggest that the photosynthetic efficiency can be preserved or restored in short time after the stress event, but resilience (measured in terms of dynamic of non-structural carbohydrates at tree level) may be more severely affected. The conservation and management activities of natural resources require monitoring the stress conditions of vegetation and predicting the possible changes in species composition and structure of communities and ecosystems. This is desirable to maintain and optimise the ecosystem services in a new environmental and future climate scenario.

1. Introduction

Plant stress studies were developed for crops (Ahmad and Prasad, 2012) to identify the environmental factors limiting their production and to adapt the cultures to unfavourable environmental conditions by taking agronomical measures or selecting more suitable genotypes, also through genetic engineering. In natural environments, on the other hands, plants growing spontaneously can be considered adapted to the conditions in which they live. The ongoing global changes exert increasing pressure not only on crops (by reducing the cultivated areas and available resources) but also on natural conditions and non-anthropized areas. Urbanization and ecosystem fragmentation, fires, environmental pollution, biotic invasions, changes in atmospheric chemistry, UV radiation, and, finally, increasing drought induced by climatic changes, alter habitats progressively and permanently, with an overall negative impact on plant fitness, biodiversity, and associated ecosystem services (Chiaibai et al., 2018).

Studies on plant stress may have two different approaches: (i) agromonomical, to sustain the crop production (including timber in productive forests) in a changing world, and (ii) ecological, to preserve the biodiversity and ecosystem services under drier and harsher environmental conditions induced by ongoing climate change. The analyses of stress events and their effects on plants include advanced omics techniques (Macedo, 2012) to address the complexity of gene expression and of molecular and metabolic pathways (Potters et al., 2009) involved in plant responses to environmental constraints. The ecological approaches consider the relationships between the components of the ecosystems, the various physiological responses of different plant species, their strategies and the ways of stress factors acting as an ecological filter on communities. Finally, an important aim of the ecological approach is to provide guidelines and tools for the management and restoration of natural resources (i.e., plant species, communities, forest ecosystems).

The present paper aims to revise the classical definitions of stress for plants living in natural conditions, and to provide a general concept

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<https://doi.org/10.1016/j.stress.2021.100013>

Received 6 April 2021; Received in revised form 7 May 2021; Accepted 8 May 2021

Available online 12 May 2021

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for the monitoring and management of plant communities under the increasing drought induced by climate change. This review is focussed on forest trees, that are long-living organisms, grown in a specific site and unable to escape from environmental changes.

2. Plant stress: pressures and responses

2.1. Definitions of stress in plants

In the physical sciences, stress is defined as the pressure, or the amount of force per unit of area, that produces a deformation (strain) on a material (Koenemann, 2014). Translating the definition of stress and the related concepts from physical bodies (materials) to living organisms, stress can be defined as an environmental pressure (from abiotic and biotic factors) and the strain provokes a response to prevent or repair the damage, by means of metabolic and physiological adjustments (Kranner et al., 2010). In a classic work, Levitt (1972) introduced the basilar concepts related to the responses of plants to abiotic (environmental) stresses. These responses may consist of injury, avoidance, or tolerance. Strain in plants consists of physical or chemical changes, which can be either reversible (elastic strain) or permanent (plastic strain) until the breaking point. Elastic responses involve reversible strain, which is repaired by internal physiological mechanisms, so that viability and function are maintained. On the other hand, plastic responses constitute irreversible damage without repair capacity, reaching the breaking point, just before the death of the organism. According to Blum (2016), strain in plants is a signal for either adaptation (by activating relevant genes) or the death of the organism.

Lichtenthaler (1988, 1996) elaborated the concept of stress in plants by differentiating between eustress and distress. Eustress is an activating, stimulating stress and a positive element for plant development, whereas distress is a severe and 'real' stress (i.e., stress in the negative sense), which causes damage. In either case, stress is dose dependent. The responses to stress do not follow a linear pattern, and a moderate administration of a stress factor may act as a stimulant of physiological performances (*hormesis*, Agathokleous et al., 2018, 2019). As explained by Lichtenthaler (1988), 'A mild stress may activate cell metabolism, increase the physiological activity of a plant, and does not cause any damaging effects even at a long duration. Such mild stimulating stress is favourable for the plant'.

