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Plant applications in space-research: effect of gravity and bio-inspiration for future space technology

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1 Introduction to Space Research

The emergent field of space research and applications development expanded the frontiers of science starting from the last Century. It has improved our understanding of many of the basic scientific principles that guide many activities performed both on Earth and in space. Basic research has driven the evolution of modern industry with the discovery of new technologies. Space exploration requests a very innovative and advanced technology to perform different operations with a great accuracy and have to face extreme conditions at a feasible cost. For this reason this field improve our knowledge about materials, try to address problems that affect astronauts' abilities or life functions during space missions or to clarify all fundamental biological processes where gravity plays a central role. In turn it helps new research activities and supports with the creation of new technologies to be used on Earth (Lee 2000, Clément 2011). The study of materials, fluid, and life sciences in microgravity and hypergravity is an exciting opportunity that covers a broad multi-disciplinary field of investigation (e.g. biotechnology, chemical processes, fluid and combustion physics, fundamental physics, human health systems, materials science, etc...) and ways in which these areas of research can be used to advance efforts to extraterrestrial exploration. As result in recent times the number of space research related studies is rapidly intensifying. For example microgravity-related patents increased constantly in the past decades powered by advances in molecular and cell biology and genetics, and substantially more information derived from flight experiments and it is expected to rise greater in the coming years. Although not all patents result in a trade or business process improvement, these increasing numbers highlight the economic value and innovation of these studies, with the subsequent needs of protection through intellectual property rights (Uhran 2012, SSB 1998).

1.1 Experiments in modified gravity condition

During a space mission the influence of gravity is predominant. The launch phase and re-entry phase are characterized by high accelerations (respectively up to 3.2 and about 1.4g) while in space we have to consider how our world is working without the constraining of gravity. In the 18th century the English scientist Sir Isaac Newton first described mathematically the universal force of gravity and extended this concept to other planets and space, beyond the domain of Earth, resulting in the Law of Universal Gravitation. Gravity is a vector, a force that has the flow and direction in all points in space towards the center of the Earth (or the nearest planet). The acceleration of an object subjected only to Earth's gravity is approximately 9.8 meters per second squared and we refer to it as one g. The mass of an object is constant and it is related to the acceleration when a certain force is applied. Thus, on the earth surface the gravitational field that acts upon an object of a certain mass defines the weight of each object, and is a parameter that affects many of the chemical, biological and ecological processes.

The gravitational force acting upon an object will affect its apparent weight. For example a boy, standing on a weighing scale in an elevator, will have different apparent weights depending on the upward or downward acceleration of the lift as shown in Fig. 1. Whilst the mass of the person remains the same, his weight increases under hypergravity condition as shown in the second step and decreases when the gravity vector is reduced as in the third step. If we study the case in which the elevator falls down as shown in the last step on the right, without considering the effects of air friction, the person and the lift drop together reaching the ground at the same time despite their different mass. In this case the apparent weight of the person is close to zero and therefore the boy is weightless into a state of free fall (and the weighing scale, elevator and boy are all accelerating downward at the same speed). The same situation can be applied to an astronaut present in a space station if he drops an apple. In this case the apple, the astronaut and the station do not fall towards Earth but around it. For this reason all objects inside of the station look as if they are floating in a condition "zero gravity" (0g), or more correctly “microgravity”. It is more correct to use the word “microgravity” rather than “zero gravity” because the gravitational attraction is a fundamental property of all entities of the universe and the gravity force is always present also if very small; anyway during a free fall, the apparent weight is reduced by several orders of magnitude that can reach a value from one percent of Earth’s gravitational acceleration (on board an aircraft in parabolic flight) to one-millionth less (10^{-6} g) (Clément 2011). The quality of the exact microgravity value depends on several factors mainly due to the method used to produce it; we generally refer to this condition as microgravity environment (Rogers et al. 1997). The previous example easily explains how it is possible to create a condition of hypergravity or microgravity simply by modifying the apparent weight. By spinning an object in a centrifuge we can increase the gravity vector thus obtaining hypergravity while by putting it into a state of freefall we achieve microgravity.

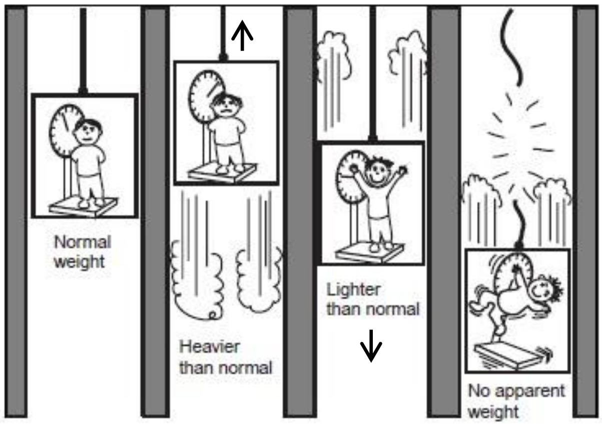


Fig. 1 Example of how the apparent weight of a person standing in an elevator can change because of the upward or downward acceleration (Rogers et al. 1997).

1.1.1 Hypergravity

Commonly when we refer to space research, we mainly refer to low-gravity environments; nevertheless hypergravity experiments can improve our knowledge of the basic influence of gravity both by studying directly the influence of hypergravity or as a complementary study of microgravity. In Europe two examples of flexible and advanced facilities used to perform hypergravity experiment are a Large Diameter Centrifuge (LDC) of European Space Agency (ESA), situated in the Life and Physical Sciences Instrumentation and Life Support Laboratory (LIS) at ESTEC (European Space Research and Technology Centre) in the Netherlands and another centrifuge in the Center of Applied Space Technology and Microgravity (ZARM) part of the Department of Production Engineering at the University of Bremen in Germany. These two facilities have a high maximum capacity (from 80 Kg for LCD up to 1000 Kg for Zarm Centrifuge) and big dimension in length, depth and height providing ample spaces, called gondolas, in which to allocate different tools for data collection allowing the acquisition of measurement points in the range from 1 to 20 g (LCD) or more (30 g in Zarm Centrifuge) depending on the payload of the experiment. Internally there are also all electrical connections necessary for all instruments and a cable connection to permit remote control from an outside scientific location.
The artificial hypergravity is accessible using a high speed rotation and the length of the high

arm (ω, r respectively in Fig. 2 left). It is necessary to use very long arms in order limit the g level which depend on the position across the radius and limit Coriolis Effect. In fact, in a small centrifuge it changes rapidly and consequently gravity severely fluctuates along the height of the sample. The equipment is artificially accelerated ("ae", experimental acceleration) due to the Earth's gravitational acceleration and the centripetal acceleration developed by the speed of the rotating arm (Fig. 2 right). In hypergravity experiments, the following relationship can be made between the centripetal acceleration and the acceleration experiment (acceleration of gravity equivalent the one the equipment will be subjected):

$$a_{\exp} = a_{centripetal} \cos(\alpha)$$

whereas the inclination angle of the gondola is related to the speed of the rotor arm and its evolution when the speed of the rotor increases.

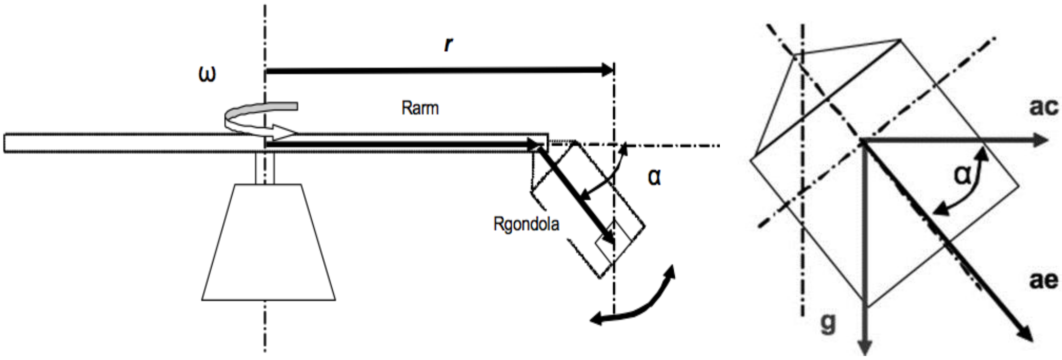


Fig. 2 Rotation scheme of the Large Diameter Centrifuge (LCD) and schematic experimental acceleration of the equipment in the Gondola. (ESA Experimenter Users Manual).

1.1.2 Microgravity

Using a method similar to the elevator example it is possible to produce a microgravity environment relied on free-fall. Unfortunately the only way to establish long-term weightless conditions is actually to go into space (Seibert 2001). Anyway there are several facilities or methods to obtain this state with the limitation of maintaining this condition for an acceptable period of time. Besides other solutions as the clinostats or the random position machine which principle consists of rotating a sample to reduce the effect of gravity all facilities can be resumed in different categories:

Drop tower or drop tube

The principle of the drop tower is often used in Amusements Park to recreate the sensation of freefall. The height of the drop is fundamental to improve the period of microgravity. The only drop tower for scientific use in Europe is located in the Zarm laboratory of Bremen. It has a height of 146 m and produces only 4.74 s of near weightlessness (and if needed, it is possible to use a centrifuge for experiments previously carried out under microgravity condition). In all drop facilities usually the microgravity period is short but it is a good quality gravity period close to level of 10^{-6} g, which cannot be achieved by more expensive methods like parabolic flights or sounding rockets. The air inside the drop tube is evacuated in order to minimize the drag effect; after the launch, the capsule falls in a specific container filled with soft materials to gently decrease the extreme deceleration that all experimental instruments undergo. To extend the experiment period to more than 9.5 seconds, a catapult system can be also utilized in the facility (Fig. 3). This method consists in shooting the capsule from the bottom to the top of the tower which afterwards falls down again. A pneumatic piston driven by the pressure difference generated by the vacuum of the evacuated conduit causes the initial acceleration of the capsule which then drops again into the deceleration container which has been moved under the drop tube in the meantime (Zarm 2000).

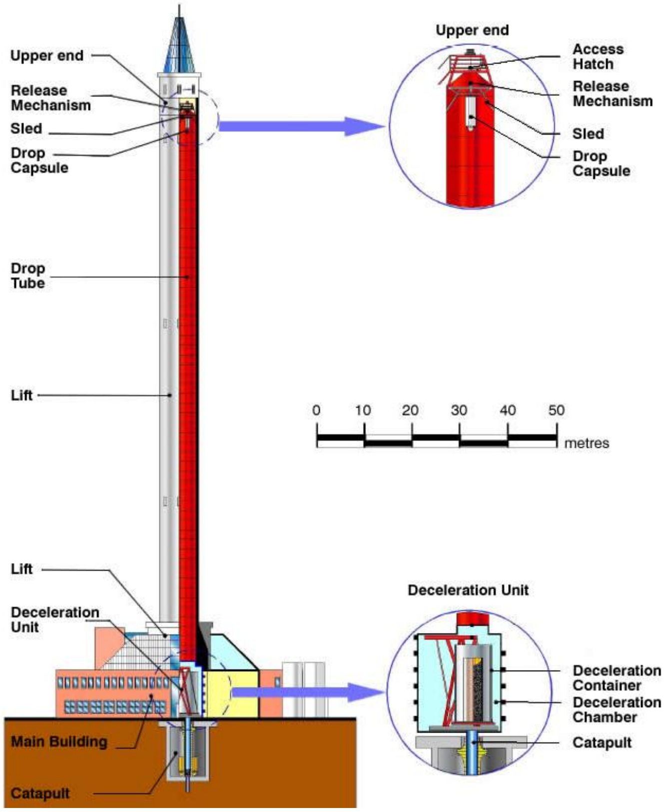


Fig. 3 The ZARM drop tower schematic view (http://www.lunartech.org).

Parabolic flight

The parabolic flights are conducted aboard a special aircraft, modified and configured in such a way that it can perform a series of maneuvers, called parabolas, useful to recreate inside around 20 seconds of microgravity, with a reduced gravity level of 0.16 g. These particular aircrafts have been used starting from the late 1950s to create a weightless environment for the training of astronauts or to conduct experiments under conditions that would not be possible to obtain on earth. Novespace, founded in 1986, operates the only European aircraft able to perform this task, the "Airbus 300 Zero-G", and manages and promotes parabolic flights

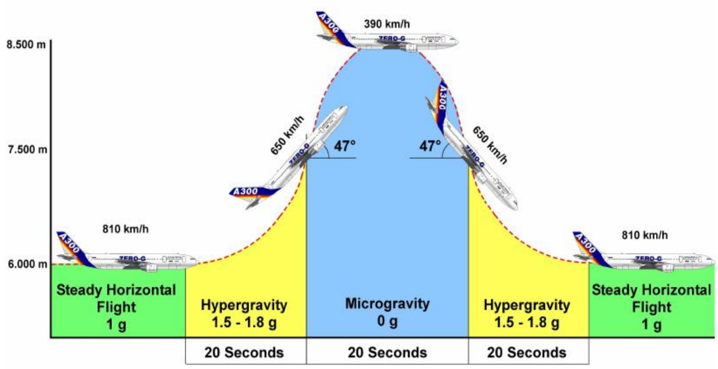


Fig.4 Schematic representation of a parabola (http://www.leem.es/imagenes/Imagen1.png)

activities in collaboration with the French CNES (National Centre for Space Studies) and ESA. The operations carried out by the plane to make the correct parabola and create 20-22 seconds of weightlessness are extremely precise and require experienced and trained pilots (Fig. 4). At the beginning the aircraft, by raising the angle of inclination for 20 seconds (pull up phase), is subjected to an acceleration of about 2 times that of Earth (1.8 g). Once reached the maximum inclination, the plane follows the parabolic trajectory of an object in free fall for 20-22 seconds during which a microgravity environment is produced. The peak of the parabola is obtained at high altitude and the plane continues the parabolic trajectory until it has to move back horizontally causing an addiction period of 20 seconds hypergravity (pull out phase) because of deceleration. Despite the lower quality level of reduced gravity and a higher price compared to the drop tower facilities, parabolic flights produce a longer period of microgravity and allow a direct interaction between experiment and experimenters, a great opportunity that can only be obtained in Space Stations. Nonetheless it is possible that during this flight passengers develop motion sickness and for this reason in the past they were referred to as "Vomit Comets" (Dempsey 2007). Anyway in recent time, a medication (usually in scopolamine injection) is given to the experimenters to limit and reduce these side effects. In addition, some private companies have decided to offer commercial parabolic flight, giving the opportunity to anyone in good health to experience the sensation of floating in space, and the proceeds of these public campaigns of parabolic flights will be used to fund space research.

Sounding rocket

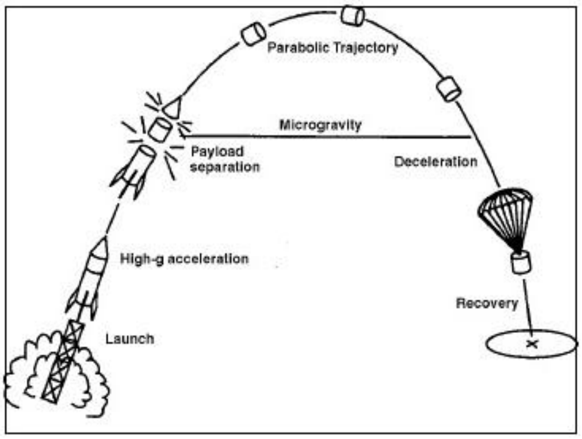


Fig. 5 Rocket parabolic flight profile (Roger 1997)

Another way to obtain a long period of reduced gravity (up to ten minutes) is the utilization of rockets able to perform suborbital parabolic trajectories (Fig. 5). These rockets are usually called "sounding rocket" that refers to the nautical term "to sound", which means to take measurements. These rockets are composed of different parts: a propulsion system, the scientific payload and other accessories for rate control, telemetry and recovery (Ceglia et al. 2005). The microgravity period is obtained after the rocket burn out and before entering the atmosphere and it has a level between 10^{-4} and 10^{-5} g. Usually missions about sounding rocket used by ESA or DLR (German Space Agency) are launched from the Esrange Space Center located outside the town of Kiruna in northern Sweden. The payload and the microgravity period can vary from about 180 to 780 seconds and it can accommodate several experiments, not necessarily proposed by the same agency (Seibert 2001).

1.2 Opportunities

All facilities previously analyzed present severe experimental constrictions and limitations and all hazards and risks must be carefully taken in consideration during the design phase of each experiment. All companies expected a detailed documentation of all operations and hardware used to fulfill the scientific object consequently the preparation phase of each campaign is a challenge for all scientists and physics. Numerous fields of study can significantly benefit from a microgravity environment, highlighting the diversity and range of subjects and the opportunities to be provided by financial support. The utilization of a modified gravity environmental can be an easy way to achieve scientific purposes and a suitable alternative to experiments in a satellite or Space Shuttles in orbit with reduced cost. The International Space Station has been developed as a microgravity research platform to explore microgravity and conduct research relatively free from the effects of gravity. Anyway because of the great expenses and the limited access to space shuttles or satellites, they should be considered as the end point of each space related experiment and it is important to consider the possibility to test ideas about materials, fluids, and life sciences on ground. Clinostats and 3D random machines can be helpful to mime microgravity but they cannot be considered as substitutes for authentic microgravity experiments in absence of gravity because the gravitational effect still occurs, but it has a continuously varying direction. With the development of the space research a large number of important effects on fluids and materials, science biology and biotechnology, human physiology and fundamental physics were discovered and required further investigations. All these studies attracted interest because, besides being important as research for long-term space missions, they enhance the quality of life on earth by finding applications in many industrial and commercial activities or health support (Seibert 2001, Lee 2000).

1.3 Plant biomimetic and its applications on space

In the recent years there has been an increased interest towards the study of nature for the construction of bio-inspired structures, and this new area of research is now defined as "Biomimetic" (Schmitt 1969, Vincent 2006). Plants are a good source of inspiration due to their ability to survive and thrive in hostile or inaccessible environments; they have evolved efficient solutions, through the use of sophisticated physiological and biochemical mechanisms, to deal with challenging and variable surroundings. Furthermore, all these strategies to cope with an ever-changing environment have evolved without a conventional locomotion system and usually with limited resources (e.g. light, nutrients) for plant metabolism. For example, the leaves of the *Lotus* spp. flower are excellent model surfaces that have "self-cleaning" properties; lotus leaves can remain clean thanks to a super-hydrophobic fine structure that completely rolls undesirable particulates off the leaf (Neelesh et al. 2004, Marmur 2004).
Others interesting behaviors are those represented by the sophisticated self-repair mechanisms in plants such as *Aristolochia* and *Ficus benjamina*; the latter can produce latex, which enables the recovery of tissue mechanical properties and prevents the entry of pathogens into the underlying tissues (Busch 2010, Bauer 2011). In addition, plants can show a broad spectrum of deformation principles that are reversible and depend on the elasticity or viscosity of their structure materials. Thus, the kinetics behavior of *Strelitzia reginae* flowers have been used to create Flectofin®, a flexible material which mimics the valvular pollination mechanism in the bird-of-paradise flower: the weight of a pollinating bird or lizard is enough to cause a deformation of the attached lamina inducing a simultaneous sideways bending by 90° which exposes the previously hidden anthers (Lienhard 2011). Seed dispersal is also target of aerodynamics studies because seeds can cover vast distance travelling in the air. They can face and module the influence of the wind with the ability of recovering their orientation in

destabilizing situations (Pandolfi 2012). All this particular structures and behaviors are attractive for the development of space technologies because are able to exploit environmental natural energies or intrinsic properties and minimize power consumption. Many inventions drawing their inspiration from plants' behavior have presented a scientific high-impact revolution and have helped the creation of a new generation of devices with innovative potential applications, even in the field of space research (Menon 2007). One of the most famous invention which is well suited to these features is that of the hook and loop fastener, i.e. Velcro. This material is generally used by aerospace industry for its technical safety advantages, e.g. to hold objects in place while the space shuttles are in zero-gravity zones. Another example of how plants have been used as model systems is the construction of a robot modeled on the Russian thistle, i.e. "tumbleweed"; this robot is capable of generating power by using *in situ* resources (e.g. wind), enabling the robot to explore planet surfaces and avoid obstacles (roughness, presence of stones, cracks or soft sand) (Southard 2007).
In others words, under the constant pressure of variable abiotic and biotic stresses, during the evolution, nature has modified its structures and strategies, in a specific and diversified manner, thus providing us with an inexhaustible source of inspiration.

2 Plant applications in space-biology

All scenarios for long-term space missions and the habitability of extraterrestrial structures need to consider the plants as a key component to support the presence and activities of man. The logic behind this statement is linked to the close interrelationship between plants and man and their complementarity. The plants, in fact, are able to recycle the waste produced by humans, to provide them with food, produce oxygen and provide psychological support. This consideration is the basis of the concept of Advanced Life Support System with which many researchers have evaluated in the past decade the ability to create a sort of 'biosphere' for long-term space missions or extra planetary colonization. Plants are able to growth and reproduce in space if several attentions are taken into account as gas convention and generally are smaller than comparable plants grown on Earth (Musgrave 1997). To achieve this goal, however, is required a long series of experiments to be able to understand and comprehend the ability of plants to grow in a very hostile environment, especially for the absence of the gravity vector.

2.1 Gravitropism in higher plants

Gravity is a constant factor in the earth's environment which has played a decisive role in the evolution of life. In particular, plants have developed a gravity-directed growth process, called *gravitropism*, that permit to orientate photosynthesizing organs towards the source of light and to optimize the root system for anchoring and exploration of the soil and searching water and nutrients. Gravity is one of the most important parameter which drives plant growth. It is well known from the 19th century from the studies of Ciesielski (1971) and Darwin (1880) that the root tip and its structure is essential for root gravitropism. The root apex and growing tip of plants have many differences, e.g. in layers of cell division, frequency of mitosis and mature cells (Strasburger, 1995), and they differs in their sensitivity of gravity perception (Poff and Martin 1989, Fukaki et al. 1998, Sack 1997, Chen et al. 1999). In contrast to the vegetative apex, the root systems are much more sensitive and complex; they have a territorial behavior, are able to discriminate between their roots and to adjacent plants (Callaway 2002, Gruntman 2004) and often establish symbiosis with bacteria and fungi implying a social nature of plants and a multidimensional signalling below ground (Trewavas 2009). The root apex is able to monitor and perceive many environmental stimuli with high sensitiveness, especially concerning gravity (Moseyko 2002, Kimbrough 2004), is particularly suits the study of the response of a living organism to gravity.