Strasser (1988) proposed a general framework for stress impact and adaptation based on inferences from open thermodynamic systems. The adaptation of plants is regarded as an expression of an optimisation strategy in the face of the environment (Tsimilli-Michael et al. 1995, 1996), driven by the thermodynamic demand for minimal entropy. When a thermodynamically optimal state (steady state) is achieved, the system is characterised as being in harmony with its environment. Any change in the environmental input is a stressor in the sense that it disturbs the achieved optimality and leads the system into sub-optimality. Stress can be defined as this disturbance, and therefore has a relative meaning, with non-stress as the reference condition. Sub-optimality is defined as a continuum from the stressed state to a new non-stressed or harmonic state, without specific ranges or directions of change. If the system reaches a new optimal state, the force driving the state change vanishes, and new stability (i.e., harmony) between the organism and its environment is established.

2.2. Multiple stresses, common responses

Plants, both cultivated and spontaneous ones, are exposed to daily and seasonal variations of environmental factors (e.g., the diurnal and seasonal changes in solar radiation and temperature, Srivastava and Strasser, 1996; Krüger et al., 1997; Strasser and Tsimilli-Michael, 2001), to which they respond with reversible physiological adjustments necessary to cope with the fluctuating environmental conditions. Any external change can be regarded as a stressful event or factor that provokes

a deviation from the non-stressed condition, regardless of the plant's ability to cope with it. Different fluctuating environmental pressures often act simultaneously, and many of them are autocorrelated (e.g., sunlight radiation intensity, air temperature, atmospheric relative humidity, drought conditions). It is therefore not possible to isolate the relative contributions of various environmental factors and the physiological processes triggered by them (Mittler, 2006; Potters et al., 2009). This means that, while the effects of various environmental stresses on plants are commonly investigated in laboratory experiments, they cannot be deconvoluted in the field (Sewelam et al., 2014).

Mittler (2006) proposed the concept of cross-resistance, meaning that the mechanisms developed to withstand a specific stress also make plants more resistant to other stress factors that activate the response of the same candidate genes (Sewelam et al., 2014) and produce a similar molecular and biochemical pathway. Cross tolerance is often associated with changes in the metabolism of reactive oxygen species (ROS) (Potters et al., 2009; Atkinson and Urwin, 2012; You et al., 2015; Sewelam et al., 2016). ROS are produced in normal cell metabolism activities and are involved in the regulation of many cell and plant physiological processes. Excessive ROS production leads to a state of oxidative stress (Apel and Hirt, 2004), which activates a ROS defence network (i.e., an antioxidative system). Moreover, ROS are important signalling molecules, involved in the perception of stress and in mediating plant responses following stress exposure (Apel and Hirt, 2004; Laloi et al., 2004), including the so-called stress-induced morphological responses (SIMR, Potters et al., 2009). Potters et al. (2009) pointed out that a maze of interchangeable molecular processes, rather than a single pathway, is responsible for the general plant response to stress, agreeing with the stress response concept proposed by Tsimilli-Michael et al. (1996) and Gaspar et al. (2002). The ability to activate the biochemical responses to cope with stress conditions is connected to the carbon metabolism, especially to starch and non-structural carbohydrates (NSC). Thus, the amount of carbon reserves (Thalmann and Santelia, 2017) and their dynamic within the plant organs (leaves, stem, roots) play a crucial role in stress defence and acclimation (Niinemets, 2010).

3. Plant stress in the "real world"

3.1. Adjustments, acclimation, acclimatization, adaptation

The responses of plants to cope with stress in the real world are defined in different ways, according to the intensity and duration of the stress application, as well to the combination of the stress factors. Often, there is no full agreement among researchers about the exact significance and application of each term. For this review, we propose the following definitions.

"*Temporary physiological adjustments*" are the responses to daily and seasonal rhythms and fluctuations occurring in a short time and are easily reversible (elastic stress).

"*Acclimation*" (occurring in plant exposed to a single stress factor in controlled conditions) and "*acclimatization*" (occurring in the field as a response to multiple stress factors) are responses of well-established singular plants subjected to a change of environmental conditions during their lifespans (Wilson and Franklin, 2002). These responses allow long-living organisms, like trees and woody plants, to modulate their phenotypes to cope with harshening environments (phenotypic plasticity, Nicotra et al., 2010).

"*Phenotypic adaptation*" (Fox et al., 2019) occurs at the population level when plants spend their whole lifespan growing and reproducing in harsh environments. A classic example consists of plant species naturally distributed along an ecological gradient such as the slope of the mountain. At higher elevation, shallow soils, high wind speed, low temperature and high sun radiation are factors conditioning the expression of the genetic potential of a plant species. The genetic pool of a species determines plant growth and physiological processes allowing survival and reproduction in a harsher environment. In Fig. 1 it is shown, as an



Fig. 1. *Fagus sylvatica* trees at the Monti Alburni site (Southern Italy). A. Tall trees growing in a fertile site (800 m a.s.l.). B. Bushy plants living on a rocky slope (1400 m a.s.l.).

example, beech trees (*Fagus sylvatica* L.) growing at a mountain slope in Southern Italy Apennine (Monti Alburni). The first plant (A) is a tall tree that grows in a fertile forest site (800 m a.s.l.); the second (B) is bushy and lives on a rocky slope (1400 m a.s.l.), with very thin soil and limited water availability. In both sites, beeches grew in their own typical environment. Phenotypes of the same species may differ in terms of structure, growth rate, and carbon allocation strategies, but they are in harmonic equilibrium with their ecological conditions, modulating their physiological functioning to reduce entropy.

“Genetic adaptation” (Anderson et al., 2011) occurs when populations persist and reproduce over generations in stressful environments, producing adapted genotypes (“genetic accommodation”, see Kelly, 2019). This response depends on genetic change by selection in the current population, and it is strongly influenced by the genetic isolation and the strategies of seed and pollen dispersal (Savolainen et al., 2007; Ghannoun and Way, 2011). The production of adapted genotypes goes through a process of mortality – regeneration (Millar and Stephenson, 2015) and a transient increase of pioneer (opportunistic) tree species before the restoration of a new climax condition. The term “genetic adaptation” is used also for the change of species composition and species migration. For wind-distributed tree species (the broadleaved genera *Acer*, *Betula*, *Salix*, *Populus*, and many conifers) the speed of migration may be high enough to follow the gradual shift of climate zones whereas, for species with heavy seeds, this may not be the case. For these species, migration occurs in a long time, but it may be favoured by direct management. Bussotti et al. (2015) reviewed the genetic variability and related functional traits of relevant forest trees in Europe, and discussed the possibility to use Southern European genotypes, more adapted to drought conditions, for the purposes of “assisted migration” in Central Europe.

3.2. Are there non-stressed plants in stressful environments?

A non-stressed plant is defined as a plant living in an environment where it realises the maximum physiological performance connected to its specific genome, including growth and reproduction. In the “real world” this condition rarely occurs, moreover plants live in dynamic environments subjected to recurrent abiotic and biotic stress factors that can differ in intensity, duration, and repeatability. All the physical factors (soil resources, water availability, climate) and biotic interactions at a specific site, together with the normal diurnal and seasonal rhythms and fluctuations, constitute the normality for a specific plant at a specific site. In a condition of stability of all the environmental factors, we can suppose that plants and plant community reach their ecological equilibrium and are able to grow and reproduce over time and generations. Such condition constitutes a relative optimality and plants can be

considered “non stressed” (at least in relative terms) according to the concept expressed by Körner (2018) “neither high mountains nor deserts are stressful for those naturally living there, contrary to common belief”.