2.1.1 Root tip anatomy

The root apex, where is situated the center of gravity perception, can be divided in several fundamental zones. In fact whilst roots gravity is sensed in the cap, the curvature occurs through the growth of the elongating zone situated few millimeters away depending from the plants species.

The root cap

The roles of the root cap are many: its primary role is to act as a barrier to protect all meristematic cells against friction between soil particles and root during penetration. Furthermore, the cap produces mucilaginous substances that help the insertion of the root by

lubricating its passage through the soil. The size of the cap remains constant because when some cells die, others are produced from the meristematic apex (Hawes 2003, Pandolfi 2009). In higher plants the gravity susceptors or statolytes, are constituted by amyloplasts that sediment in specialized cells called statocytes. Shoot statocytes are less sensitive than root statocytes, which do not possess large vacuoles (Perbal et al. 1997). In the root a group of amyloplast-containing cells called Columella is situated adjacent to the root cap (Fig. 6).
These cells are mainly responsible for the gravity perception. In fact the ablation of the central

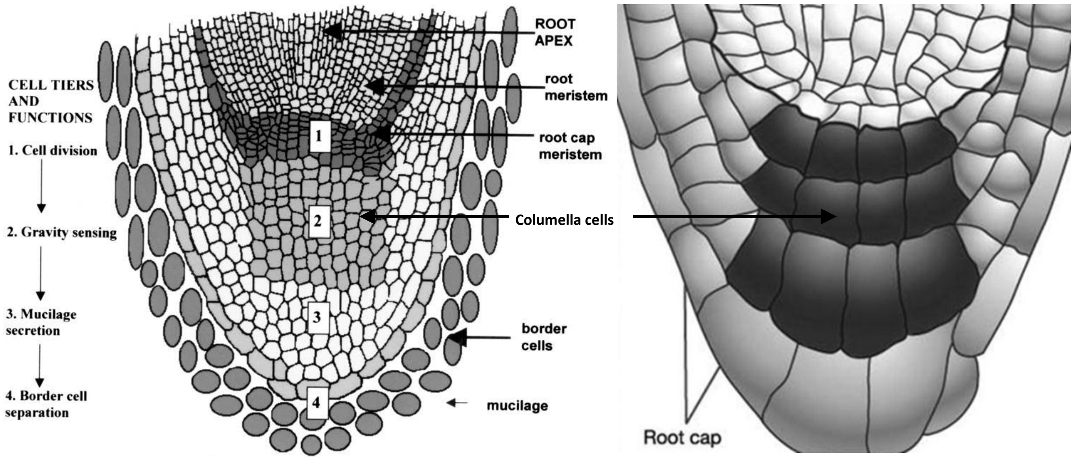


Fig. 6 Root cap structure and development. Columella Cells group is highlighted on the right (Hawes 2003, Barlow 1975, Bancaflor 2002).

Columella cells by a laser has been demonstrated to cause the strongest inhibitory effect on root bending (Blancaflor et al. 1998).

The meristematic zone (Me)

This zone is located immediately above the columella and protected by the cap. It is composed of meristematic cells active and in rapid cellular division which later become specialized and can differentiate into the new tissues of root structure. The meristem sustains growth by continuously adding cells to the youngest end of the zone of elongation (EZ).

Elongation Zone (EZ)

The zone of elongation merges with cells division and it is the part where cells elongate. The elongation of each cell can be more than ten times their original length and is responsible for pushing the root tip, including the meristem, forward. In this zone usually the nucleus is pressed between the wall and the large central vacuole.

Transition Zone (TZ)

The transition zone includes cells between the meristematic and elongation zone which are still ready for the cellular division and can develop in a new tissue if necessary. During the 1990s the vision of root apex changed with the discovery that cells go through a phase of slow isotropic growth, after leaving the meristem (Baluska et al. 1990). Later this zone has been called “Distal Elongation Zone” (DEZ) (Ishikawa and Evans 1993, Fig. 7), mainly because there is not a real elongation. The cells of the DEZ present a particular architecture and show

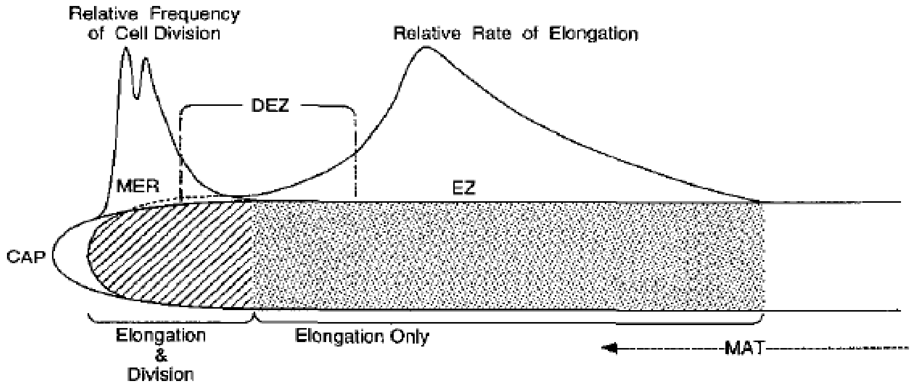


Fig. 7 Schematic diagram of specialized zones in the maize root tip. CAP, Root cap; MER, root apical meristem; DEZ, distal elongation zone; EZ, elongation zone; MAT, maturation zone (Ishikawa and Evans1995).

a reorganization of cytoskeletal elements required for the elongation phase (Baluska et al. 1996 b, Baluska et al. 1997, Baluska et al. 1992, Baluska et al. 2001 Baluska et al. 2003). For this reason it was then introduced the term “Transition Zone” (TZ) to define this unique part of the root apex (Baluska et al. 1994, Baluska et al. 1996, Baluska et al. 1997, Baluska et al. 2001,). It is also possible to distinguish the cells of the TZ from the cells belonging to adjacent anatomical regions because they have the microtubules and actin filaments not yet perfectly aligned in bundles (Baluska et al. 1992; Baluska et al. 1997; Baluska and Hlavacka 2005, Pandolfi 2009).

In recent years, with the advent of plant neurobiology, the study of this particular part of the root has stimulated the interest of many researchers because of its own unique features. This region is therefore an important center for the calculation of the root, which leads the plant to "process" information and "make decisions" (Baluska et al. 2006, Pandolfi 2009). The cells of the transition zone not only possess a strong sensory ability but also show certain plasticity in their behavior. Plasticity is defined as the capacity of an organism to change in response to environmental signals, in physiological and/or morphological terms. The particular cyto-architecture of the TZ, in which the nuclei, placed in central location, are surrounded by peri-nuclear microtubules that radiate towards distant zones, makes it particularly suitable to signals transmission; furthermore, these cells do not perform consuming tasks such as cell division or rapid elongation and therefore can actively use all their resources in the perception and processing of the signals received from the environment (Baluska et al. 2004). As support of this theory, the reconstruction of the oxygen absorption profile in the root apex zone shows that the TZ absorbs a quantity of oxygen much higher than in the meristematic cells and the elongation zone, without any kind of stimulation (Mancuso and Marras 2006). In fact it is the TZ which highlights this high oxygen demand (Baluska et al. 1990). This peak of oxygen consumption coincides with the position where the transport of synaptic auxin is more active (Mancuso et al. 2005; Santelia et al. 2005; Bouchard et al. 2006; Schlicht et al. 2006); in addition it is also the position where there is a greater sensitivity to aluminum, a neuro-toxic element (Sivaguru et al., 1998). These facts suggest that the TZ can act as a "command center" that, through synaptic activity, processes the sensory information and makes decisions on the future exploratory behavior and adaptation of roots and plants.

Maturation zone (MZ)

The last zone of the root is called Maturation Zone (MZ), in which cells gradually decrease the rate of growth, differentiate and become functionally mature; here begins the secondary growth of the plant that forms the various tissues and it is characterized by the presence of root hairs that increase the absorptive surface of roots. Once cells elongated and mature, the root become stationary and no further extension takes place.

2.1.2 Perception of gravity

All parts of a plant respond to gravity in different ways. Stem and shoots grow upwards, against the gravity vector (negative gravitropism), whereas roots grow downwards along the gravity vector (positive gravitropism). The gravitropic response in roots of plants can be divided into (Kiss 2000, Fig. 8):

1. gravity perception
2. transduction of the information in a signal
3. signal transmission
4. growth response and carry out of curvature through differential cells growth.

When the orientation of a root changes and it is subjected to a different gravity vector, this modification is perceived in less than 1 second (Hejnowicz 1998). Nevertheless, only a repetitive stimulus can induce the gravitropic mechanism response and it must be repeated several times or applied continuously for about 10 seconds. Once activated, the gravity perception initiates an asymmetrical signalling inside the statocytes (i.e. the transduction

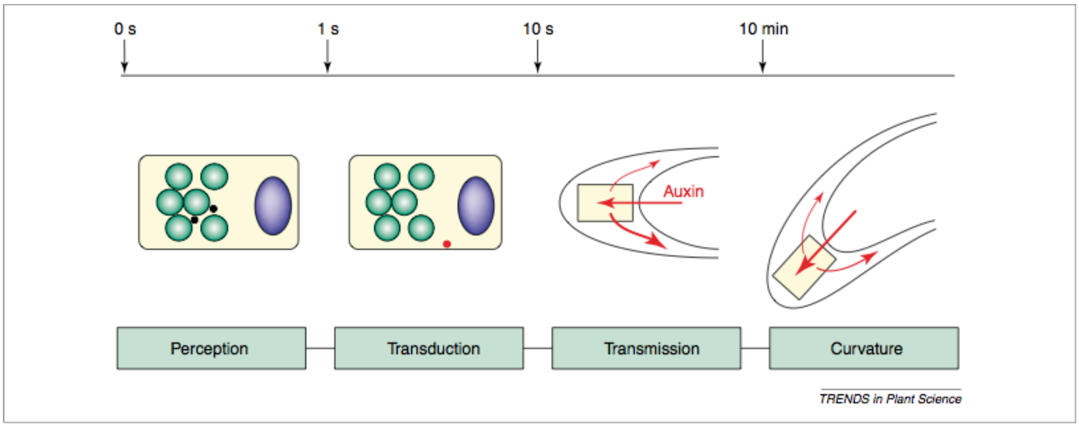


Fig. 8 Different phases of root gravitropism and their timing. The red circle represents the start of the relocation of auxin efflux carriers. The green spheres represent the amyloplasts and the purple sphere represents the nucleus. (Perbal e Driss-Ecole 2003).

phase). The phase of transduction is carried out with the dissipation of the potential energy produced by the gravitational stimulation of amyloplasts, which sediment and produce chemical signals; this generates a downward and lateral distribution of auxin called transmission phase. This asymmetric auxin distribution in gravistimulated tissues is the cause of the differential cell growth which subsequently leads to the root curvature. After gravistimulation, a lateral (downward) transport of auxin occurs from the Columella cells toward the zone of reaction. This lateral transport is required for the gravitropic curvature but it cannot be considered the sole mechanism responsible for the gravitropic response but only for the differential growth. The early stages of the curvature take place in the transition zone (TZ) and only subsequently in the elongation zone (EZ) (Wolverton et al. 2002, Perbal Edriss-Ecole 2003). Artificial applications of exogenous auxin in the TZ do not stop growth but stimulate elongation (Ishikawa and Evans 1993). Indeed, exposure of the root to high levels of auxin does not affect the gravitropic response (Ishikawa and Evans 1993, Katekar et al. 1992, Muday et al. 1994); in these conditions the curvature is carried out only by the TZ, while the cells of the EZ are inhibited and do not contribute to the response (Ishikawa and Evans 1993). All these facts underline the complexity of this mechanism, and lead the hypothesis of the existence of two distinct areas where gravity is perceived (Wolverton et al. 2002, Mancuso et al. 2005, Baluska et al. 2006, Pandolfi 2009).

2.1.3 Electrical activity in plants related to gravitropism

The conduction of electrical signals is used for the transduction of environmental stimuli, which are perceived by sensory systems, into information and biological processes. It is well known that action potentials (AP) exist in all plants since pioneer studies on carnivorous plants in the 19th century (Burdon-Sanderson 1873, Darwin 1875). There are two main stimuli-induced changes in membrane potential: action potentials (AP) and variation potentials or "slow-wave" (VP). These potentials are involved in the transmission of electrical signals in plants and regulate several important physiological processes as photosynthesis and respiration (Malone 1996). The VP is generated as response to deleterious stimuli, while the AP is produced by not dangerous stimuli, e.g. light or touch. The main difference between AP and VP is that the second do not exploit "voltage-gated channels". These channels determine, once surpassed a certain threshold, a rapid variations of the membrane potential where, in particular, the channel of Ca^{2+}, Cl^- and K^+ are involved (Davies 2006). The generation of an action potential can be summarized in four steps (Fig. 9):

1. depolarization: entry of Ca^{2+} ions;
2. repolarization: exit of K^+ and Cl^- ions;
3. refractory period;
4. rest (resting state).

The role of the electrical signals in plant is crucial, especially in processes involving the propagation of signals, as the gravitropism. Several studies show the presence of electrical currents in the root; in particular there are considerable electrical currents in the meristem and TZ, that become weaker in the elongation zone and MZ, meanwhile close to the root cap currents are extremely variable and dynamics (Behrens et al. 1982; Weisenseel et al. 1992, Collings et al. 1992, Pandolfi 2009).

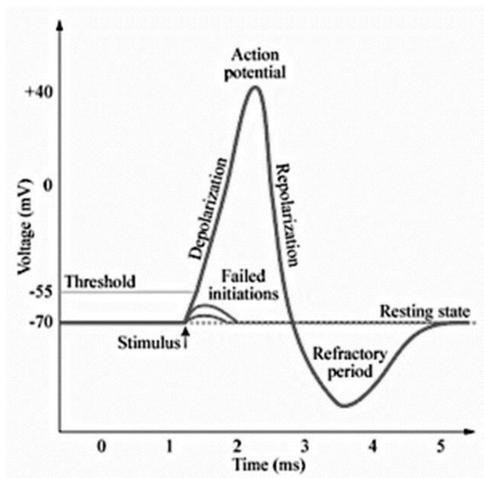


Fig.9 Representation of the phases of an action potential.

2.1.4 Reactive oxygen species and gravistimulation

Reactive oxygen species (ROS) are involved in many important processes in plants (Elstner et al.1994), including gravitropism. ROS are partially reduced or activated derivatives of oxygen. They are formed during some redox reactions and during both the incomplete reduction of oxygen and the oxidation of water by transport electrons chains in mitochondria or chloroplasts. The singlet oxygen formation subsequently stimulates the production of other ROS as the superoxide anion, hydrogen peroxide and hydroxyl/perhydroxil radicals. The superoxide anions are also produced in the chloroplasts when the electrons are transferred directly from the photosystem I to the oxygen. These molecules are highly reactive and therefore toxic for lipids, nucleic acids and proteins, and can lead to the oxidative destruction of cells (oxidative burst) (Asada et al. 1997). Consequently, the evolution of all aerobic organisms has been dependent on the development of efficient ROS-scavenging mechanisms (Mittler et al. 1994). The generation of ROS is unavoidable for aerobic organisms, and occurs at a controlled rate in healthy cells. In plants, ROS are continuously produced as byproducts of various metabolic pathways that are localized in different cellular compartments (Foyer et al. 1994). Under physiologically steady-state conditions, these molecules are scavenged by different antioxidative defense components that are often confined to particular compartments (Alscher et al. 1997). The balance between production and scavenging of ROS may be perturbed by a number of adverse environmental factors; as a result, intracellular levels of ROS may rapidly rise to toxic levels (Malan et al.1990, Elstner 1991, Prasad et al. 1994, Tsugane et al. 1999). External conditions that adversely affect the plants can be biotic, i.e. imposed by other organisms, or abiotic, i.e. arising from an excess or deficit in the physical or chemical environment, such as changes in the direction and/or magnitude of the gravity vector. The role of ROS in plant gravitropism is currently not well understood but they have recently been identified as new signaling molecules in plants (Hapel e al. 2004, Pitzschke et al. 2006), for control and regulation of many biological processes (Mahalingam 2003). They also actively participate in gravisensing and graviresponse of plants together with nitric oxide (Hu et al. 2005). Joo et al. (2001) investigated the role of auxin in root gravitropism. Their results suggest that the redistribution of auxin by gravity induces an increase in the gravitropic curvature in maize root through the generation of ROS. A significantly higher concentration of ROS was detected in the TZ compared to that in the MZ immediately after gravistimulation. Due to the rapid diffusion capability of these molecules, after a continuous stimulation, an accumulation of ROS was also observed in the MZ. Interestingly, the synthesis of these signaling molecules, all involved in early stress signaling in plants, seems to be related to an improved oxygen consumption, which is also one of the earliest metabolic response detected in plants during microgravity exposure (Mugnai et al. 2008). Gravistimulation elicits a transient increase in intracellular ROS, which plays a role as a downstream component in the auxin-mediated signaling pathway. Furthermore, unilateral application of hydrogen peroxide to the root elongation zone of vertically positioned roots induced curvature, whereas ROS scavenging by antioxidants inhibited root gravitropism. Other works underline an accumulating evidence that Ca^{2+} regulates hydrogen peroxide homeostasis in plants (Neill et al. 2002). Conversely, K^{+} and Ca^{2+} permeable channels are also activated by free oxygen radicals that are involved in promoting a large Ca^{2+} influx in the elongation zone of Arabidopsis roots (Demidchik et al. 2003). This would suggest that a cross talk between ROS and Ca^{2+} contributes to the regulation of the auxin-induced differential growth responsible for gravi-curvature in roots. How molecular mechanisms involving ROS are integrated into a physiological signal that leads to the gravitropic curvature remains to be elucidated.

3 Purpose of the thesis

The effect of hyper-and microgravity on the development of the plant is a topic of great interest for space companies that support and promote research in this field. Despite many studies have been conducted to understand the molecular, cellular and physiological mechanisms underlying plant responses to the absence of gravity and the surrounding environment, there are several questions that remain to be elucidated. Furthermore, we evaluated the possibility to use plants as a font of inspiration and exploit their characteristics and strategies to develop new technologies. With the supports of several space related projects, this work has been structured in two parts:

- in the first part, through the possible use of plants in space biology, various studies have been conducted to clarify the mechanisms involved in the early stages of the chemical and electrical response to gravity in root tips, center gravity perception in plants, focusing on situations of hyper-and microgravity. With an active participation in several campaigns in collaboration with the European Space Agency which offered the participants the possibility to use various space facilities (listed below), it has been possible to investigate gravity-related phenomena:
  "Spin Your Thesis 2010-2011": Large Diameter Centrifuge in ESTEC;
  "Drop Your Thesis 2011": Drop Tower in ZARM;
  "Fly Your Thesis 2012": Parabolic Flight Campaign with Novespace.

- in the second part we have evaluated possible biomimetic applications in systems that take their inspiration from the plant world for space research, in particular the exploration of extraterrestrial soils. This section focuses on the possible biomimetic utilization of *Erodium cicutarium*, a plant that belongs to the family of *Geraniaceae* and possesses a unique mechanism of seed dispersion; its seeds have a hygroscopically-active tail that enables them 'to drill itself' into a crevice. With the contribute of the Advanced Concept Team (ESA) and the Italian Institute of Technology we have examined the self-burial performances and dispersal dynamics of *Erodium Cicutarium* seeds in order to evaluate the possibility of a biomimetic transfer of its strategies, focusing on space exploration.

4 Acclimation to hypergravity in plants

4.1 Abstract

In stress conditions, plants have the ability to respond to environmental changes by a process called 'acclimation', consisting in the alteration of the expression of complex gene networks through sensing environmental cues, signal transduction, and modification of biochemical pathways. These transcriptional changes can result in successful adaptations leading to an improved stress tolerance. Plants have evolved under the constant force of gravity and its presence strongly influences their growth and development.

Changes in the gravitational field strength (hypergravity or microgravity) can be considered a source of stress that is perceived at the whole plant level and in particular at the root level, where it is transduced and then transmitted to the other organs by signaling chains, thus leading to an adaptation. Plant responses to environmental stimuli and excitation can be quickly dispersed throughout the entire plant body by electrical signals. It is worth noting that electric fields must be regarded as one of the most universal properties of living organisms used for intercellular and intracellular communication in the presence of environment changes.

Hypergravity generation through the Large Diameter Centrifuge (LDC) is an easy and effective method to modify directly the magnitude of gravity, and can have a direct influence on growth and metabolism of plant cells and organs. In order to confirm and understand the role of hypergravity as a source of stress for plants, the main objectives of the experiment described below has been, following the preliminary acclimation of maize seedlings to hypergravity, the study of the different response of acclimated *vs* non-acclimated plants in terms of electrical signals generated in responses to increased gravity.

4.2 Background

Due to their immobile nature, higher plants have to cope constantly and continuously with various abiotic and biotic stresses. Therefore, organisms experiencing stresses have to physiologically adapt to the conditions of their surroundings. All living beings survive in equilibrium with the external environment that influences and affects their organization and their behavior (De Weese and Zador, 2006). To increase their chances of survival, in fact, all biological systems must continuously monitor and obtain information from the environment and use it to predict the changes and drive their growth or physiological modifications to improve their fitness (Barlow 1993).

Plant acclimation to any environmental changes represents the temporal integration of short-term and long-term fluctuations in an environmental signal. In stress conditions, plants have the ability to respond to environmental changes by altering the expression of complex gene networks through sensing environmental cues, signal transduction, and modification of biochemical pathways. These transcriptional changes can result in successful adaptations leading to tolerance through a process called "acclimation". In the literature there are numerous studies on plant acclimatization to the most common causes of stress in plants (high and low temperatures, salt stress, drought etc.).

Plants have evolved under the constant force of gravity and its presence strongly influences their growth and development. Thus, changes in the gravitational field magnitude, both hypergravity and microgravity, can be considered as a source of stress that is initially perceived at the root level and then transmitted by signalling chains to the other organs in order to adapt plant physiology to these changes. Traditionally, the gravisensitivity of growing root apices is associated with their root caps which cover the extreme tips of root apices and protect them from mechanical damage (Mancuso et al. 2006). It is undisputable that statocytes, located in the center of the root caps are critical for the high gravisensitivity of root apices, and nowadays the starch-statolith theory is the leading theory on this important topic of plant cell biology (Sack 1997). Although it is obvious that statoliths are closely related to those elusive cytological processes which allow gravisensing in lower and higher plants, the molecules and the processes in which they participate to accomplish the perception of gravity, and the diverse responses to different gravity levels are still not clear.

All processes of living organisms generate electric fields that must be regarded as one of the most universal properties used for intercellular and intracellular communication in the presence of environment changes. Plant response to environmental stimuli and excitation can be dispersed throughout the entire plant. Gravity-changing conditions generate strong and rapid responses in root of plants and many evidences lead to the hypothesis of an intrinsic capacity of the root apex to generate functional electrical networks.

The MEA (Multi Electrode Array) system has been recently and for the first time used on plant tissues by Masi et al. (2009), allowing to record extracellular signals from in up to 60 sites simultaneously, thus enabling the study of any distributed/synchronized electrical activity of whole cells and tissue, highlighting the connection between electrical response and physiological and metabolic reactions to external stimuli (such as that of gravity-changes). The sample can be monitored for up to 3-4 hours. In the field of space plant biology, for example, Masi et al. (2008) performed a series of short-term preliminary experiments in micro- and hypergravity during an ESA parabolic flight campaign, in order to detect any changes in electrical activity of root cells during the changes of gravity. Roots showed a clear and detectable overall electrical activity during each experiment. Differences in rates were observed both during the same flight and among different ones. In the first case, differences seemed to be related to changes in gravity conditions whereas in the latter one these were probably due to differences in the sample biological spontaneous activities. Roots electric activity can be registered starting from few second after the exposure to hyper/micro g and the generation of action potentials can keep going for many hours.

Hypergravity generation through centrifuge is an easy and effective method to modify directly the magnitude of gravity, and can have a direct influence on growth and metabolism of plant cells and organs. For example, hypergravity treatment inhibited the elongation growth of various stem organs and, this growth inhibition was accompanied by a decrease in the cell wall extensibility (Soga et al. 2005). Recent studies have also shown that exposure of Arabidopsis seedlings and callus cells to hypergravity induces changes in gene expression (Moseyko et al. 2002, Yoshioka et al. 2003, Centis-Aubay et al. 2003, Martzivanou and Hampp 2003, Kimbrough et al. 2004, Martzivanou et al. 2006). Changes at the transcript levels were, however, found to be largely treatment-specific. While some transcripts were not affected by any treatment, some were altered in an opposite manner by hypergravity conditions, or desensitized to hypergravity after extended clinorotation, and vice versa (Centis-Aubay et al. 2003). Callus cultures derived from stems of *A. thaliana* seedlings responded to hypergravity (between 2g and 10g) by up-regulating the expression of approximately 200 genes (Martzivanou and Hampp 2003). Although changes in transcript levels are not necessarily related to the expression of the corresponding proteins (owing to proteolytic processing, posttranslational modifications etc.) such changes should - with some delay in time – also affect protein expression patterns.

4.3 Multi Electrode Array (MEA) system

The Multi Electrode Array (MEA) system is a planar multi-electrode arrays that allows monitoring non-invasively spontaneous and evoked activity as a change in the electrical potential of the intercellular space surrounding the cell at up to 60 sites in the tissue (Fig.10), thus it allows the analysis in terms of electrical network.

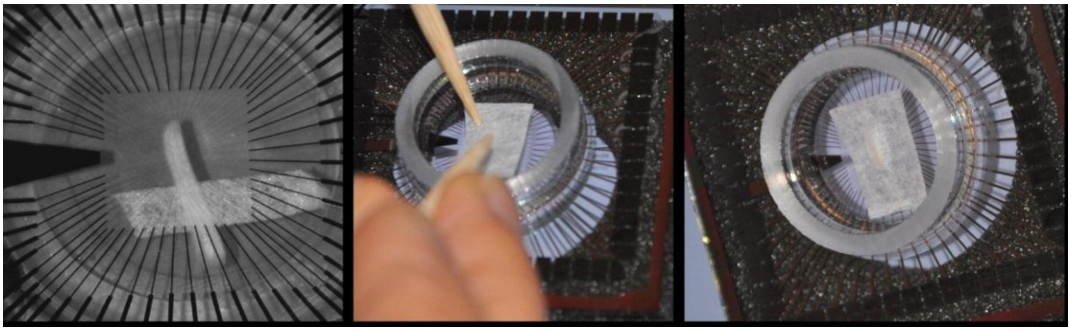


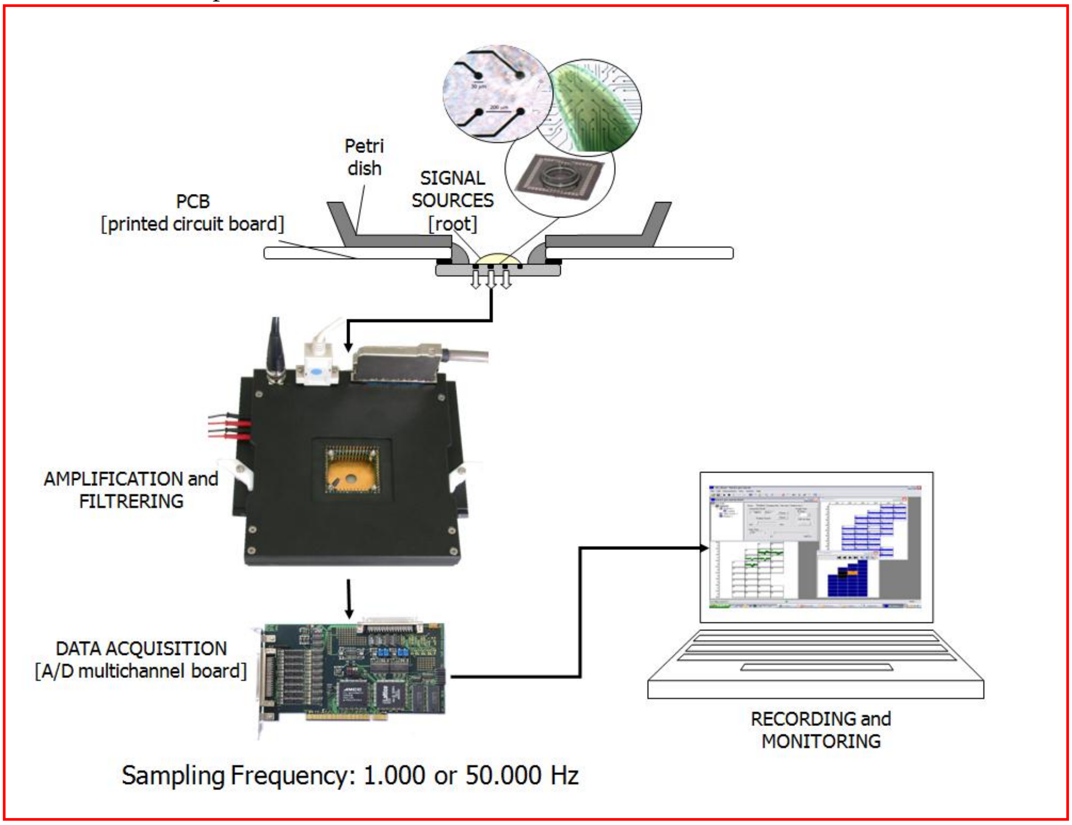
Fig. 10 Root slide fixation on a Multi Electrode array. A special surgery micro-pored tape permits a correct and stable adhesion of the root to the electrodes and at the same time allows liquids and gases exchanges.

The system is characterized by the following component parts:

- a Multi-Electrode Array (MEA) for the measurements of electrical field potentials composed of a glass substrate and 60 thin film titanium electrodes (tyN), glued onto printed circuit boards by a screen-printing technique and seated on the bottom part of a Petri dish in which a hole was stamped for access of the electrode array;
- a discrete-element preamplifiers and filters located close to the MEA device;
- a multi-wire cable that conducts the pre-amplified analog signals to a data acquisition system which is embedded in a personal computer where a special software runs allowing on line monitoring and recording of data got from each electrode;
- a temperature controller for the maintenance of the right conditions during the measurements.

Signals can be recorded continuously at 1-50 KHz rate of sampling frequency.

MEA schematic representation:



4.4 Experimental procedure

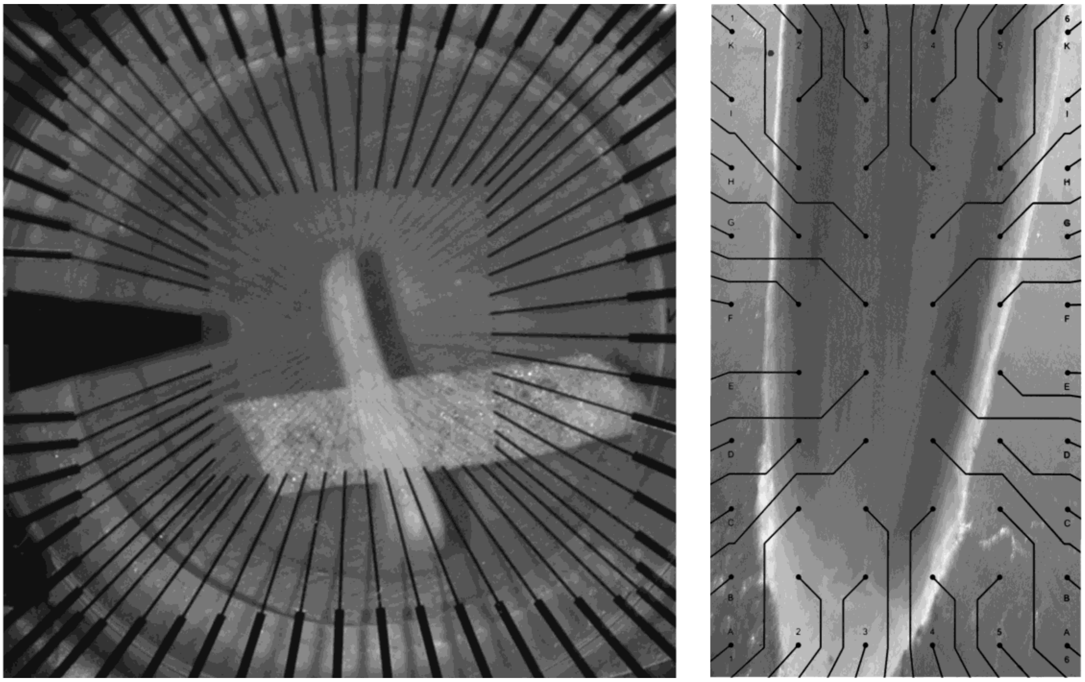
Objectives

In order to confirm the role of hypergravity as a source of stress for plants and understand the physiological responses in plants subjected to hypergravity, the main objectives of this experiment were:

1) to verify the acclimation of maize plants to hypergravity through the use of the LDC for different time-length and hypergravity levels
2) to monitor the response of acclimated *vs* non-acclimated maize plants in terms of electrical network at root level.

The experiment was mainly divided into two steps:
1) acclimation of maize plants for a period of 4 hours at 2g, conducted prior the MEA experiments;
2) after acclimation, MEA experiments were immediately performed at 5g with acclimated plants, in order to detect and measure the electrical network activity of root apices under hypergravity. We recorded data for at least one hour for each experiment.
More, data from non-acclimated plants were recorded respectively at 2g and 5g to obtain reference control samples used to compare data of acclimated *vs* non-acclimated plants.

During the experiment all data were collected with a dedicated software (MC Rack, Multichannel Systems GmbH) that enabled the recording of the electrical activity and the post-processing analysis of the data.



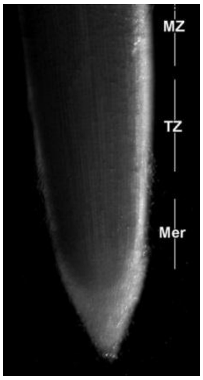


Fig. 11 On the upper left and right part images of a maize root apex fixed on a Multi Electrode Array. Below an image of a maize root apex with the different zones highlighted. **Mer** = meristem, **TZ** = transition zone and **MZ** = mature zone

The software recorded all the electrical signals (spikes) generated by the root apex in gravity stress condition. It was set with specific parameters to detect spikes occurring in each of the 60 channels of the MEA's array. Every channel was correlated with a specific zone of the root as shown in Fig. 11.

4.5 Data analysis

Sources: (Potter 2001, Ecken et al. 2003, Stett et al. 2003, Jeggo et al. 2005, Mouw et al. 2007, Masi et al. 2009, Multichannel Systems guide website).

For the spike detection, a threshold of -35 μV was set as the bound value. This value was sufficient to avoid the average noise of about ±15 μV and, at the same time, to detect every spike. Data were acquired at a sampling frequency of 10 KHz with an input voltage range of ±410.0mV. According to the root anatomy, electrodes were divided in two groups covering respectively the transition zone (TZ) and the mature zone (MZ) of the root apex (Fig. 11). In

average, the electrical activity recorded by around 20 electrodes for each zone was available, and data from the 7 more active electrodes for each zone were used for the statistical analysis. Analyses were performed both considering the two root zones and considering the whole root sample.
For the analysis of data, a dedicated software (Neuroexplorer, Nex Technoloigies) was used. NeuroExplorer is a powerful data analysis program for neurophysiology and it supports several data types: spike trains, behavioral events, time intervals, continuously recorded data and other data types. The most commonly used data type is a spike train who represents only the spike timestamps. A special waveform data type is used to store the spike waveform values. It performs many statistical analyses such as rate histogram, burst analysis, interspace interval, crosscorrelogram etc., and can export all the numerical results or a summary of these in file excel. NeuroExplorer allows also to export waveform data and timestamps data that have been used for the analysis of shape and speed of propagation of signals.
The mean values were used for statistical analysis using GraphPad Prism 5 (GraphPad Software, San Diego, CA).

SPIKE DURATION

Spikes with similar amplitude (50-70 μV) recorded during each experiments were selected and used for shape analysis: the duration of each waveform was calculated using a fixed threshold (chosen as -5 μV) and measuring the distance between x_1 and x_2, where x_1 and x_2 represent the two points of intersection of the waveform with the threshold (Fig. 12).

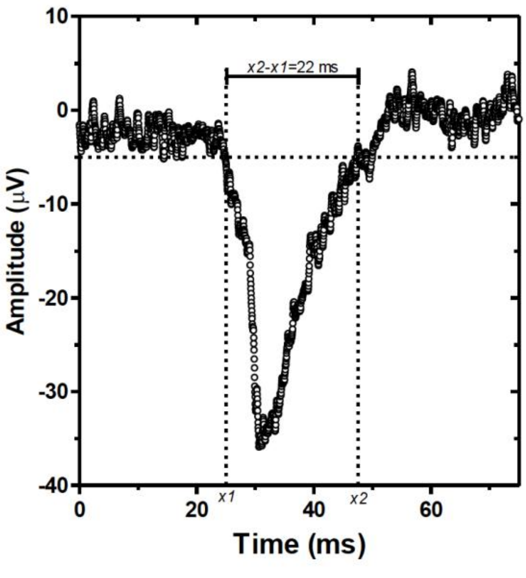


Fig. 12 Example of a spike where duration was calculated using a threshold method

SPEED OF PROPAGATION

Some signals propagating from one region to the other were extracted and used for the computation of the speed of propagation.

INTERSPIKE INTERVAL (ISI) Histograms

Interspike interval histogram shows the conditional probability of a first spike at time t0+t after a spike at time t0. For the analysis, a temporal bin of 0.01 s was considered.

SPIKE RATE

The spike rate analysis displays firing rate versus time. The time axis is divided into bins of 1 s. For each bin the number of spikes is calculated and thus expressed as spikes/sec.

BURST ANALYSIS

This analysis identifies bursts in spike trains. Burst is a continuous sequence of spike (Fig 13). For each spike train, a new interval variable is created that contains the time intervals corresponding to bursts.

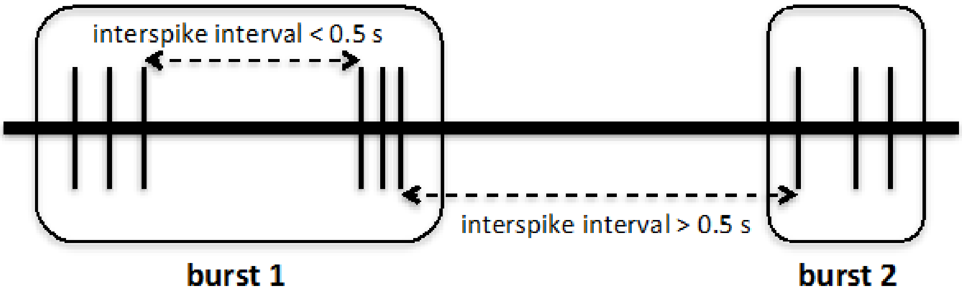


Fig. 13 Examples of how can be the general dynamics and features of a Burst.

The algorithm scan the spike train until an interspike interval is found that is less than or equal to maximum interspike interval in a burts; spikes with interspike intervals that are minor than it, are included in the burst. Maximum interspike interval in a burst was set to 0.5 s. Analyses were performed with a temporal bin of 0.01 s.

CROSSCORRELATION

In order to better characterize the nature of the spike activity in the two zone of the root apex under different gravity conditions, we obtained crosscorrelograms that showed the conditional probability of a spike at time $t0 \pm t$ on the condition that there is a reference event at time $t0$ (t = 50 ms), calculating the probability between spikes occurred at the two different groups of electrodes. The lag time of 50 ms was chosen to be larger enough to allow to highlight synchronizations involving the whole recording area (the maximum distance between two electrodes is 5 mm).

4.6 Results

Results refer to data obtained with experiments performed during "Spin Your Thesis" campaigns in 2010 and 2011. Data analyzed have revealed interesting features and patterns of electrical spike generated by roots subjected to different levels of gravity. Many results have been confirmed in both campaigns, some have shown additional trends and occasionally they have been in contrast.

SPIKES DURATION

Spike duration was calculated for all the spikes generated during the experiments. Fig. 14 shows that average spike durations of roots monitored under 2g and 5g conditions were shorter in respect to control conditions (averaged spike duration were around 20 ms).
Spikes generated by acclimated plants (2-5 g) had variable durations highlighted by the big standard deviation. Whilst 2-5 g acclimated plants presented a various trend with a high error which sometimes was more similar to the one detected during 1g condition, even if some spikes tended to last shorter.

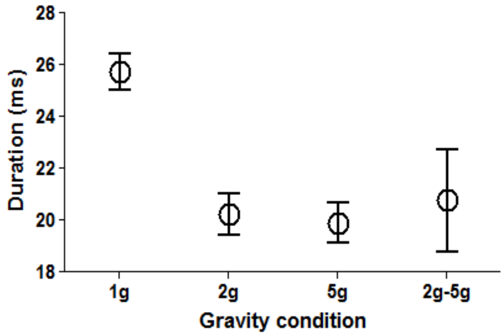


Fig.14 Total spikes duration (ms) of the three experimental conditions monitored where bars represent SD.

SPEED OF PROPAGATION

Higher-temporal-resolution analysis of each frame composing synchronized events allowed the visualization of a spread of the impulse across the root apex. For the analysis, all signals spreading from the TZ to the MZ and *viceversa* were considered. In all gravity conditions, the velocity of the signal propagation decreased starting from the origin of excitation (Fig. 15). This phenomenon is always observed in control experiments and supposed to be an intrinsic property of signal propagation in plant cells (for further reading, see Masi et al. 2009). Interestingly, this decrease was much more pronounced in 1g. On the other hand, 2g and 5g conditions had the opposite effect on the speed of propagation of signals that were able to cover the same distance in less time. Moreover, quite strong signals were observed in 2g and 5g able to propagate for relative long distance (2.5 mm) while in control condition the maximum distance recorded was of 2 mm. The curve of acclimated plants showed an intermediate trend between that of 2g and that of 5g.

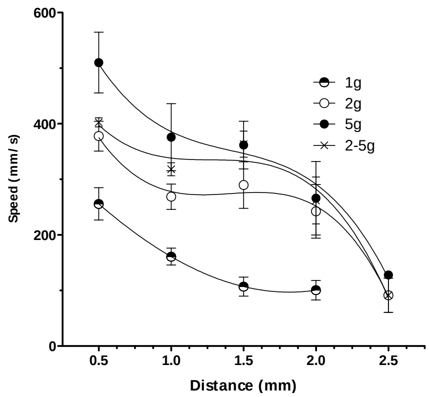


Fig. 15 Speed of the propagation of signals that originate in the TZ and propagate in the MZ.

INTERSPIKE SPIKE HISTOGRAM AND INTERSPIKE BURST HISTOGRAM

We did not observe significant differences in the ISI histogram data (data not shown). This was probably due to the very low spike activity recorded during each experiment that did not help in performing a good statistical analysis.

SPIKES RATE

Differences in spike activity were observed in both experiments and among the same experiment. The overall spike activity, thus spike rate, was comparable with that recorded in control conditions on ground, showing, nevertheless, interesting differences among the altered gravity conditions considered by the study (Fig. 16). In all data collected we observed that roots subjected to hypergravity showed a different electrical activity in comparison to control plants (1g). In particular, considering all the measurements under hypergravity conditions it is possible to hypothesize that the root transition zone (TZ) could be most affected by environmental modifications of gravity, especially when no acclimation was performed, as decreasing spike rates were observed when hypergravity increased. Interestingly, the acclimated samples 2g-5g present an electrical activity which in most of the cases was between the results obtained from the two gravity condition, respectively 5g and 2g.

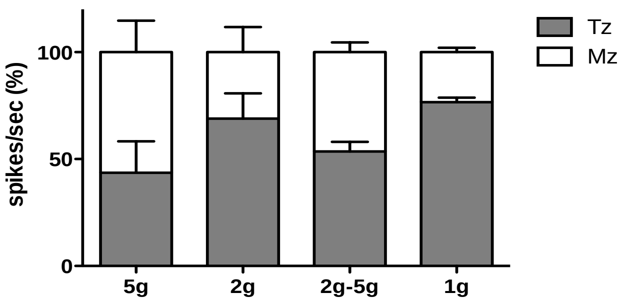


Fig.16 Spike rate distribution (%) and SEM among the two most important regions of a root apex deriving from SYT2010 and

BURST ANALYSIS

A clear increasing trend of the burst activity, starting from control experiments (1g) in which is recorded the lowest frequency (1 burst *per minute*) up to 5g which showed the maximum frequency (over 10 bursts *per minute*) was observed (Fig. 17). Although the reported value of 2g-5g is lower than expected, it can be stated that the treatment had a positive influence on the production of this type of signal. The average number of spikes in a burst has not been reported because not any differences have been noticed among treatments but it always significantly increased when compared with the control.