The challenge for plant stress studies in the climate change era is therefore the definition of the starting points (or relative non stress conditions) on which environmental pressures act, and responses are expected to be variable for each species and specific situation.

3.3. Fitness and resilience in a changing environment

The equilibrium of a plant at a specific site can be defined by its fitness (i.e., capacity to grow and reproduce at a specific site, Körner, 2003; 2018) and resilience (i.e., the ability to restore the ecological and physiological equilibrium after a disturbance, Lloret et al., 2011). In the context of climate and global change, the overall conditions in the sites where plants are acclimatized or adapted, are likely to change progressively. Forests and natural ecosystems have already been subjected to increasingly stressful conditions for many decades (including lower precipitation and rising temperature and air pollution levels). Environmental factors can act as filtering effect, affecting selectively plant species and ecological niches within a community. In this way, species composition, structure and ecosystem functioning are currently slowly changing; and it can be supposed that this process continues in the future (Martínez-Blancas and Martorell, 2020).

After a transitional period, during which the metabolic pathways are reorganised, plants and plant communities reach a new dynamic equilibrium with a new sub-optimal stable condition, with a species-specific behaviour. This new ecological and physiological state is characterised by a new set of structural and conformational parameters, both at plant and community levels. Maintaining this new state under harsher environmental conditions may be more expensive for the plant in energetic and metabolic terms, because of the increased metabolic costs of sustained growth and physiological and reproductive functions. There is observational and experimental evidence that a population of trees adapted to stressful conditions perform better in terms of photosynthetic efficiency than a population grown on mild environments. It is the case of *Quercus ilex* L. (higher photosynthetic efficiency in trees growing at a more xeric site, Bussotti, 2004) and *Abies alba* Mill. (higher photosynthetic efficiency at higher elevation, Konôpková et al., 2020). These observations suggest that a hormetic behaviour occurs in natural plant populations under harsh conditions (Agathokleous et al., 2018). Nevertheless, there are uncertainties if plants growing on harsher sites are prone to environmental changes (because of environmental metabolic and physiological limitations) or are more resistant than those growing on ecologically optimal sites (because of the activation of cross-resistance mechanisms, Mittler, 2006).