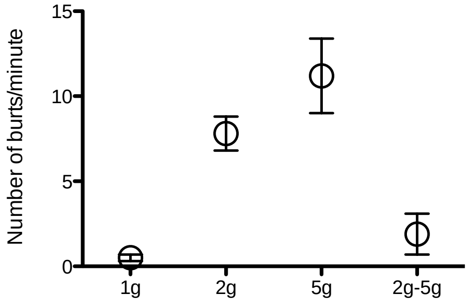


Fig. 17 Representation of the number of bursts per unit time (min) detected during the treatment and in the control

CROSSCORRELATION

The analysis showed that in control condition (1g) the probability of correlated events was lower than in all the other experiments: only few correlated events were, in fact, observed when comparing each pair of different zones and among the same zone of the root apex. On the other hand, in condition of increased gravity, the occurrence of correlated events was higher: the highest peak at $t = 0$ observed in these cases shows that increased gravity conditions enhanced synchronized electrical activity (Fig. 18). In particular, in the case of 5g treatment, the mature zone showed the higher frequency of correlated events, confirming the involvement of this region in the response to gravity changes (Fig. 18, right). On the contrary, the correlated events were more frequent in the transition zone of acclimated roots (Fig. 18, left). Moreover, in general, any king of treatments increased the number of synchronized events recorded over the two different zones of the root (Fig. 18, center).

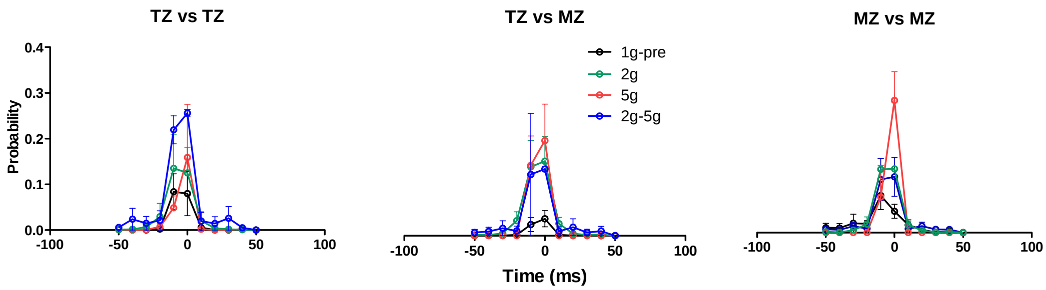


Fig. 18 Pairwise correlation of any possible pair of electrodes firing in the transition zone (left), in the mature zone (right), and in both zones (center)

4.7 Conclusions and discussion

Concluding, our experimental system was able to monitor successfully the electrical signals in roots at different g level. The MEA-system was able to continuously record the electrical activity of roots and to prevent relevant noise interferences. The electrical activity was significantly altered compared to the control in all treatments tested, particularly in that more stressful (5g). Acclimation at 2g, in our experimental conditions, attenuated the stress derived from treatment at 5g resulting in signals more similar to those recorded in 2g or 1g conditions. The process of acclimatization carried out during the experiment for a period of 4 hours at 2g seems to have attenuated the stress perceived by the root tips when subjected to gravity with a magnitude of 5g. In fact, in the analysis of electrical activity, except for a few exceptions (where the analysis of the data was probably altered due to the excessive noise in the signal), the acclimated samples showed an activity quite different from that of the samples subjected to 5g without acclimatization. The spike rate and the duration analysis show interesting features of the electrical response of plants in hypergravity conditions. In brief, by observing the spike rate the root transition zone (TZ) was highly affected by the hypergravity. This part of the root apex is very sensitive to environmental modifications, and especially when no acclimation was performed, a decrease of the spike rates of TZ respect MZ was observed. The mature zone, that in the absence of stress appeared to be less active than TZ, appeared to increase the response capacity in proportion with the intensity of the stress, and this increase in activity was also confirmed by a greater interaction between the two zones (see crosscorrelation analysis). The activity of bursting appeared to be in direct proportion with the increase of the gravity level. These results demonstrate that changes in the force of gravity are interpreted by plants as a stress factor so they respond to this stress by changing their signalling chains in a more active one. The reactivity is demonstrated by an increase in the speed of the electrical signals in the root apex and a capability to spread these signals in more distant zones of the root compared to controls plants in normo-gravity. A real physiological acclimation to hypergravity could occur, when plants were preliminary subjected to 2g prior to the treatment at 5g. At the same time we can observe in the majority of the cases that acclimated plants had a different behavior respect to non-acclimated plants, suggesting that something happened during the four hours of acclimation period. This is particularly confirmed in the analysis of spike duration and spike rate: in fact acclimated plants showed higher spike duration and also a significantly higher spike rate distribution in the TZ at 5g. The data derived from these experiments will help to better understand the physiological and electrical response of plants to hypergravity and the role of acclimation on plant subjected to hypergravity, modifying themselves to survive in unfriendly and extreme environmental conditions.

5 Chemical signalling in roots under microgravity conditions

5.1 Abstract

Among the chemicals produced by roots under abiotic stress, nitric oxide has a leading role in redirecting plant metabolism in order to overcome environmental constraints, such as microgravity. The experimental objective of this project was to investigate the production and release of nitric oxide and other related reactive oxygen species from roots during the period of microgravity. This objective was achieved by using a simple collection system: water samples in which roots were placed during the capsule fall were collected just before the end of the microgravity period. The concentration of reaction oxygen species and nitric oxide in these water samples was determined by a fluorimeter on ground, after the recovery of the capsule. Despite the number of drops and the amount of roots used, our data show very low statistical differences before and after the microgravity treatment. For this reason, in the final section of this chapter, we have discussed the possible causes behind these results and some considerations have been made on how to plan future experiments.

5.2 Background

In recent years nitric oxide (NO) has emerged as an important endogenous signalling molecule in plants that mediates many developmental and physiological processes including xylogenesis, programmed cell death, pathogen defense, flowering, stomatal closure, and gravitropism (Lamattina et al. 2003, Neil et al 2003 and 2002). Furthermore, one of the undesirable consequences of the physiological stress is the concurrent production of reactive oxygen species. One of the most important physiological process activated by ROS with the participation of NO is the Programmed Cell Death (PCD). This process is developed by specific cells both as a response to environmental stresses and as a response to an excessive production of substances which results in an uncontrolled cell proliferation. Moreover, due to the morphological and functional variation of cells located in the tissue environment, NO activity may be limited to specific cells or even certain intracellular compartments (Mugnai et al. 2012). In fact, NO is strictly associated with transition zone of the root apex, especially during the onset of abiotic stress, such as hypoxia (Mugnai et al. 2012) or gravistimulation (Hu et al. 2005). In a parabolic flight campaign, ROS (i.e. hydrogen peroxide, NO) have been detected by the use of specific microelectrodes, but this technique has been shown to have limited applicability due to technical constraints (Mugnai 2008 et al.). Interestingly, the synthesis of these signalling molecules, all involved in early stress signalling in plants, seems to be related to an improved oxygen consumption, which is one of the earliest metabolic response detected in plants during microgravity exposure (Fig. 19).

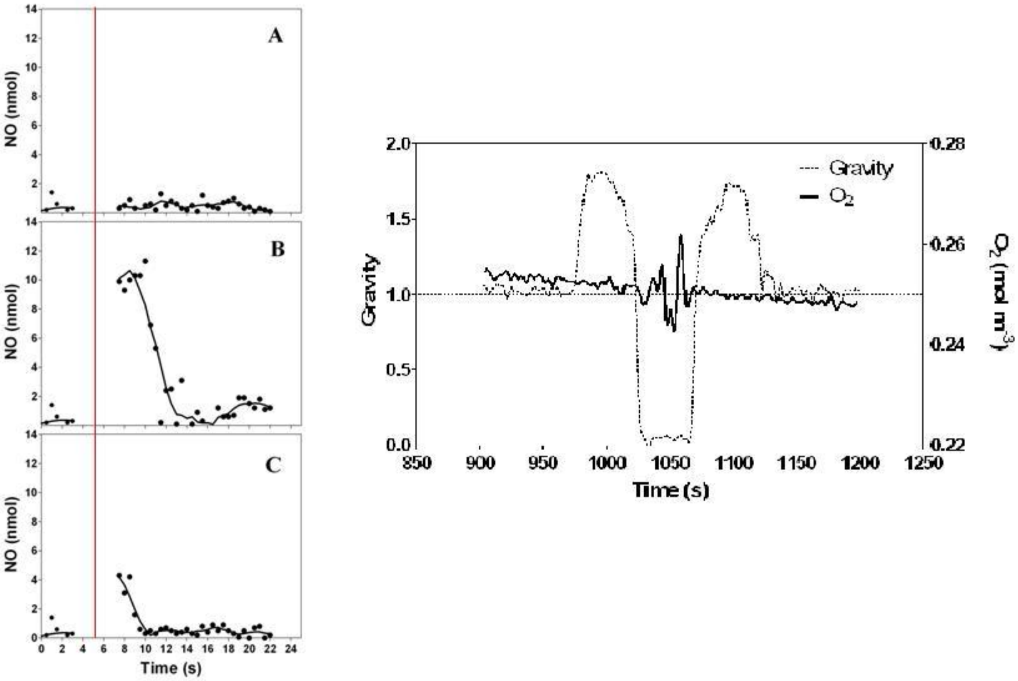


Fig. 19 LEFT: emission of NO after gravistimulation. The red line represents the moment in which maize roots have been placed horizontally with a 90° rotation. Measurements were made with specific NO microelectrodes in three different zones of the root: mature zone (A), transition zone (B) and meristem (C) (Pandolfi 2009).
RIGHT: Oxygen spikes detected during a parabola (Mugnai 2008 et al.).

The missing information of all the reported papers is the behavior of NO production and release by root apices subjected to a real microgravity stress. For this reason, the main objective of this project was the detection of NO production from maize roots with the use of the Drop Tower.

5.3 System description

The experiment consists of 35 syringes placed in a box and a two plunger system as shown in Fig. 20.

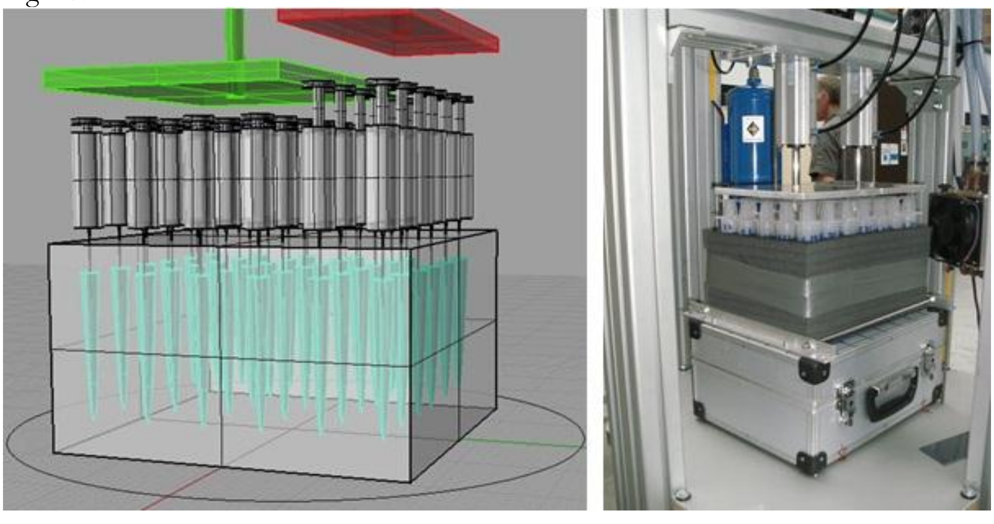


Fig.20 LEFT: schematic drawing of the capsule from the design phase.
RIGHT: picture of the actual complete set up in the capsule.

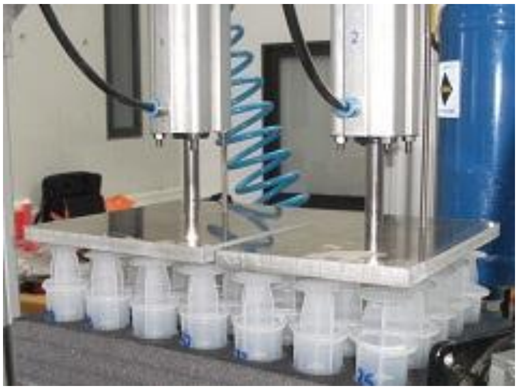


Fig. 21 Pictures of the two automatic plunger systems.

Two automatic system plungers (Fig. 21) were connected to two different pneumatic motors that could be activated separately. They could move about 3 cm. The two plungers were connected and controlled externally to collect samples when activated on ground. Automated software has been developed with the help of the Zarm Laboratory Staff to sequentially depress the two plungers: the first one before the drop and the second one after 3 seconds of microgravity.

An aluminium box coated with an insulator (Fig. 22) contained several collection tubes immerged in dry-ice. The box was coated with polystyrene to avoid temperature changes. The collection tubes were headspace vials able to be injected by needles without losing any substances.

Fig.22 Three pictures of the Aluminium box and the collection tubes inserted in dry ice.

A set of 35 syringes containing water and root samples were fixed into a plastic support and attached to the top of the aluminium box (Fig. 23). All the needles reach the tubes through a hole in the aluminium box and penetrate the headspace vials.

Fig.23 The 35 syringes and the polystyrene support on the top of the box. The needles reach directly the collections tubes.

Metallic clamps were used to fix the plastic support of all syringes in the top of the aluminium box and the box to the bottom of the capsule. A probe for the temperature was inserted into the dry-ice to ensure the rapid freezing of the samples once in the vials. A thermostated serpentine was also used to maintain the temperature of the roots at about 24°C for all the loading time (Fig. 24).

Fig.24 From left to right: overview of the set-up fixed on the capsule and of the metallic clamps, the thermostated serpentine and the probe to monitor the temperature inside the capsule.

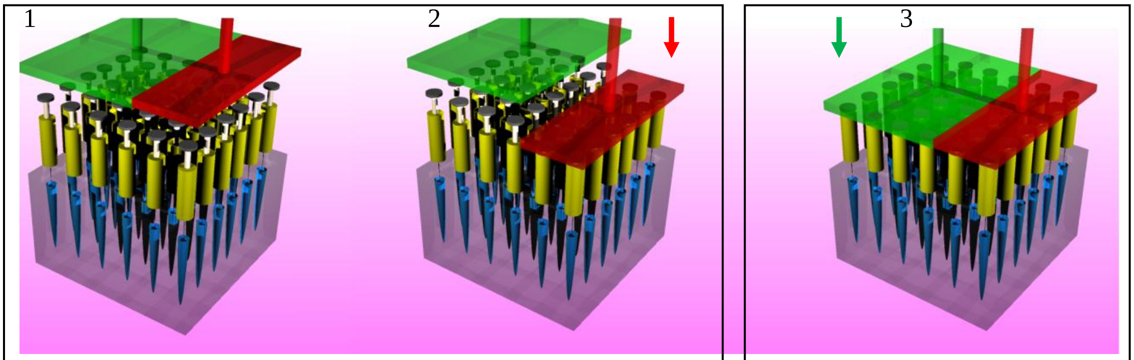
5.4 Experimental procedure

Objectives

- Detection of nitric oxide and hydrogen peroxide production using maize seedlings roots after a treatment of 3 seconds of microgravity with the use of a drop tower.

During the experiment, after 3 sec of microgravity, the medium where the root samples could have released possible compounds was collected. For this purpose we used 35 syringes placed in a box and the two plunger system. Once loaded and before each drop, the first plunger system pushed down the first group of 15 syringes to collect the first samples as Negative Control (Fig. 25 – step2). This operation was performed to avoid false positive as stress-related compounds production coming from samples preparations and load procedures (temperature changes, different pressure, different waiting time etc. may be a source of stress for roots). As a following step, 3 seconds after the beginning of drop, the second plunger pushed down the remaining 20 syringes to collect the liquid and compounds related to microgravity treatment.

Steps required for each drop		
Three days before each drop we spread some seeds in wet paper to germinate in a growth dark chamber	Before loading our set-up, each syringe was filled with root samples and physiological solution. The needle of each syringe ended inside a collection tubes. We collected a first set of liquid as control trial.	Once loaded we operated the procedure as depicted in the figure below:



One minute before the drop

After 3 seconds of the microgravity period during the drop

Fig.25 Collection procedure. Here an indicative scheme is reported whilst in the real system the syringes were arranged slightly differently due to the rectangular shape of the box. It was made by 7 rows and 5 columns and not in a 6x6 square as in this scheme. For this reason the first and second group of syringes were respectively of 15 and 20 as shown in fig.20, 21, 23.

Preparation steps:

1. 3 day-old seedling plant roots were cut at a distance of about 0.4 mm from the apex;
2. successively roots were washed twice for 15 minutes in a physiological solution (potassium chloride 0,5 mM) to eliminate ROS derived from the cut;
3. the 35 syringes were numbered and all groups of roots were weighed before being inserted in a syringe to calculate the biomass present inside each syringe; each syringe was then filled up with 5 mL of physiological solution;
4. insertion of each syringe in the plastic support;
5. insertion of each numbered vials in one needle;
6. connection of the upper part of the box (syringes and vials) to the lower part containing the dry ice;
7. fixation of the box in the capsule.

5.5 Discussion, conclusions and future perspective

The data from DYT2011 campaign are omitted because there was no significant difference resulting from the microgravity treatment. In all vials where NO production was examined there were very low signal levels, comparable with the absence of analyte, as the production of hydrogen peroxide, which is more stable and can resist degradation for more time (few minutes). The absence of any signals in our samples suggests that there could have been some problems during the collection of the samples or during the following analysis. We therefore report here all the possible causes that could have led to this lack of signals.
The first issue to consider is the freezing speed of our samples once the liquid, pressed by the syringe, reached the headspace vials immerged in the dry ice. Even if this process only took few seconds, this step may have played a key role in the loss of information, especially with regard to NO which is very responsive and has a very short time-life. During the freezing time, NO could have reacted, with the consequent reduction of the chances to obtain good signals. In addition, a further loss of compounds may have occurred in our laboratory during

the thawing phase required to analyze the sample with the dye: even if this phase was done carefully and rapidly, each single step could have led to a loss of material. In general, for this analysis, it is advantageous to work in real-time or with protocols that can act on the product directly frozen. The possibility of using liquid nitrogen to instantly freeze our samples has been considered but its utilization is still limited due to its fast evaporation and all safety restrictions required. If this can be solved, novel experiments can be easily planned to understand molecular production in living tissue.

As a second issue, new strategies should be considered to improve the signals. For each drop, about 4 mL of sample were collected for each syringe. This amount is derived from the experimental constraint of not being able to collect all the liquid inside the roots without damaging them, pressing the syringe to the end of the tube. Furthermore, as the roots apices inside the syringe were free-floating in microgravity, they could have accumulated on the piston side and consequently all compounds would not have been able to spread towards the needle but would have been retained in the residual liquid. Moreover, despite the large amount of roots used for each drop, the recorded signals were very low. This may be due to the fact that all compounds were diluted in 4 mL; reducing the volume of liquid to be collected could increase the concentration of substances in the solution. For example a smaller volume of 0.5 mL could be used, which would increase by 8 times the signal.

The last consideration is about the production of NO. Although in gas form, NO is mainly derived by intracellular productions: after its production can spread out as a gas form. Therefore, the monitoring of its presence is quite achievable in vivo (monitoring it directly inside cells or tissues) while its detection is more difficult in the external solution, and requires much more intricate and sensitive methods. For this reason it would have been better to use a system in which we could freeze directly all roots and biomass and, later, analyze all the frozen roots as an alternative of collect all liquid excreted. During our project design we evaluated and proposed a solution where some dry ice was pushed down directly into some roots. This operation would have enabled us to directly collect all biomass and also analyze all the NO produced inside the tissue. Unfortunately, the whole set-up would have been much more difficult to build as during the microgravity period all materials are floating. For this reason we came back to the first experimental set-up and we decided to use a large number of roots to increase the signal.

6 Self-burial mechanism of *Erodium cicutarium* and its potential application for subsurface exploration

6.1 Abstract

Erodium cicutarium plant is a worldwide distributed flowering species which possess a unique mechanism of seeds dispersion. Moreover, seeds are provided of an additional movement thanks to its hygroscopically-active tail that permits the seed 'to drill itself' into a crevice. The aim of the experiment described below was to investigate the self-burial performances of the *Erodium cicutarium* seeds in order to evaluate the possibility of a biomimetic transfer of its strategies, focusing on space exploration. The seeds of this plant are able to improve their chance of penetration in the soil, by exploiting the characteristics of the material of which they are made out. Their particular structure enables the seed to use environmental natural energies as changes in humidity during night-day cycles. This is a good source of inspiration to build device able to minimize power consumption and capable of exploiting *in-situ* resources.
In order to validate the potential arising for future technical applications in unmanned planetary exploration, a first step toward understanding the self-burial performance in *Erodium cicutarium* was undertaken. Self-burial experiments were performed on different types of soils, which were selected for their mechanical properties to be similar to extraterrestrial soils (Moon and Mars regolith and a very coarse soil to represent an asteroid). Time-lapse images of the seeds were recorded for 24 day/night humidity cycles and additionally landing phases were analyzed using high-speed video equipment.

Objectives

1. Evaluation of the seed self-burial performances of *Erodium cicutarium* seeds in different types of soil and identification of the contribution of its accessory structures to penetration.
2. Analysis of the dynamic of landing phase to understand its influence in the mechanism of penetration.

6.2 Background

Basic research has driven the evolution of modern industry with the discovery of new technologies. Space exploration requests a very innovative and advanced technology to perform all operation with more accuracy and at lower cost. For this reason this field helps in turn new research activities and supports the creation of new technologies to be used on Earth. In the future, spacecraft and probes will go farther into space for longer duration of time, and a larger autonomy is an attractive feature while searching for deeper mysteries of the universe or evidences of existence of extraterrestrial life forms (Lee 2000). It is important to characterize and describe with accuracy the geophysical properties of extraterrestrial bodies as planets or asteroids, and is a priority research area for future space mission. The possibility to widely distribute several probes, at different locations on the surface that can reach a deep penetration for an extensive ground tests and measurements is one of the demanding challenges for success (Sheeres 2003, Blitz 2006, Bombardelli 2007). Space missions possess many constrictions relate to mass and volume due to the launch phase; thus meanwhile it is therefore necessary to use material with an efficient mass, at the same time an efficiency packaging of the whole construction is requested. These requirements, essentially opposites (lightweight materials *vs* materials and packaging extremely resistant), suit plants obligation to carefully define the relationship between fruit mass, dispersal structure size and morphology through a sophisticated cost-benefit analyses because of the limitation of available resources (Eadie 2011, Pandolfi and Izzo *not published*). The *Erodium cicutarium* self-burial mechanism shows several features that are contained in a very simple structure which is very attractive for future robotic space exploration: it is able to launch itself from a stationary plant, subsequently to move and roll on the surface of the soil, to brush the soil grains with its hairs, and at the end it can both penetrate and/or extract itself from the soil with coiling and uncoiling movements. However, space exploration places unique requirements regards engineering which actually increase the desirability of capturing the characteristics of biological organisms in general and of plant seeds in particular.

6.2.1 Self-burial behavior of *Erodium cicutarium*

A wonderful example of an efficient and brilliant strategy used by plants to disperse their seeds is use the unique self-burial mechanism of *Erodium cicutarium* that makes the seeds able to propel into the ground. A similar mechanism based on a humidity-driven movement is seen in the wild the wild wheat plant (*Triticum turgidum ssp. diccocoides*) and its awns; nevertheless the friction forces and the structures used by the two species are different, confirming the well-known statement that the natural selection can find different solutions to solve similar problems. The worldwide and abundant presence of *Erodium* clearly underlines the importance of its simple but effective mechanism to thrust soil, by exploiting natural source of energy such as the humidity-cycle occurring during day-night changes (Mensing 1998). These features can well suit and support the main aim of the study: a novel approach in digging systems for under-ground planetary exploration, focused on the idea of minimal power.

Erodium cicutarium is a winter herbaceous plant, annual or biennial, belonging to the family of *Geraniaceae* and is considered as a flowering weed. It is characterized by a high capacity for adapting itself to different conditions, and can therefore be found worldwide (Europe, Asia, Africa, and North and South America). The optimum growth temperature for *Erodium* plant occurs at 15-25 °C but it can be found in arid and semi-arid areas and it grows successfully in calcareous, sandy, loamy and in mechanically decomposed granite soils. The seeds germinate in soil with temperatures between 21°C during the day to 4°C at night (Mensing 1998). *Erodium* plants produce several stems, usually, red in color, haired and branched. They grow along the ground and can ascend with stalks 10-50 cm long. The structure of the inflorescence is composed of groups of flowers, generally from two to eight, gathered in clusters. Every individual flower consists of five, pink to purple, petals and five sepals. The sepals of the flowers are somewhat pointed and hairy. The fruits of *Erodium* have the form of a bird's beak and sizes that vary from two to five centimeters. The developing fruit splits into five segments, each with an elongated, spirally twisting style with a seed attached at the base. The awn twists hygroscopically and shoots the seed into the soil.

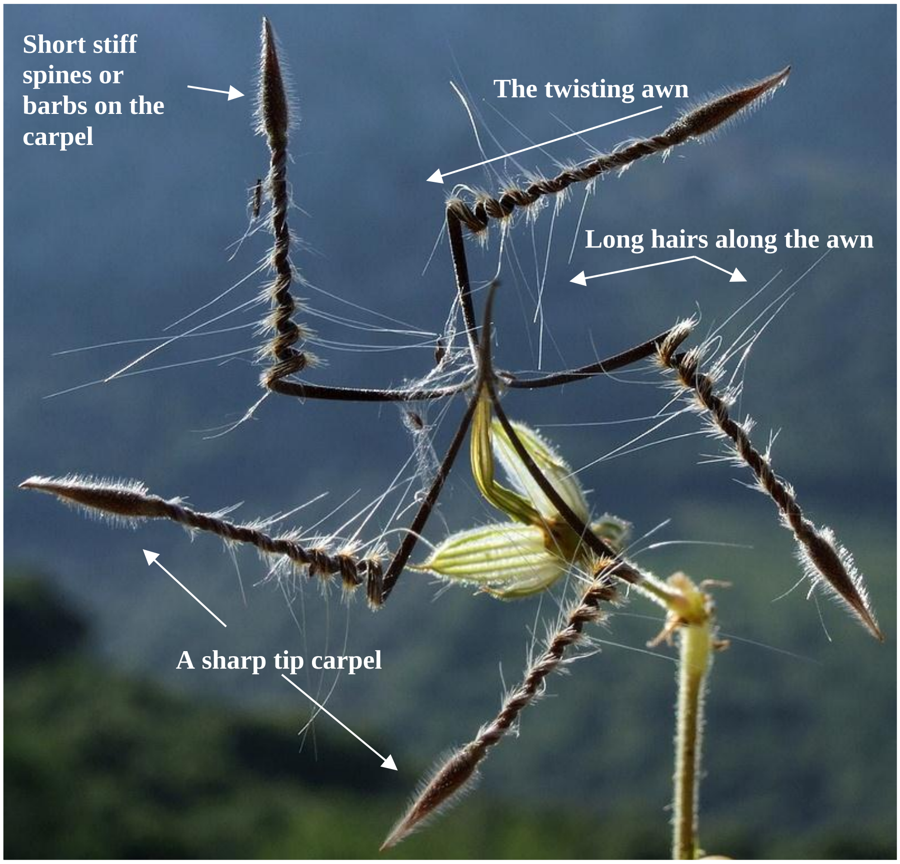


Fig.26 The five seeds attached at the base of the flower and their structure (http://es.treknature.com/gallery/Europe/Spain/photo53405.htm).

Erodium cicutarium seeds (Fig. 26) possess a typical wedge-shaped hairy carpel with barbs similar to stiff spines. The awn, with white long hairs along the longitudinal axes, ends with a hygroscopically active tail sensitive to humidity and that is able to twist. A single mother plant can shoot from the fruit their seeds up to 0,5 m through an explosive dispersal system. Furthermore, the seeds are endowed of additional movements derived from their hygroscopically-active tail and can also be dispersed by animals, birds and insects. The tail responds to the natural wet-dry cycle occurring during day and night by reversibly uncoiling when wet and recoiling when dry (Fig. 27); this surprising structure makes the seeds able to move by decreasing and increasing tension and drills itself into the soil. The self-planting system needs soil surface irregularities for self-burial: the seeds roll on the soil and establish

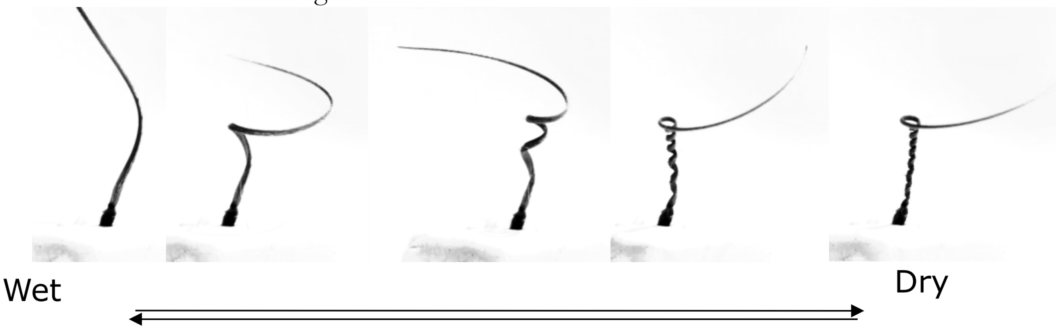


Fig.27 The seed configuration results in a coiling and uncoiling motor: the awn is linear when wet and it turn into helical when dry.

themselves in fissures and crevices of the soil whilst the barbs on the carpels act as anchorage allowing the seed 'to drill itself' into the ground (Stamp 1984).

6.2.2 Extraterrestrial soils

Several planets and asteroids or satellites of our solar system possess a cover of dispersed and granular material which is called regolith (from Greek rhegos, "blanket", and lithos, "rock"). Despite it is incorrect to refer to extraterrestrial terrain as "soil" because it is a pedo-genetic term used only for Earth, which also implies the alterations produced by biological activity, it is very common to mention Martian or Lunar soil and this term is widely used. The study of extraterrestrial regolith and dust, which cover the surface of planets and asteroids, has been deeply investigated in the past. The requisite to understand soil composition, in order to permit an optimal functionality of landers and probes which usually need to act on the surface (i.e. drilling, sampling or landing), is tightly linked with the limited possibility of directly using extraterrestrial soil sampled during past missions for its very limited availability. The impossibility to use test materials from planets therefore has forced people to use different components to mime their key features. In this case soil simulants can be used, i.e. any material, both synthetic and/or natural whose purpose is to imitate the chemical/physical properties of the extraterrestrial soil (i.e. dusts, rocks, regoliths).

Mars

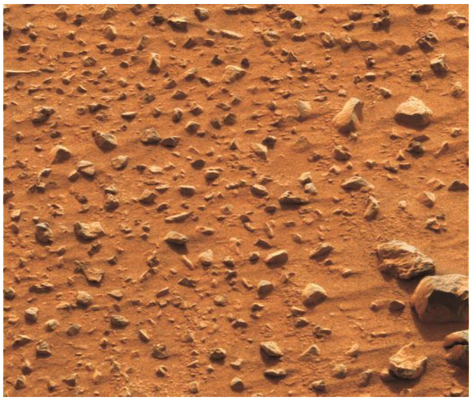


Fig. 28 A Pancam color image of soil in Gusev crater of Mars, showing regularly spaced clasts and coarse sand, all coated with dust (Squyres et al. 2004)

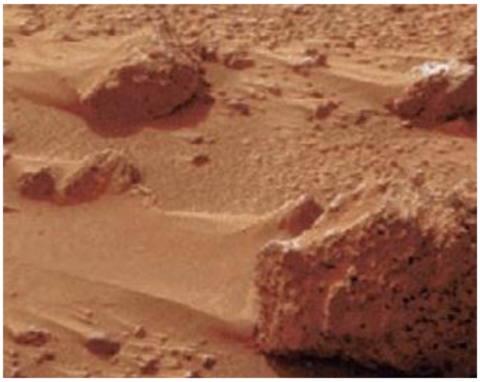


Fig. 29 Images of Mars soil powder cemented near the Pathfinder lander site (Janice et al. 2002).

Mars exploration can be expected to be the best candidate application for such probe, thanks to its abundant desert soils which are quite similar to those where *Erodium cicutarium* seeds are naturally found. Moreover, if compared to the Moon or asteroids, this planet has less extreme constraints (i.e. the temperature, gravity level or atmospheric pressure). Martian regoliths are composed of a complex and heterogeneous mixture. The entire surface is characterized by volcanic rocks of basaltic composition partly pulverized by impacts and by sedimentary rocks of possible marine origin formed by silicate and rich of sulphate (Knauth et al. 2005). Similar gravels to these dusted stones can be found at the confines of the sunny districts in Arabia and Tharsis (Murchie et al., 2000). In this rocky substrate a layer of fine and grainy powder that accumulates or forms a crust covers most part of the surface (Binder et al. 1977, Janice et al. 2002). A great difference in temperature between its two hemispheres induces strong winds, up to 400 km/hour, for this reason soil is transported from one place to another as powders. Thus, the composition of most of solid materials on the rock and in space between rocks is formed by all particles transported in the atmosphere and released on the ground as showed in fig. 29. The surface has been deeply analyzed in several mission from different probe (i.e. Viking Lander and Mars Pathfinder) and we know the surface possess 5% of rock of about 1cm. Numerous areas are covered by fragments of a dimension that reach 10 cm, up to 50% compared with the total rock-covered area and powder (Fig. 28 and Fig. 29).

Moon

Moon soil is composed mainly of regoliths (Fig. 30). Space missions were mainly focused on craters which can guarantee a more favorable landing place for safety and exploration, and thus the majority of the samples we currently have come from lunar mare sites. The surface of the planet is covered of a sandy heterogeneous layer that can be from 4 to 15 m-thick. Going below this layer, there is a region formed of "megaregolith" composed of plaques or fractured bedrock created by larger impacts. On the moon the presence of craters has been known for a long time and the presence of many interesting places where to perform soil exploration is well known (e.g. the Shackleton crater which hypothetically is one of the most promising site for the first moon base. Unfortunately, on the Moon the atmospheric pressure is negligible and this can cause constraints, in particular on anchorage phase of a drilling probe. Temperature is hostile too and rarely exceeds about −173 °C. Lunar soil can be used as synonym of Lunar regoliths for their abundance and usually refers to the finer fraction of the soil. This fraction is composed of grains of about 1 cm in diameter or less. Finer grains are indicated as "Lunar dust" and can reach the diameter of less than 100 micrometers that give them an efficient consistency. This fine grain powder has been mainly molded by continuous impacts by meteoritic bombardment.

Fig. 30 Example of lunar regolith composed of fractured rock material ok parts with a size <1cm to 2-4 mm size. (photo by Randy Korotev, Mancuso et al. 2008)

Asteroids

On asteroids the atmosphere is absent and there are great temperature differences, which vary with the distance from the Sun. However, the surface temperature can vary significantly depending on the side exposed to solar radiation and shadow during the rotation. Finally, asteroids also have regoliths mainly derived from meteoroid impacts. The soil properties are generally unknown, and can vary greatly among different asteroids. The surface is covered by dust and generally its shape is very irregular. Recent studies suggest that the composition of many asteroids can be different: some can have a composition very similar to their analogue meteorites, with high bulk density and limited porosity, meanwhile other asteroids can present a very significant porosity produced by a fractured surface (Britt et al. 2003). This mainly depends on the violent collisional history of each asteroid that strongly influences its surface composition, which can in some instances become a “rubble pile”. Chapman (1978) first describe the “rubble pile” as a weak aggregate of inhomogeneous particles held together by gravitational bounds rather than material strength that is formed as a result of high speed collisions; these collisions cause extensive fracturing and destruction, resulting into a “rubble”.

6.4 Evaluation of the self-burial performances of *Erodium Cicutarium* seed in different types of soil.

Different soil compositions have been used to mime extraterrestrial soils only in terms of “physical behavior” rather than mineral composition because the aim was to understand the functionality of the self-burial mechanism of the seeds. For this reason in this project we have mainly considered density, grain size and shape of different materials. Subsequent experiments have been done to mime different extraterrestrial soils or to explore similar substrate compositions, in order to understand the possible use of this kind of penetration. Three soils were tested, as they are the best candidates for such inspired probe, and have been called: Mars, Moon and Asteroids. Another composition, named “Test soil”, was used to calibrate and test the set-up and the behavior of the seeds and understand the influence of the fine particles in the penetration mechanism.

6.4.1 Starting materials for the soil mix

All starting material used for this project have been chosen not only for their physical characteristics but also to reflect other advantages on the basis of the analysis techniques used. All estimation of penetration was performed by image analysis; for this reason we had to consider the problem of the contrast between the seed and the background color of the soil. Therefore mainly white materials were used to highlight seed movement; in particular talcum was always employed instead of silt and clay. Talcum is a non-toxic material with very small particles which is able to cover all external part of the other rocks, resulting in a clear background. Second, to better connect the aim of this work with potential applications in the future and to understand how to scale-up this device, very light materials were used to focus more on the dynamic property of the penetration as the seed weight is very limited. This choice was also done because the gravity pull on other planets is lower than on Earth, and this can modulate particles adhesion and forces. As consequence the bulk density of the soil simulants used for this work usually resulted lower than the ones commonly calculated; this guide it is therefore not going to be a perfect simulation of extraterrestrial soil but a simplified and flexible version of it on the base of the experimental requirements. In addition a crevice or at least a hole is necessary for the seed to bury itself; thus some materials were used to this purpose and can be intended as big rock on the surface.
To assemble all soil mixture employed in this study, different composition of expanded clay, perlite, pumice, sand and talcum were used, as shown in the table below (Tab. 1), where we have listed each material one by one and described their physical properties.

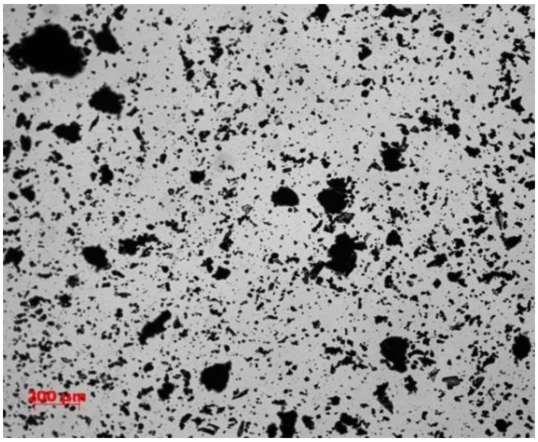
Tab.1Particle size and shape

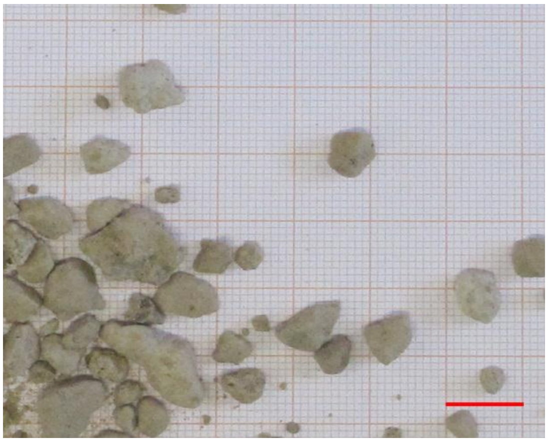
Expanded clay

All particles of expanded clay used to mimic the soil, as representatives of bulky boulders had a volume in the range of 0.8cm to 1.8cm. The majority of the granules had a rounded shape. The surface presented a roughness with sporadic crevices in the rock.

Pumice

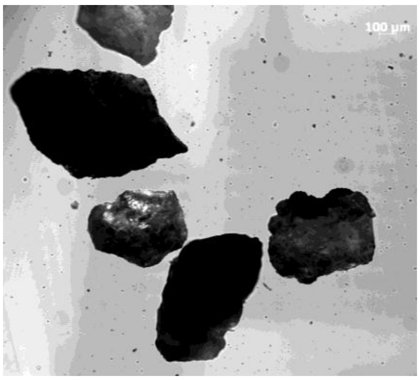
All particles of pumice presented irregular shape with a good variability in size from 0.1 cm to 1.2 cm. A good amount of fine particles was presented in the residual with a dimension sometimes lower than 5 µm as shown below.





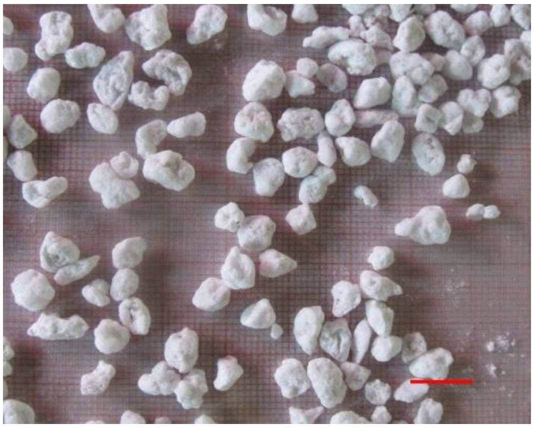
Sand

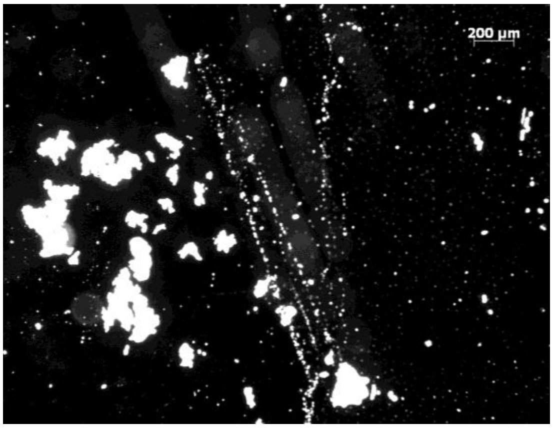
Sand used in this work presented regular round particles of about 0.8 mm of coarse grains and very limited residual.



Perlite

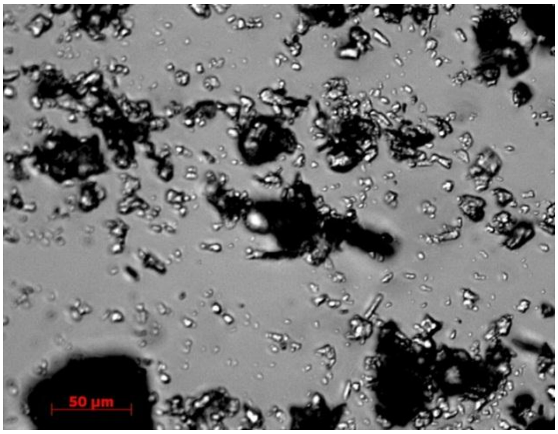
All particles of perlite presented a shape similar to the pumice with dimension from 0.2 cm to 0.8 cm. The residual was rich of very small powder of few μm.





Talcum

All particles of talcum were less than 50 μm.



6.4.2 Soil mix

Four soil mixes have been used to test the dynamic of penetration of numerous *Erodium Cicutarium* seeds (Fig. 31). Here below is a short description of each soil and the reason that led us to work with certain materials.

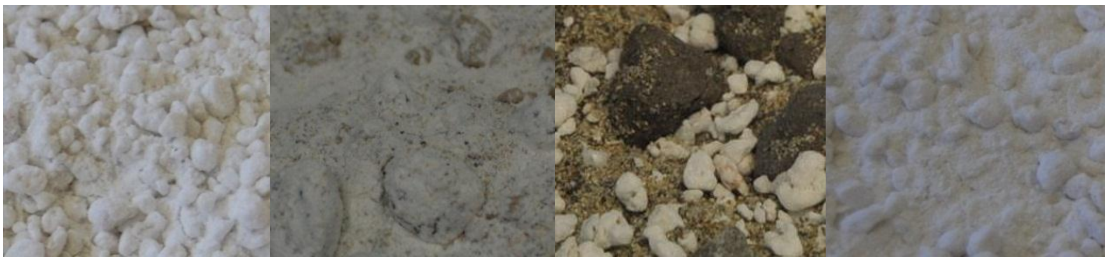


Figure 31 Overview of all the soils used to test the self-burial performance of *Erodium Cicutarium* seeds. From the left to the right: Moon, Mars, Asteroid and Test soil.

Moon

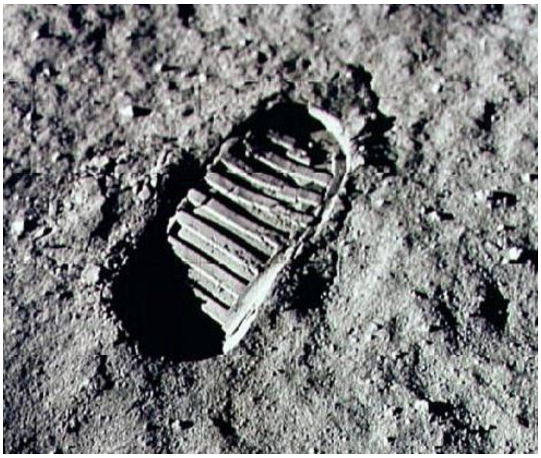


Figure 32 Footprint of Neil A. Armstrong on July 20, 1969. This image shows the powdery nature of lunar surface (NASA).