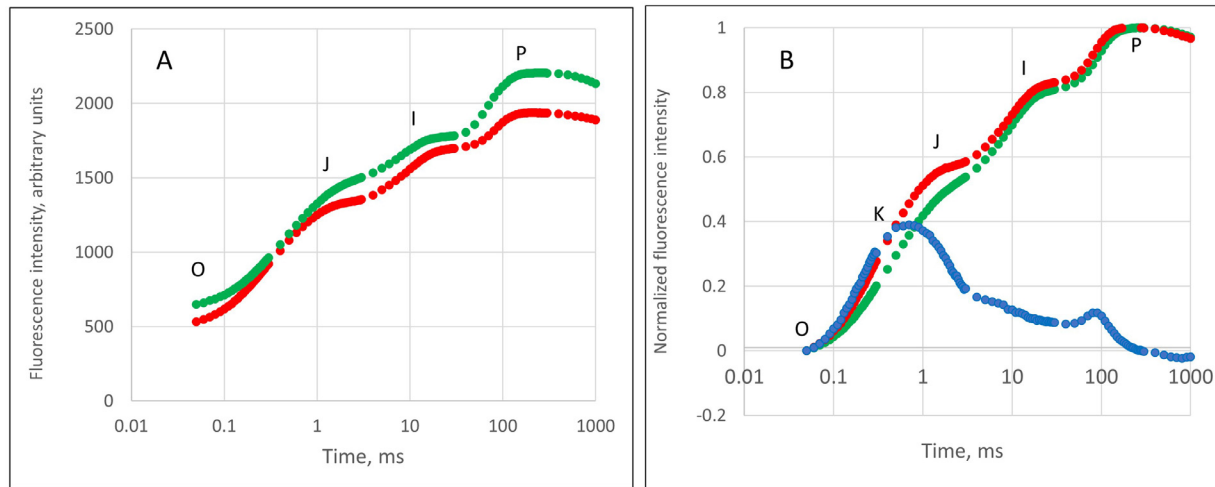


Fig 2. (A) Examples of OJIP transients for unstressed (green) and stressed (red) plants. The curves refer to an experiment of poplar trees subjected to ozone and drought (Pollastrini et al., 2014). The rising OJIP curves (ChlF transients) are induced by a short pulse (1 s of duration) of saturating red light. Plotted on a logarithmic time scale, the ChlF transients show a polyphasic shape. The label O refers to the initial fluorescence level; J (2 to 3 ms), I (30 ms) and P (500-800 ms - 1s) are, respectively, intermediate and the peak levels of the fluorescence emission. The latter indicates the highest, or maximal, fluorescence intensity, when saturating light is applied to the leaf. (B) The same ChlF transient normalized between 0 (F_0) and 1 (F_M). The differences between the are shown in the ΔV_{IP} curve (blue), obtained by subtraction at each ChlF point (F) and magnified per 4 [$(F_{\text{stress}} - F_{\text{non stress}}) \cdot 4$]. With this operation a K-band (300 μm) was evidenced. The K-band indicates the inactivation of the oxygen evolving system.

The most effective driver of dramatic change could be, rather than a progressive environmental change, the recurrence of occasional extreme events (Jentsch et al., 2007), like severe heat and drought waves (Carnicer et al., 2011; Pollastrini et al., 2019; Schudt et al., 2020). The time needed to restore the photosynthetic efficiency and growth rate (fitness) to the level before the stress event could be quite short (Rohner et al., 2020). In the years after the event, however, the plants are especially vulnerable since the recovery of the defence capacity to cope with subsequent stress can be compromised from the delayed recovery of non-structural carbohydrate reserves, which take many years after the stress impact (Wang et al., 2020). Observations in Mediterranean evergreen forests (Galiano et al., 2012; Lopez et al., 2009) show that 10 years after of drought event, starch stocks in *Quercus ilex* trees have only recovered to half their former amount. Galiano et al. (2012) claim that more frequent droughts may lead to a loss of resilience, leading to mortality in the long term (Hartmann and Trumbore, 2016; Trugman et al., 2018). That behaviour suggests a possible trade-off between fitness and resilience.

4. Monitoring the stress

Several methods have been proposed to assess the overall plant stress conditions, both for an individual plant or at the community level (Niinemets, 2010), depending on the objectives of the study and the environmental factors involved. The combination of lab and field experiments with observational studies within large forest monitoring programmes is therefore highly desirable (Bussotti et al., 2018).

Independently from the causal factors, the stress is quantified through the reduction of the photosynthetic function (Lichtenthaler, 1998; Tsimilli-Michael and Strasser, 2013), which led to the reduction of the growth and overall physiological functioning. Such reduction depends on the loss of foliar surface and on the photosynthetic efficiency of the remaining leaves. These attributes can be measured in monitoring programmes with the application of reliable indicators, fast and easy to achieve, both in remote sensing and field surveys.

Remote sensing surveys assess the optical properties of the foliage to assess parameters such as leaf area index, chlorophyll content and photosynthetic efficiency (Serbin et al., 2012). The Sentinel 3/FLEX programme assess the passive chlorophyll fluorescence (ChlF) emitted

by vegetation for evaluating the condition of vegetation across Europe (Mohammed et al., 2019; De Grave et al., 2020). The functionality of the photosystems is considered a proxy for photosynthetic efficiency (Baker and Oxborough, 2004). In remote sensing surveys, ChlF parameters are related to the net primary production of both terrestrial and aquatic ecosystems (Norton et al., 2019).