For the composition of the moon we try to understand the influence of a dusty soil without modify too much the test soil, in order to understand if the small particles fraction can affect seed performance. As shown in Fig. 32 "Lunar dust" can reach the diameter of less than 100 micrometers, which gives it an efficient consistency. We therefore used:

- 2/3 part as the portion of the regolith: size <10 mm (medium size grain 2-4 mm).
- 1/3 part as the portion of lunar dust: a mixture of silt and clay.

Composition: 2/3 part perlite, 1/3 talcum.

Mars

As show in fig. 33 several simulants have been proposed to recreate the desert soil of Mars. These simulants are artificial desert soil with at least a 30% of silt and 20% of fine sand, whilst the rest is medium or larger size sand and coarse. We used:

- 1 part of sand, 2 part of silt, 1 part of clay (to mime desert soil),
- 1 part rock from 1 to 5 cm (to mime rocks on surface).

Composition: 2 part talcum, 1part sand, 1 part pumice (desert soil) and 1part expanded clay (stones).

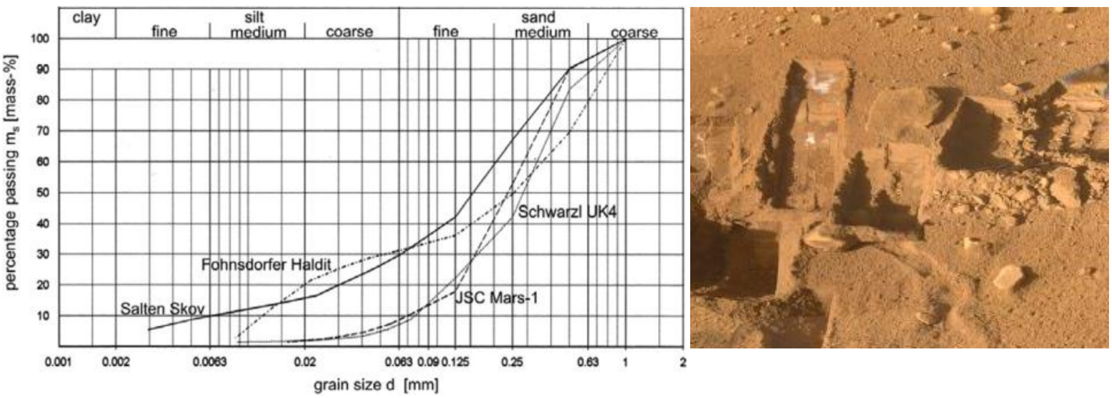


Figure 33 LEFT: examples of particles size composition of four common analogue of Martian soil (Seiferlin et al. 2008).
RIGHT: example of mars soil profile (Certini 2010, picture of NASA/JPLCaltech/UniversityofArizona/TexasA&MUniversity).

Asteroids

To mime primitive asteroids, which are fundamentally weak and more probable to be loosely agglomerated, with a big fractured part on surface as rubble piles. This choice has been forced to try to explore the behavior of a seed in a soil with coarse gravel with features opposite to others simulant as show in Fig. 34. We used:

- 1 part sand, 1 part grain size of about 5-10 mm, 1 part big grain size of about 10-30 mm

Composition: 1 part perlite, 1 part sand, 1 part expanded clay.

Test Soil

A test soil was used to perform few preliminary tests on the set-up, i.e. time of cycle of wet and dry condition. A test soil similar to our simulant called Moon was employed to understand if the fraction of small particles could affect the dynamic of penetration. We used:

Composition: 1 part perlite, 1 part talcum.

This simulant was assembled also to study soils with different features and to cover different position in the texture triangle; this enabled us to monitor the seed behavior in different and contrasting soil composition. The only exception was that the Test soil was deliberately mixed with characteristics similar to those of the Moon simulant. The composition of the gravel and the position of each soil are shown below in Fig. 34.

6.4.3 Physical and mechanical properties of the soils

Granulometric Composition

The granulometric composition of each soil used in this study has been calculated and referred to soil texture as shown in Fig. 34. Here below the composition of each soil divided by gravel and fine particles and the composition in clay, silt and sand of each mixture.

Here below the proportion of different materials that we use to create the soil mix:

- Moon: 66% gravel, 33% fine particles (50%clay, 50%silt)
- Mars: 40% gravel, 60% fine particles (33%clay,33%silt,33%sand)
- Asteroid: 66% gravel, 33% fine particles (100% sand)
- Test soil: 50% gravel, 50% fine particles (50%clay, 50%silt)

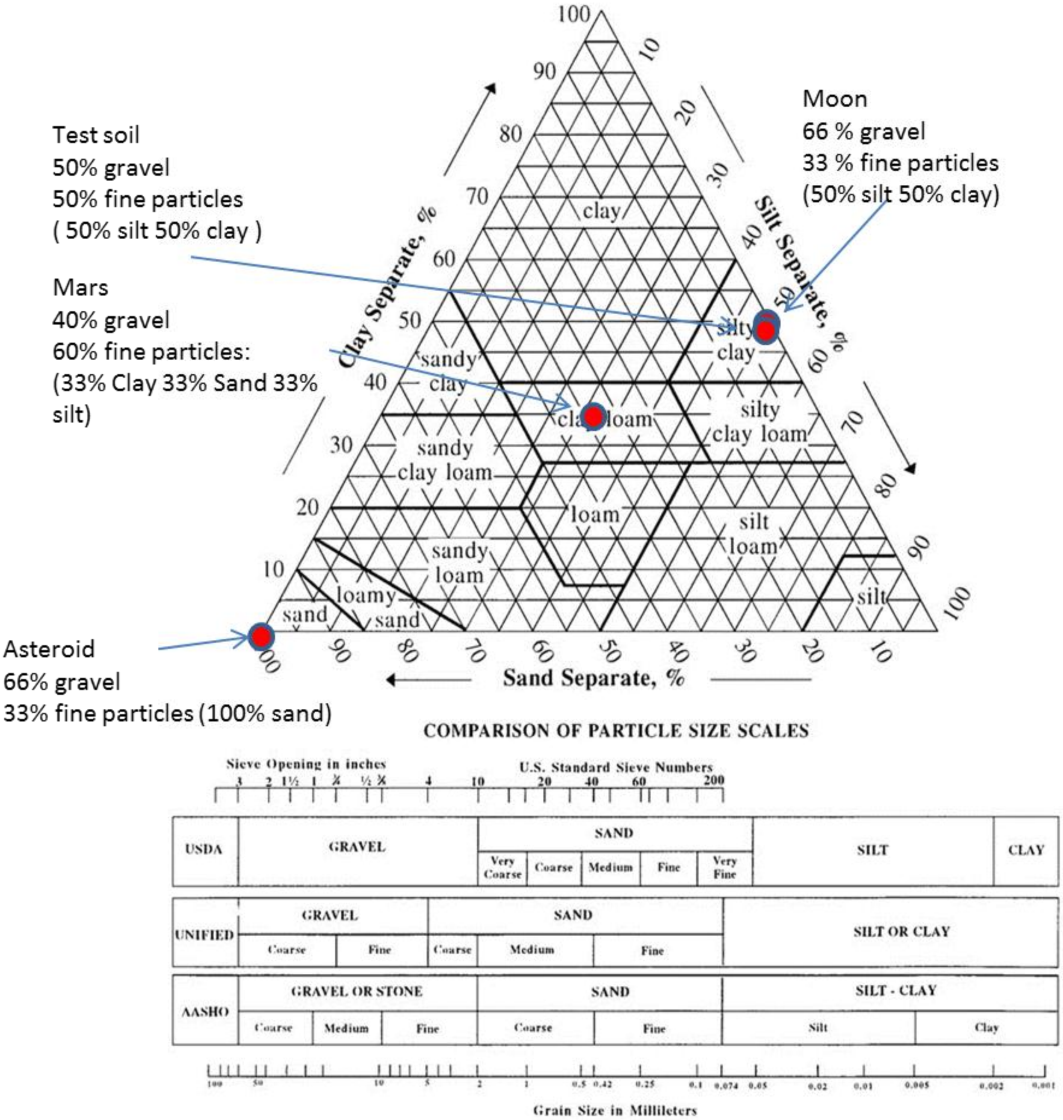


Fig. 34 All simulants compositions are resumed in this scheme and the position of each simulant is showed respect the soil texture triangle.

Bulk density

Bulk density is a property of a soil mixture and/or a material which possess a mass as powder or granules. For this reason it depends on soil texture and on its packing organization. It is an indicator of soil compaction, expressed as the ratio of the dry weight of a material and its volume, comprehensive of the empty space of pores among soil particles. In general a loose and porous soil possesses a lower bulk density. On the other hand, high bulk density usually indicates low soil porosity and is linked with high soil compaction. Soils with a big amount of sand have relatively high bulk density because the inter-particulate empty volume is less compared to silt or clay. Soils rich in silt and clay, with a smaller particles size composition, have higher void volume and a lower value of bulk density.

The bulk density is expressed as:

Bulk density= mass (weight) of soil/total volume occupied

It is commonly presented as g ml^{-1} but also as kg m^{-3} or g cm^{-3}.

The bulking property of a powder is not always an intrinsic property of a material but it depends on several factors such as preparation, treatment and storage of the sample. All materials can pack in an altered way also with very small differences in the perturbation of the system. For this reason, the bulk density is typically described in two methods:

Freely settled: is obtained simply adding a quantity of soil in a specific container.

Tapped: is referred to a powder inserted in a container "tapped" by a mechanical compaction process.

In this study all soils have been measured in different containers (100mL or 200mL of total volume) and soft tapped, by repetitively beating each vessel for 30 seconds on a bench to generate vibrational forces. Measurements have been done at least two or three times for each soil.

Porosity and particles density

The porosity of a soil or sediment is defined as the fraction of the volume that is not occupied by solid material. In particular it defines empty spaces among all particles of a mixture.

Porosity= volume of void space/ volume of soil sample, including both voids and solids.

This property is expressed either as a fraction with values between 0 and 1 or as a percentage. Porosity range is not linked with pore sizes and distribution or connections. For this reason also soils with similar value of porosity may possess different physical characteristics.
In sedimentary soils the porosity depends mainly on grain dimensions, shape and the degree of arrangement and cementation. In stones, the porosity depends upon the amount, configuration and positioning of fissures and crevices. Mixtures of particles of similar size usually have lower porosity than distributed sized organized materials because all void spaces between larger grains are occupied by small elements.

In this study total pore volume of each soil was calculated. All materials were weighed once inserted into a container with a known volume after being saturated with water. They were then maintained for three days at 50 ° C to dry and reweighted.
Particles density can be defined as the weight of all particles of the soils on the volume occupied by the soil without considered any empty space as complementary of the porosity of the soil:

Particles density: Weight/Volume occupied by soil (total volume-volume of void space)

Angle of repose

The angle of repose or "critical angle" for soil is usually defined empirically by pouring a granular material on a surface and measuring the angle of the resultant conical pile. It is the sharpest angle of decay or the inclination of the slope between the surface of the heap and the horizontal level. There is a minimum angle (or a maximum slope) where grains maintain a pile due to the forces of gravity and the effect of friction among all particles. This angle is always more than 0° and less of 90° and is usually in the range of 30-45°. This property is linked to several soil particles density, surface area and shapes and naturally it depends to the coefficient of friction of the material. Irregular granules or elements with a strong grip power have a tendency to produce piles with steeper sides and a higher angle of repose. Recent studies also have been demonstrated that it is influenced by gravity conditions (Kleinhans et al. 2012).
There are several methods used to measure this property and in this study we used the "Fixed Funnel Method": it consists in the insertion of all material through a funnel to create a conic heap as show in Fig. 35. Gradually when the pile of material grows, the outlet funnel is moved

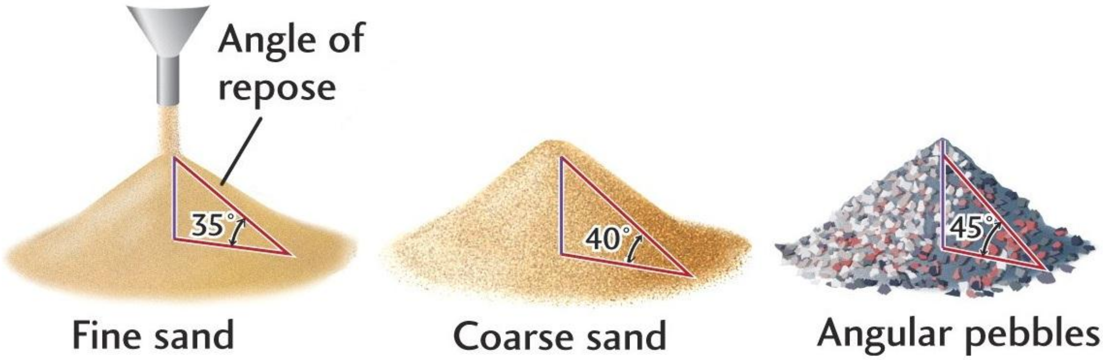


Fig.35 Images of the angle of response of three different soils (http://hays.outcrop.org).

upwards very slowly without interfering with the formation of the heap and in the same time reducing the effect of falling particles. A picture from the side of the cone has been taken with the camera objective disposed not too close to the pile to avoid perspective mistakes.

6.5 Experimental setup

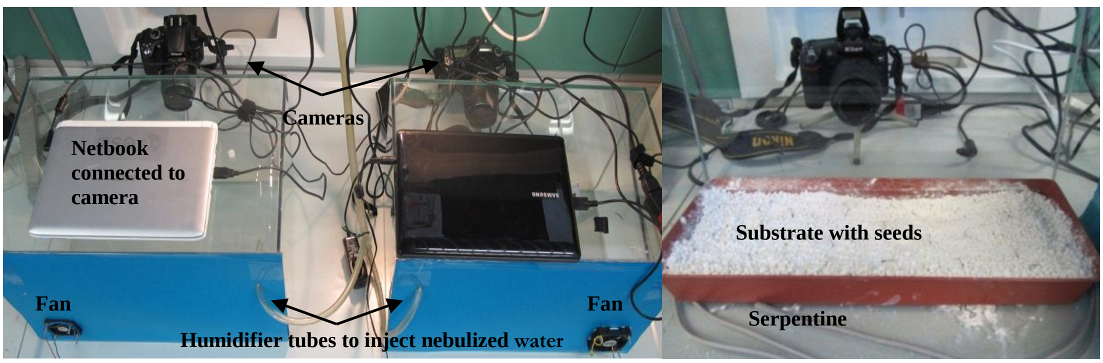


Fig. 36 Pictures of the experimental set-up

For the analysis of penetration performance two chambers were assembled. In each chamber a container with several seeds and the soil were placed (Fig. 36). Using two cameras connected to a notebook a sequence of image was collected as shown in Fig. 37. All stored images were used to create a time-lapse video of each experiment and to analyze accurately the movements. To monitor the penetration activity of all seeds several wet-dry cycles were used to mimic day and night humidity changes. All seed were placed in the container with soil and the image capturing started whilst dry and wet cycles alternate for several hours. Images were collected with a frequency of one picture every ten seconds.

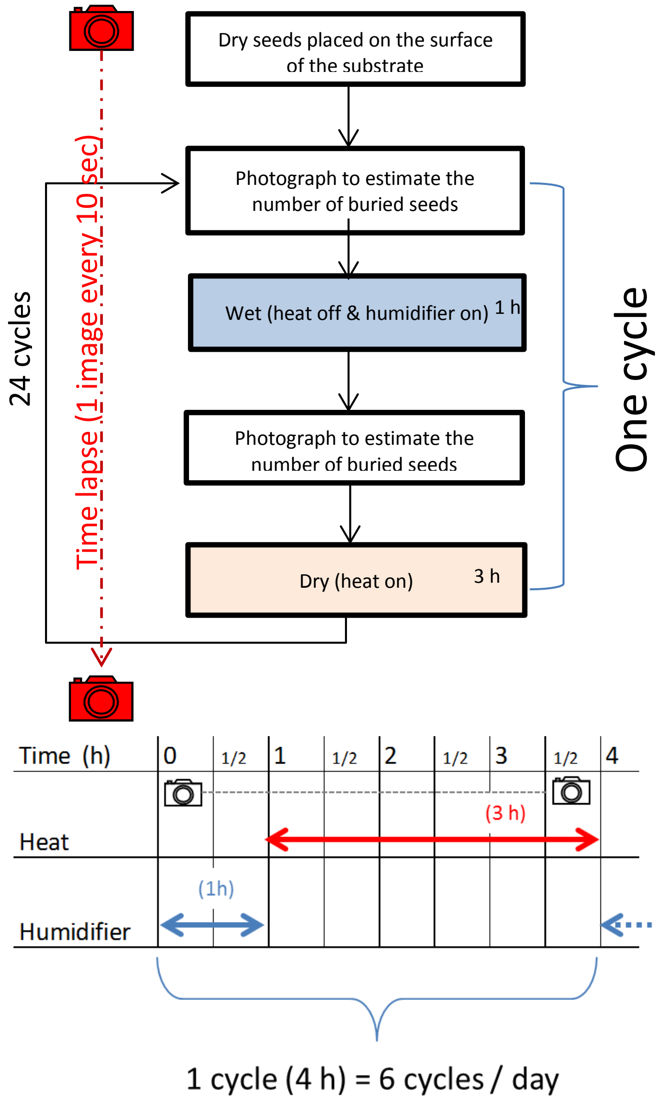


Fig. 37 Scheme of the set-up where duration and a brief description of each operation performed during all stages is showed.

WET CYCLE

During the wet cycle a humidifier was activated and a humidity of about 100% was reached in a few minutes. The humid cycle was maintained for 1 hour to allow, a period sufficient for all seed to completely uncoil.

DRY CYCLE

During the dry cycle the serpentine and the fan were activated in order to dry quickly the soil and air. Usually about 3h of dry cycle were required to completely dry the soil and permit the complete coiling of the seed, except for very coarse mixture (i.e. Asteroid mix).

With this set-up we registered only the first and the last part of the wet-cycle because the dense vapor associated with 100% humidity prevented us from obtaining clear images. About 20-25 seeds have been used for each experiment for at least four days; as we collected a high number of images, the analysis of the data allowed us to investigate in details all movements made by seeds.

6.6 Results

6.6.1 Characterization of the soils

Bulk density, Porosity and Particles density.

The bulk density, porosity and particles density have been calculated for each soil for three samples. In the Tab. 2 all average value are resumed.

Pure soil
Sand had the higher bulk density value, showing a high level of compaction and a high particle density followed by talcum and pumice. On the other hand perlite and expanded clay are lighter materials with a lower value of bulk density and particles density. Porosity was found to be higher than 60% in talcum and perlite, 58% for pumice and about 45% for sand and expanded clay.

Soil	Bulk value	Porosity	Particles density
Mar	0,98	0,44	1,78
Moon	0,38	0,53	0,76
Test	0,48	0,50	0,97
Asteroid	0,81	0,37	1,29
Pumice	0,57	0,58	1,33
Talcum	0,71	0,62	1,87
Sand	1,34	0,46	2,51
Perlite	0,11	0,65	0,31
Expanded clay	0,37	0,46	0,70

Tab.2 Bulk value, Porosity and Particles density calculated for each substrate.

Bulk density: Sand > Talcum > Pumice > Expanded clay > Perlite
Porosity: Perlite > Talcum > Pumice > Sand = Expanded clay
Particle density: Talcum > Sand > Pumice > Expanded clay > Perlite

Simulant
Mars and Asteroid presented the higher level of bulk density, for this reason the soil was very compact and the level of porosity was lower than in the other soil. This feature was associated to the presence of sand, which intercalates in the empty space to limit porosity of each soil. For this reason Asteroid did not have the level of porosity expected, as it was assembled only with particles of big size. Moon and Test soil were similar as planned with a difference in the bulk value due to the greater presence of talcum.

Porosity: Moon > Test > Mars > Asteroid
Bulk density: Mars > Asteroid > Test > Moon
Particle density: Mars > Asteroid > Test > Moon
Percent of Gravel: Moon = Asteroid > Test > Mars

Angle of repose

Critical angle was measured for all materials used in this study once piled (Fig. 39). In Tab. 3 all values calculated on three different replicates are shown.

Pure soil
Pure material showed a variable angle of repose (Fig. 38). The highest value was found in the talcum which permitted a maximum angle of 60° due to the grip among the small particles of the material. Perlite had an angle of approximately 38° followed by sand and pumice respectively at 35° and 34°. Expanded clay, due to the round and big size of its particles, showed a very low value of angle of repose.

Angle of repose: Talcum > Perlite > Sand > Pumice >> Expanded clay

Soil	Angle of repose	SD
Asteroid	33.02	0.52
Mars	38.95	0.67
Moon	40.99	0.93
Test	40.69	0.82
Perlite	38.78	2.05
Pumice	33.74	1.06
Sand	35.51	1.43
Talcum	60.81	1.34
Expanded clay	26.11	0.89

Tab. 3 Angle of repose of all materials used. (SD derived from three repetitions)

Simulant
The angle of repose of the simulants had similar values to the pure material and a lower standard deviation due to the ability of soil to form more regular pile. Asteroid presented the lower value of about 33° for the contribution of the expanded clay and the sand whilst Moon and Test had a similar angle of about 40° and a value in a range of 3 degrees of difference with Mars whom was a bit lower at about 39°.
Angle of repose: Moon > Test > Mars > Asteroid

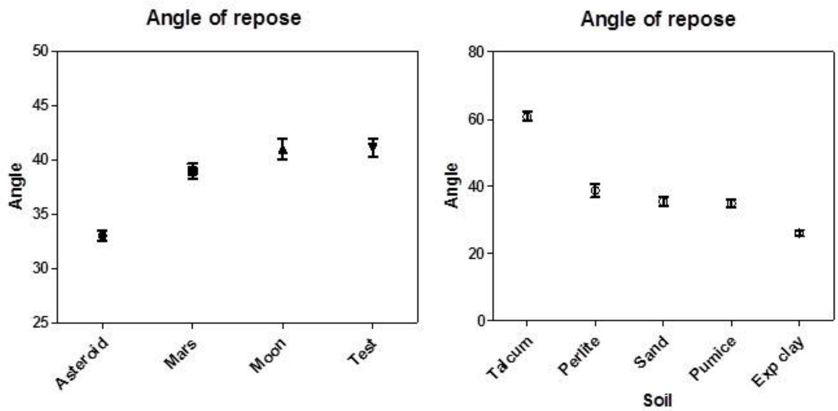


Fig. 38 Angle of repose showed in graph for each material used.

Fig. 39 Pictures of pile derived from the four soils. From left, respectively, Moon, Mars, Asteroid and Test soil.

6.6.2 Establishment of the seeds into the soil

The establishment of the seed in the soil has been calculated after 24 cycles for about 40 seeds for each soil. Two conditions were considered to discriminate the successful strategy of burial. The seed was considered as established when the carpel was completely inside the ground that is the prerogative for the seed to have reached its objective. When a seed continued to penetrate the soil until only the last part of tail could be observed, it was considered as completely established as shown in Fig. 40. The percentage of all established seeds for all different soils is shown in Tab. 4. The performance of all four groups of seeds was very similar, between 82 and 87%. On the other hand the ability to penetrate deeper into the ground presented a good variability among the four soils. More than half of the seeds were buried in asteroid, about 1/3 of the seed were completely established in Mars whilst in Moon and Test soils only 7-11% of the seed showed a similar trend. The incremental number of seeds buried increase drastically during the initial cycles where more than 50% of the seeds established.

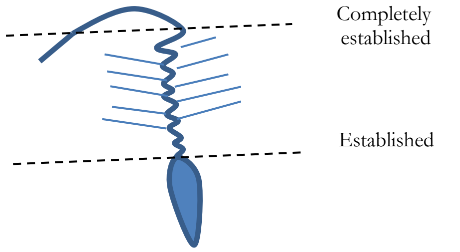


Fig. 40 Seed establishment conditions respect the ground

Established	Tot	Established	Established completely	% IN	% fully in
Asteroid	46	38	31	82,6	67,4
Test Soil	41	34	3	82,9	7,3
Moon	46	40	5	86,9	10,8
Mars	39	32	14	82,05	35,9

Tab. 4. Percent of seeds buried during all experiment

Subsequently the number of buried seeds has been counted after every cycle, in order to identify any difference in the dynamic of penetration. This analysis is reported in Fig. 41.

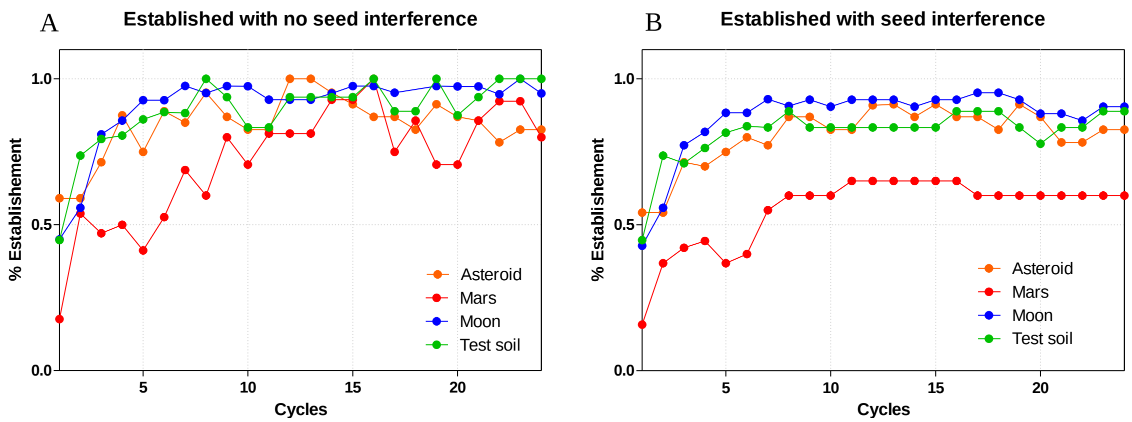


Fig 41 A: number of buried seeds has been counted after every cycle without any interference consideration.
B: number of buried seeds where interference where considered.
C: number of fully established seeds during 20 cycles.

C

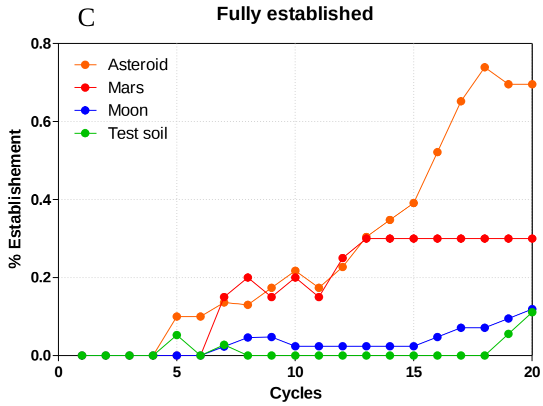


Fig. 41A does not consider the interference among seed that caused the exit of neighbor's seeds during coiling and uncoiling procedure; Fig. 41B shows the full set of data. All soils except Mars showed a similar trend of establishment, whereas Mars had the lower initial increase. The high rate of interaction registered in Mars can be interpreted as a higher mobility of the seeds onto the soil, and a lower amount of crevices or obstacles on which the seeds could start the drilling. If some seeds were too close, they started to interfere one with the other, preventing the penetration. A certain distance among all seeds was set before starting each experiment; anyway the distance covered by a seed could be even more than 10 cm so we could not totally prevent this to happen. All interferences had a negative effect on the penetration of the seeds, causing the extraction of one or both seeds involved in the contact.
In Fig. 41C the fully establishment of the seeds has been analyzed (also cycle by cycle), and it is interesting to notice the high rate of establishment registered for the soil asteroid. After only 5 cycles some seeds were completely buried in the soil. Then, in the Test soil and Moon, the number of fully established seeds remained very low, probably due to the high amount of fine particles that tend to fill all the empty spaces of the soil. Asteroid and Mars increased the number of completely established seeds from the 5th to the 15th cycle. However, from the 15th cycle, whilst in Mars there was no increment, a large amount of seeds started to penetrate in the asteroid soil.

6.6.3 Cycle length and start condition (wet-dry)

Start Condition: Dry *vs* Wet

Two experiments were run for 10 cycles to understand the difference between the two starting positions: wet and dry. As showed in the Fig. 42 on the left, both penetrated with the same trend already the second cycle. However if we start the cycle from the dry (coiled) state, there is a delay in the establishment in the first cycle.

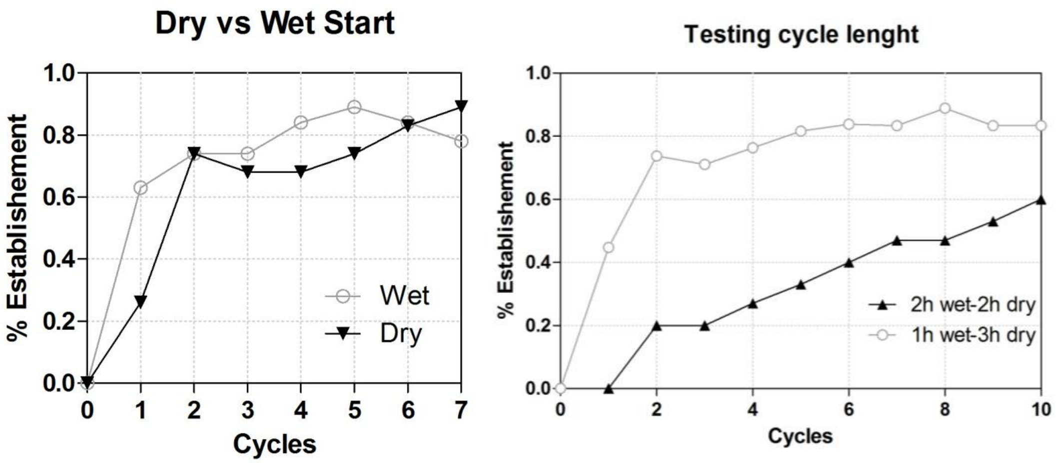


Fig. 42 Seeds establishment observed during 10 cycles where (on left) seeds present different start positions (Wet/Dry) and (on right) the duration of humidity cycle is different.

Cycle Length

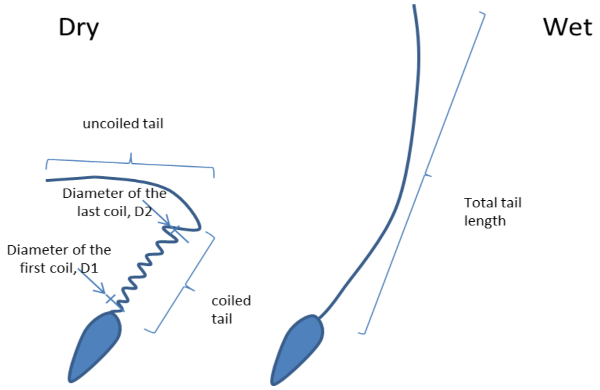
Several tests were performed to understand the time necessary for each group of seeds to completely uncoil and coil. If the humidity level was not sufficient the seeds were not able to perform at least one turn of the tail, and all seeds did not move from their starting point and did not penetrate the soil. A short length of the wet cycle did not permit the seed to totally uncoil its tail. In Fig. 42 on the right two different length cycles are shown in the test soils and were maintained for a total duration of 10 cycles. The difference between the two performances is to be interpreted as an inappropriate length of the Wet/Dry cycles, i.e. 2h Wet - 2h Dry in black and 1h Wet - 3h Dry in light grey. This experiment showed that if the dry cycle was too short, the seed did not dry completely, and then there was a decrease in the number of seeds that could settle into the ground. This clearly highlights the importance of both cycles following each other properly.

6.7 Behavior of the seeds and accessories structures contribution

The time-lapse sequence of images every 10 seconds permitted to better understand the movements and the strategies performed by the seed to bury itself.

6.7.1 Morphological traits

In Tab. 5 all the general characteristics of *Erodium cicutarium* seeds are resumed. The seeds had



Measurements	Average (14 seeds)	SEM
Wet		
Tail + seed	52.58mm	0.98
Seed	7.34mm	0.15
Tail	45.23mm	0.90
Dry		
N coils	6-9	
uncoiled tail	12.55mm	0.29
coiled tail	18.40mm	0.46
Diameter D1	0.300mm	
Diameter D2	1.5-2.0mm	

Tab.5 The table presents a schematic representation of the seed on left and its main features on right.

an average total weight of about 1.4 ± 12 mg and about 40-50% depend only on the carpel with a certain fluctuation among the ecotypes. This may indicate its importance for the anchorage phase and balancing, or preserve and protect the embryo. The spiral shaped by the coil is not regular and its diameter is half when close to seed head respect the diameter in the final part of the tail. Contracting fibers are unequally distributed along the tail in order to have the final shape of the spiral which is tighter closer to the seed and looser towards the end. The number of coil can vary among ecotypes from 6 to 9.

6.7.2 Carpel function

First of all the carpel plays a key role in this process because if it do not encounter an obstacle it keeps on moving and turning with a rotation, which may be clockwise or counterclockwise, without any effect on penetration. For this reason in very flat or soft soils, i.e. pure sand or talcum, the seed is not able to block the carpel as show in Fig. 43. This means that if the soil it too flat or the seed head is not able to grip, the penetration strategy is not working. However,

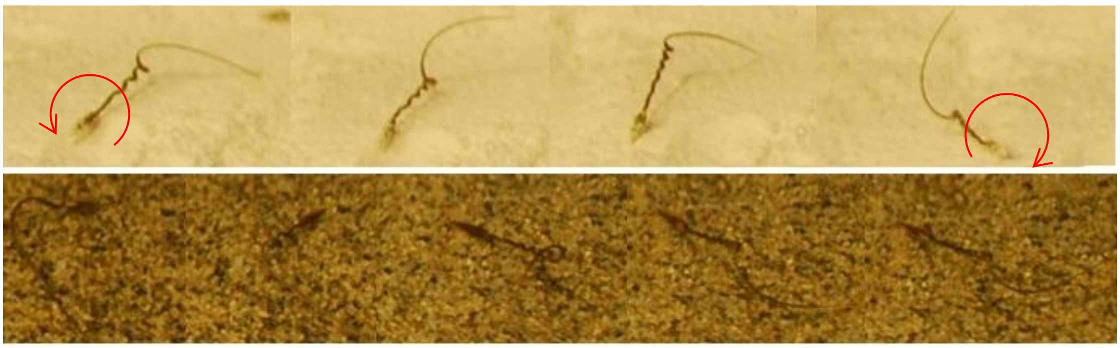


Fig.43 Erodium Cicutarium seed turning without be able of penetrate in talcum (top) and sand (bottom).

when the carpel is free, the seed moves into the ground crawling and rolling until it finds a

crack. Also the angle of the seeds is influenced by the conformation of the ground but is also affected by hairs in the twisting awn, accessories structures which give the seed a good angle of penetration.
An excessive and continuous rotation performed always in the same stone can cause the perforation and the consequent stuck of the carpel. This event is fairly rare but has been observed for all types of granules used, i.e. perlite, expanded clay and pumice as showed in Fig. 44.

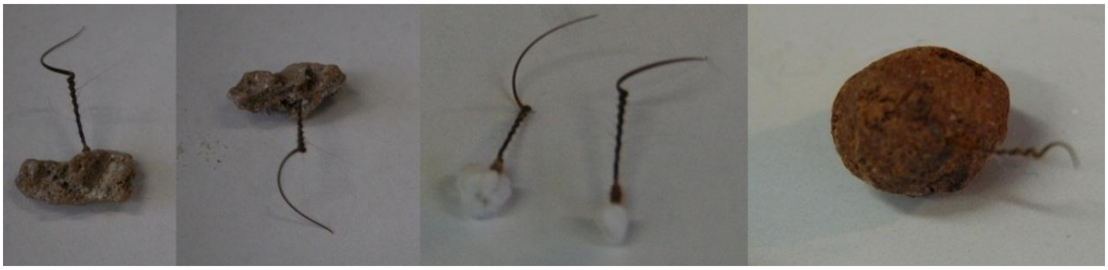


Fig. 44 Examples of carpels stuck in rocks.

The example shown in Fig. 44 shows the movement of a carpel stuck in a perlite grain. The seed once outside the soil with the free carpel blocked, has been free to roll and move on the surface covering several centimeters every cycle:

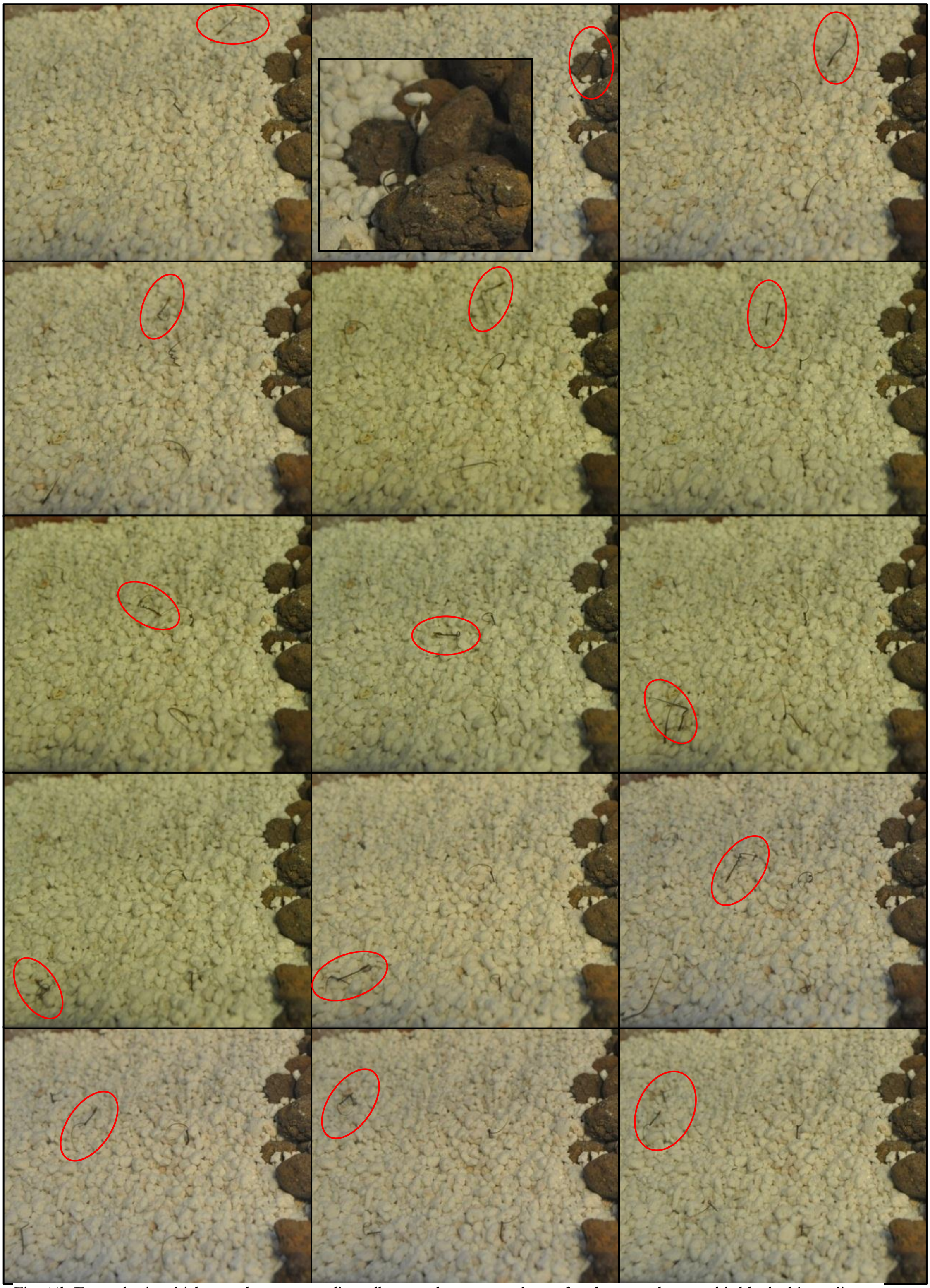


Fig. 44b Examples in which a seed on pure perlite rolls on and moves on the surface because the carpel is blocked in perlite stone (highlighted in the second frame).

Structure

The carpel has a conic structure with short stiff spines or barbs along all its length. As shown in Fig. 45 spine have different sizes, with an average of about 480μm and 180 μm for long and short spines, respectively (Tab. 6). Looking at the disposition of the spines, there is a bilateral symmetry in the carpel. It can be described as a harpoon shaped as two half screws glued together (Fig. 45). The carpel with the pressure of the tail on the soil can rotate in both directions, which may be clockwise or counterclockwise. This asymmetric distribution permits its anchorage equally for each turning with at least one side as shown in Fig. 46.

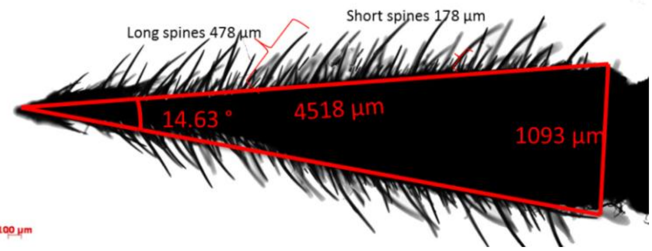


Fig. 45 Enlarged view of the carpel with a microscope.

Length of the spines of the carpel (μm)				
	Length	SEM	Angle	SEM
Long	478.59	21.66	47.78	2.78
Short	178.58	15.81	52.73	2.78

Tab. 6 Length of the short stiff spines or barbs

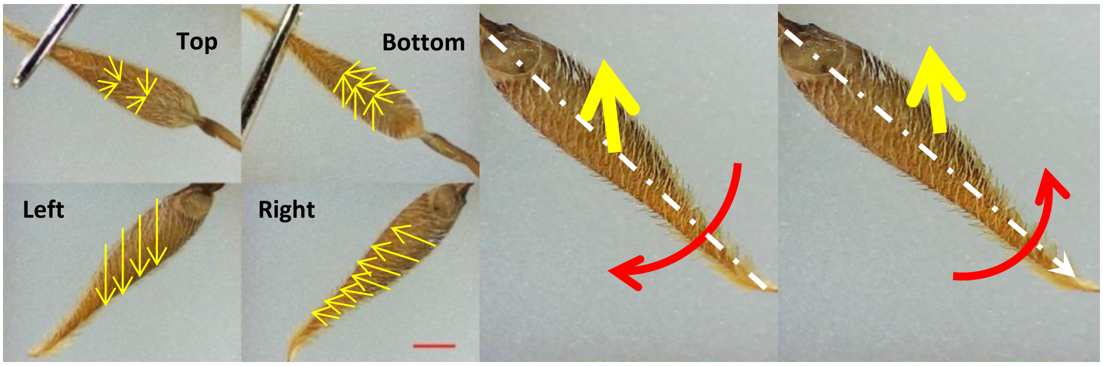


Fig. 46 Short stiff spines or barbs on the carpel are shown from all sides in the left. On the right the mechanism of anchorage is revealed. The asymmetric distribution permits at one side to be always opposite to the direction of extraction. At the same time one has no function when the seed turn back and vice versa.

6.7.3 The hairs along the awn and the angle of penetration

When the tail exert a force into the soil, rotating the carpel of the seed as a screw to penetrate the soil, in the same time the elastic structure of the twisting awn is going up and down in a circular motion (Fig. 47). This task is achieved also when the tail is not touching the soil thanks to the presence of the hairs. These continuous changes in the support position cause a helpful effect. This movement is the same of that used by a person in a beach that inserts the umbrella in

seed 01	seed 02	seed 03	seed 04	seed 05
18,556	11,355	22,249	6,729	14,249
13,296	18,096	12,676	18,702	20,729
12,929	15,533	24,77	8,873	5,643
18,408	9,864	15,637	7,531	13,967
15,79725	13,712	18,833	10,45875	13,647
Average angle				14,4896

Tab. 7 Measurements of the angle of four seeds in different positions.