Terrestrial surveys to monitor the conditions of ecosystems under climate change and air pollution stress have being carried out within large scale international programmes in Europe, North America, and Asia (Ferretti and Fisher, 2013), although the methods used in these programmes were designed under different environmental and management scenarios and are not always well suited to address the emerging climatic problems (Bussotti and Pollastrini, 2017a, b). In forest monitoring programmes, tree crown defoliation is visually measured as indices of stress (Eichhorn et al., 2016), but the real physiological significance of this parameter is still under debate (Pollastrini et al., 2016a). In general terms, we can assume that defoliation indicates a reduction of the photosynthetic surface of a tree, although the growth and physiological functioning may be sustained by the remaining leaves with the so-called "compensatory photosynthesis" (Desotgiu et al., 2012). The actual stress condition may be therefore described by the combination of the loss of photosynthetic surface (crown defoliation or, at stand level, leaf area index) with the photosynthetic efficiency in the residual crown (Bussotti et al., 2003).

In this context it is useful to have a tool to assess objectively the photosynthetic efficiency of trees, and to bridge the field observations with remote sensing. The collection of a great amount of comparable data in forest tree communities is therefore crucial to provide early diagnoses of plant vitality changes, allowing to screen many samples *in situ* in a short time. Among ChlF techniques, the JIP-test is a powerful tool for the analysis *in vivo* of plant stress (Strasser et al., 2000, 2004), widely used for many decades in plant physiological and ecological research, and applied in forest ecology research (Pollastrini et al., 2016a, b, c, 2017; Gottardini et al., 2014). The JIP-test is based on the energy flux theory (Tsimilli-Michael and Strasser, 2013) and the analysis of the fluorescence emission transient (Kautsky effect, Kautsky and Hirsch, 1931) in dark-adapted samples. The shape of the polyphasic curves (transient) of the induced fluorescence emission is interpreted through a series of parameters describing the photosynthetic events related to the light

absorption (ABS), energy trapping (TR), electron transport (ET), dissipation (D), and reduction of the end electron acceptors (RE) in the sample. All these events are expressed as yields and probabilities, specific fluxes of energy, phenomenological fluxes, and performance indices (Strasser et al., 2000, 2004, Bussotti et al., 2020). An example of ChlF transients in stressed and unstressed plants is shown in Fig. 2.

The informative potential of the ChlF assessment can be further enhanced with the assessment of indices and functional traits, such as foliar morphology, chlorophyll and macronutrients content, stable isotopic composition, growth etc., as listed by Bussotti and Pollastrini (2015). The combination of such indices with ChlF parameters may provide mechanistic insight about the determinants of stress conditions in plants.

5. Conclusions

The knowledge of the ability of trees and forest communities to reach a new optimal state under increasing stressful conditions is an important issue for the management of natural resources, to preserve the existing ecosystem services and to adapt them to the new environmental conditions. More research is needed on the genetic, physiological, and ecological processes driving the persistence or the dynamics of plant communities under stress induced by climate change.

Compared to agricultural crops and planted trees, spontaneous plants are characterised by higher intraspecific genetic variability within populations, which increases the adaptability at the population level (Bussotti et al., 2015). Furthermore, the phenotypic plasticity can facilitate the persistence of plant populations at their native sites, by promoting physiological and metabolic adjustments. Persistence is also facilitated by the ability of plants to recover after extreme events (resilience). When the persistence and recovery capacity is overwhelmed, ecological dynamics can lead to a change in the composition and structure of plant assemblages.

To study the responses of plants in extensive monitoring programmes, a small number of reliable selected indicators must be assessed according to standardised methods. Photosynthetic efficiency can be evaluated using by using remote sensing techniques at stand level, or on individual components with terrestrial surveys. Meanwhile, plant resilience can be measured by means of non-structural carbohydrate analysis. The drafting of strict protocols for sampling and analysis and the harmonisation of field and laboratory procedures are challenging tasks for a new generation of ecophysiological surveys (Pérez-Llorca et al., 2018).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We are grateful to Prof. Reto J. Strasser (University of Geneva, CH) and all friends and colleagues for the fruitful discussions concerning the physiological aspects of plant stress, their assessment and measurement, as well the application in large scale surveys.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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