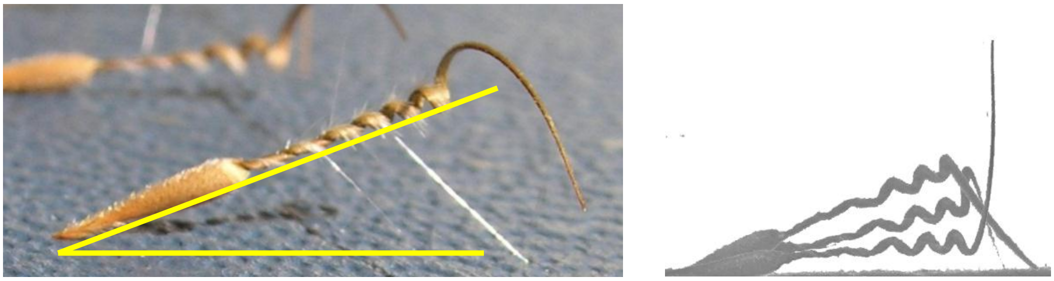


Fig. 47 Upper part: angle of penetration of the seeds and its position respect the soil. Down: hairs inclination along the tail

the sand; the umbrella is inserted not only by pushing it down but also by rotating the main pole. This operation contributes to reduce soil hardiness. This behavior remind the same movement of a root which try to penetrate inside the soil, process called circumnutation, in a variable helical movement of the root tip common among plants (Stolarz 2009). This angle was measured and it varied from about 6° to 24° with an average of 14,5°. The hairs along the tail have a crucial importance to keep the seed at a certain angle with the substrate together with the tail. Although they do not have a big role when the seed is wet, because they are aligned with the tail, during the coiling they come out tangentially as shown in Fig. 47. This disposition depends on the mechanical distribution and bending of the tail and it is not dependent on the humidity. These hairs also contribute in sweeping away the dust and particles away from hole created by the seeds during the penetration, which twist together with the awn spiral.

6.7.4 First coil of the tail

The tail is doing the most of the work turning continuously and permits the seed to literally drill the soil, when it is blocked into the ground. This can be misleading and suggest that when the seed is placed in a vertical position this tail turn without any effect. The first or second coils of the tail can result in a very irregular trajectory which is usually a good way to be able to grab a support to improve the depth of the excavation as shown in Fig. 48.

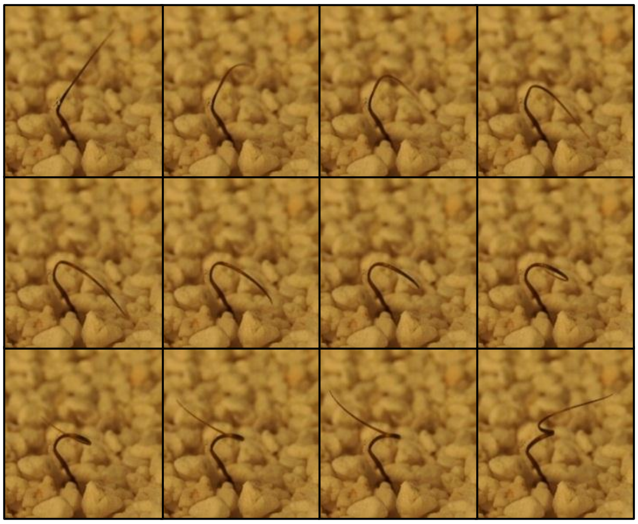


Fig. 48 First coil of the seed after a wet cycle. The last portion of the tail curved and grasp directly into the soil.

This behavior is very important and for this reason if the wet cycle is too short and the tail does not uncoil completely, the seed can just remain in its inclined position. This movement is a direct consequence of the alignment of the fibers distributed along the tail with different inclination. These fibers in fact contribute to create the final helical shape of the spiral, with the tilt angle larger on the proximal part compared to the distal part of the hydroscopic region of the awn; in addition it also create the particular initial movement. In Fig. 49 this difference has been highlighted by tracking the trajectory during the winding and unwinding movements

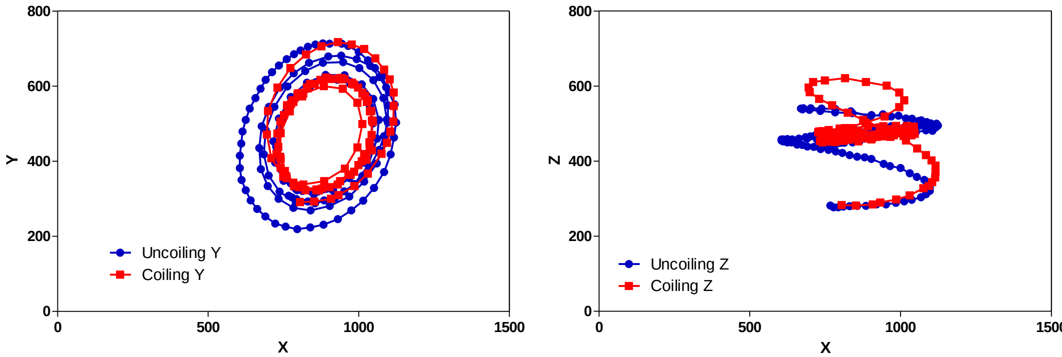


Fig. 49 3D trajectory of the tail built by Stereovision during the coiling and uncoiling where x, y, z are the axes of the Cartesian coordinate system. In red is marked the trajectory during coiling movement and in blue during uncoiling.

using the stereovision method. The method consists in placing a mirror on a side of our set-up which, with the help of a camera, enabled us to obtain simultaneously the two views required to create this diagram. The points refer to the final part of the tail. Both the diameter of rotation and the course are different. In particular the first dry coil tends to be very inclined and out of the scheme.

6.8 Landing phase

6.8.1. Importance of the landing for biomimetic transfer

The dispersal phase is a very dynamic phase and it is considered one of the most important for survival and colonize empty habitat, increasing their chance to find new suitable places (Cain 2000). This part of the project focused on the landing phase. An analysis of the speed before landing and the ratio of velocity of several seeds were measured to understand how the initial elastic dispersal mechanism affects the impact on the soil. In fact when a seed departs, the explosive elastic movement of the tail causes a very fast spinning of the seed (Evangelista 2010). Here we tried to elucidate if this mechanism has been developed only to increase the dispersal distance or has any effects on the penetration of the seeds. The landing phase is responsible of the initial state of the drilling performance of each new seed. The study of this mechanism directly by observing the nature is an opportunity to analyze features evolved by nature to help the development of a device.
Compared with a seed of the same mass without the hairy awns, drag is expected to significantly influence the trajectory, causing a decrement in the maximum dispersal range. In addition the awn is very important both for the initial elastic force, which drives the lunch phase, and for the subsequent burial phase by drilling the soil once the seed has landed. However, it is important to consider that species with intricately branched, hairy awns with a high-drag are mainly dispersed by wind and this result, occasionally, in a very long dispersal distance. This lunch mechanism has been deeply studied by Evangelista et al. (2010) but its

effect on the landing phase and how it affects seed burial performance has not been considered.

6.8.2 Experimental procedure

Experimental setup

The *Erodium* landing phase was filmed using a high-speed video recording camera, operating at 100-125 frame s^{-1}. The setup consisted of a Charge-Coupled Device recording camera (CCD), a tray filled with a substrate where seeds landed. Four incandescent lights, each of 200 watt, were used, with three situated in front of the camera and one above the tray. As landing substrate we used talcum powder which formed a white and clear contrast besides enabling us to observe the imprint left by each seed when landing. Shutter speed was set at 1500 µsec to limit the blur effect, in a well illuminated place (with a resolution of 800x600 a clear frames were obtained with four lights each with a power of 200 Watt). Next to the set-up a second video camera (HD camera operating at 25frame s^{-1}) was installed to monitor a tray adjacent to the first one, and filled with ½ talcum powder and ½ perlite (Fig. 50A). This addition was performed for two reasons: first to increase the chances to film a landing although with less time-definition and second to observe the behavior in a different substrate.

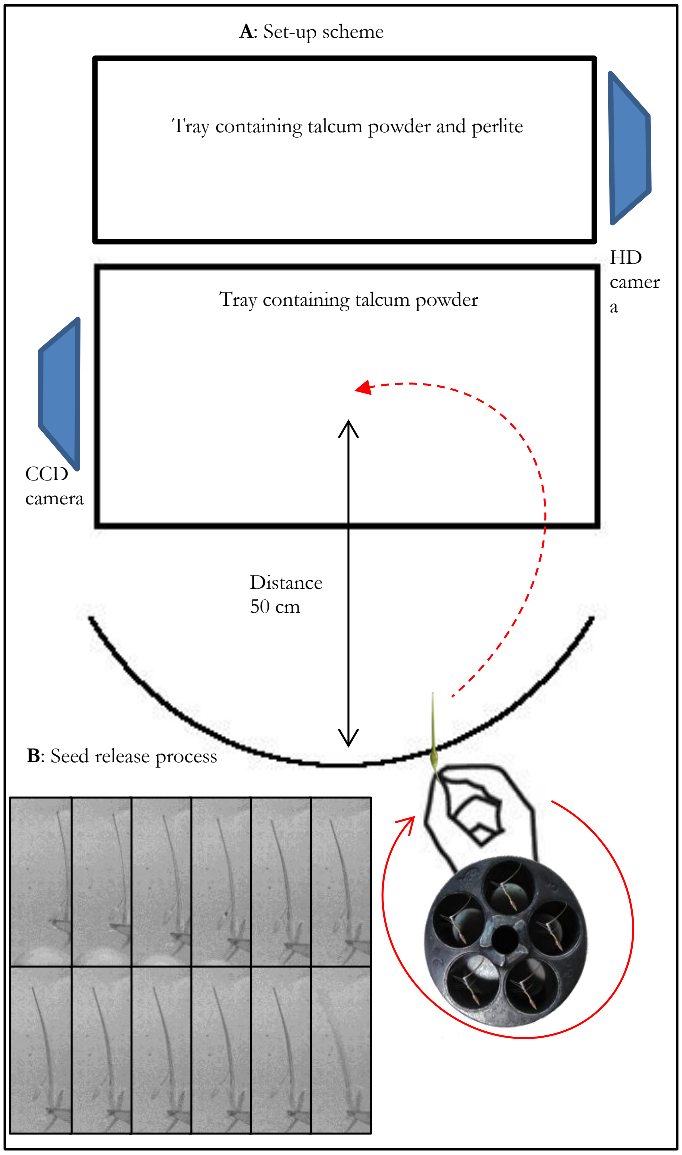


Fig. 50 A: experimental set-up. B example of a detachment phase resume in 12 pictures (about 20fps).

The first approach for the study of the landing implied the problem to register exactly the end point of the seed trajectory where a still CCD camera is continuously recording. The use of natural plants does not suit this approach for two main complications: the first one is the waiting time of the launch and the second one is the difficulty to predict with accuracy the impact site. Furthermore, the acquisition needs an optimal contrast and a very good illumination. Collecting data at 125 fps generated a large quantity of information not easily manageable, depending on quality of the image, which besides creating files with huge size also limited large dimension (i.e. limitations due to the speed of data exchange between the camera and computer). Moreover, a large amount of light and a good contrast were required by the camera. For this reason, it was not possible to film a very large site with the consequent decrease of the possibility to identify the point of landing. As result it was necessary to perform a very high number of launches to achieve our goal. Because of these limitations, in our project the fruit was detached from the mother plant and a fracture between the carpel and its linking structure was manually made. The last force to detach was usually given by the temperature of the illumination, which dried the awns to the point of launch. This method is an easy way that avoid recording the launch of a seed directly from the plant but at the same time can be used to mimic as much as possible the natural conditions

6.8.3 Detachment phase

Our launching procedure was achieved using a harvested bird's beak fruit at a good grade of maturation. The fruit was grabbed with the fingers to the base, taking care not to touch the carpels. Once placed at a distance of about 50 cm using a cutter, the carpel was scraped from its point of attachment to the base (see Fig. 50B). Using this technique the tail started its internal bending and the seed was fired in a moment, helped by the heat of the lights. Simply by rotating the hand and detaching sequentially all remaining seeds, several takeoffs could be perform with a single bird's beak fruit. Each previous launch in this “five bullet rifle” (Fig. 50A) was used to predict and estimate the subsequent trajectories, increasing in this way the possibility to center the point of landing. In fact usually the launch dynamic of a group of seeds belonging to the same fruit was found to be very similar. Using this procedure an average distance of approximately 50 cm between the tray and the starting point was selected in accord with previous studies (Stamp 1989, Evangelista 2010). The starting height has been kept at 15±5 cm as we observed in the majority of the plants. In several cases, the height of the fruit has been demonstrated to reflect weak statistical effects on dispersal, with the exception of plants under natural conditions, were ballistic dispersal interfered with neighboring plants (Stamp 1989).

6.8.4 Factors affecting the dynamic of the launching phase

As flying characteristics of each seed are unpredictable, some considerations should be reported since flying distance is strongly influence by several factors. First of all the inclination of the fruit can generate various types of parabolas as shown in Fig. 51 based on the inclination of the fruit.

This behavior has been shown for similar dispersal mechanism and it has been modeled in the past (Swaine 1979, Lucas 1982). A straight or acute angle results in the fairest distance respect an obtuse angle. A right angle usually results in good combination between the distance traveled and the height of the trajectory, while an acute angle in a launch direct but in the most of the cases, except when the angle is very low, can be the fairest one. An obtuse angle instead creates an upward flight that result in a great height respect to the distance covered. By exploiting this feature, it is possible to increase the chance to throw the seed in the place captured by the camera. After the first lunch, if the seeds were found to fly over the tray or the launch was too short, we adjusted the starting degree position simply by bending the hand. In nature the most part of the seed are disposed nearly in a vertical position. This fact can be in conflict with the logical idea that evolution privileged long dispersal mechanism, but it has been demonstrated to be a suitable solution to limit the interference of neighboring plants (Gross 1982, Stamp 1989). Nevertheless some of them have been found to be inclined and the launch angle can differ in accord with the observed value in Tab. 8. This

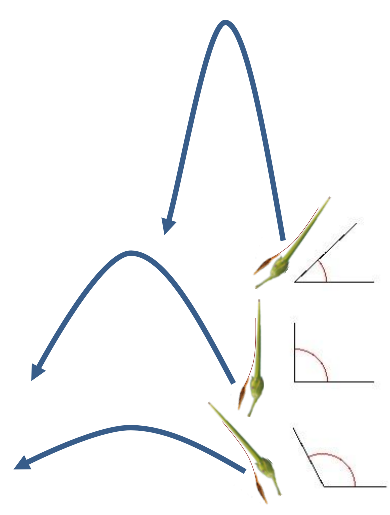


Fig.51 Relation between the trajectory of the seed and the angle of inclination of the fruit.

Parameter	Observed (mean ± s.d.*)	Predicted
Initial launch speed ($m\,s^{-1}$)	4±2	5.1
Initial angular velocity ($rad\,s^{-1}$)	200±100	182
Launch angle (deg)	40±30	set to 40
Desiccation at launch (estimated from shape)	0.1±0.05	set to 0.1
Distance thrown (m)	0.51±0.08	0.50

*Means of eight launches.

Tab.8 Kinematic data of *E. cicutarium* initial phase of launch and predicted values by Evangelista et al. (2010).

change in the starting position generates different trajectory. When a fruit tilts, it prefers the use of an effective ballistic strategy that reliably vehicles seeds as far as possible from close relatives, since it spreads five seeds in opposed spatial positions. These trajectories, derived from selection pressure, can be often observed in the natural environments with an average of at least two events (one is the seed nearest the soil and the second is the one on the top of each fruit). The paths that are derived from acute angles generate takeoffs that can have a long distance but have more probability to impact tall obstacles. While in the case of an obtuse angle, as well as for the vertical position, the plant is able to disperse its seeds in the air reaching a superior height. This behavior also if reduces the flight distance of each seed possesses other advantages, such as the ability to "climb" objects and reach high places like fissures in rocks or in taller plants, limiting space competition with other plants or greatly increasing the dispersion in situation of strong winds (Zeide 1976, Stamp 1989). All this observations are in accord with previous study performed by Evangelista (2010). In Tab. 9 we show the results on the detachment phase that has been taken in account to understand the dispersion of energy after the impact. A very high angular velocity was detected during the detachment phase but it is not clear how it affected the landing phase.

6.8.5 Seeds conditions

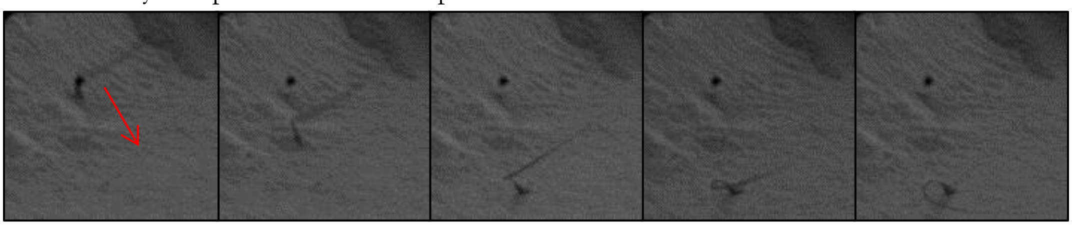
Both fresh Diasporas, which consist in the whole plant dispersal unit comprising of a seed and any additional structures that support dispersal, were used directly from fresh plants or few days after harvesting them. Both seeds collected from plants and those that were cut and preserved until maturation for several days were significantly different in the power of dispersion depending on exposure conditions. Interestingly, both in plants suffering from rot and in seeds which were kept in a wet condition, the initial elastic force of the launch was low, resulting in a very weak launch. Contrary, a fast drying during the maturation phase in few cases caused the entanglement of the last portion of the tail in the central fruit structure. This effect is showed in nature when all seeds in a fruit twist completely before being launched (i.e. Fig. 26). For this reason all seeds used in this study have been subjected to the same condition, harvested in the final state of maturation, and preserve for a maximum of 2-3 days at room temperature attached to stems, where all leaves were cut off to prevent leaf rot. In some cases, a pre-treatment of desiccation at 40°C for 10 minutes before the procedure was used to increase the explosive power of seed dispersal, e.g. it was not necessary when the fruit was ripe to avoid any risk of accidental release. Coiled Seeds maintained at different temperature of 24°C, 30°C and 50°C for one hour did not show any increase or decrease in the speed of uncoiling once immerged in water. No evidence of any relation between seed weight and flying distance was noticed. This observation lead us to the hypothesis that desiccation factor and the subsequent change in the elastic power explosion during the launch is the most important factor affecting the trajectory of the seeds. In addition, it is well known that for ballistic physic of object of very small dimension, as the seeds of *Erodium*, both size and weight frequently are of secondary importance because of small differences derived by effects of gravity and air friction. Those contributions instead should be taken in account; especially for weight of the carpel during the design phase if the scale-up device will be produced.

6.9 Data collected

Here below you can find the list of some examples as representative for each impact position deeply analyzed in the results chapter, when possible an example of resolution derived from different acquisition speed is shown to underline differences and problems to detect such a fast landing. The red arrow in the first frame indicated the trajectory of the seed before landing.

Category 1 seeds which impact the soil burying themselves immediately.

Tab. 9 Several examples reported frames by frame of seeds burying themselves immediately.

Collected by 125fps CCD camera on pure talcum:


Collected by 100fps CCD camera on pure talcum:

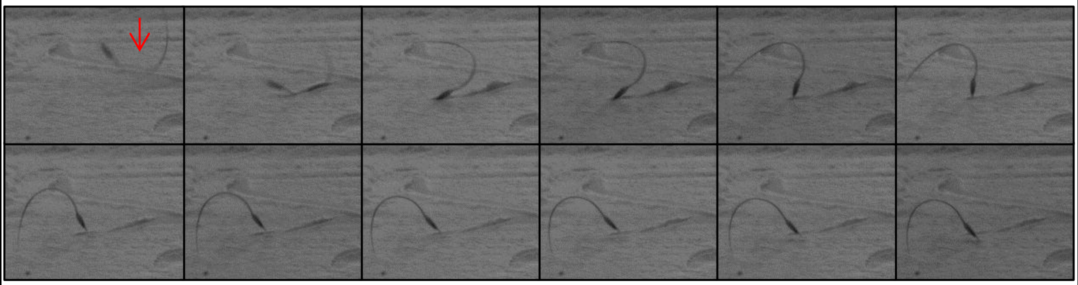
Collected by 25 fps CCD camera on a mix of ½ talcum powder and ½ perlite:

Collected by 25 fps HD camera on a mix of ½ talcum powder and ½ perlite:

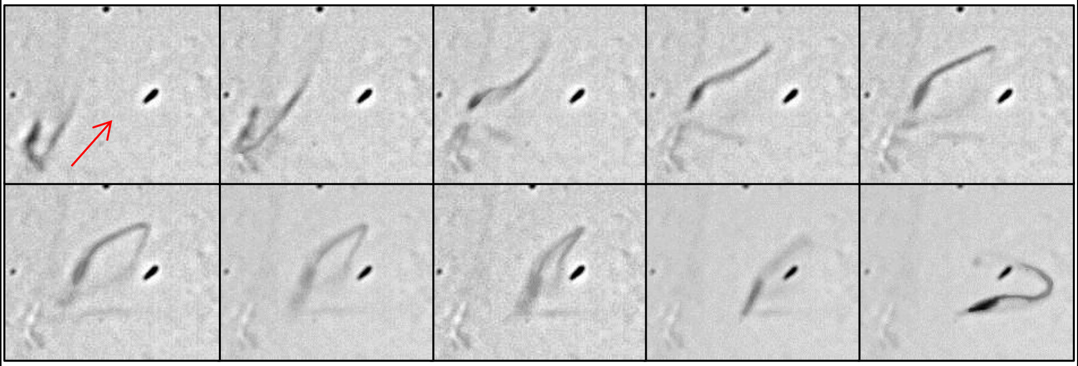
Category 2: seeds which impact and the tail revolution caused a turn movement (Tab. 10).

Tab. 10 Several examples reported frames by frame of a seed revolution movements (category 2).

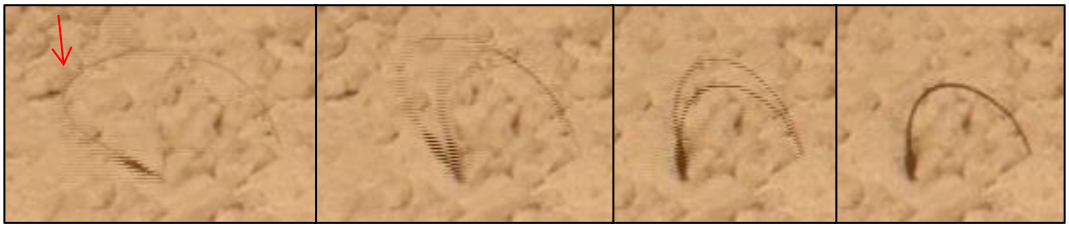
Collected by 125fps CCD camera on pure talcum:



Collected by 100fps CCD camera on pure talcum:



Collected by 25 fps HD camera on a mix of ½ talcum powder and ½ perlite, the red arrow indicate the trajectory of the seed:

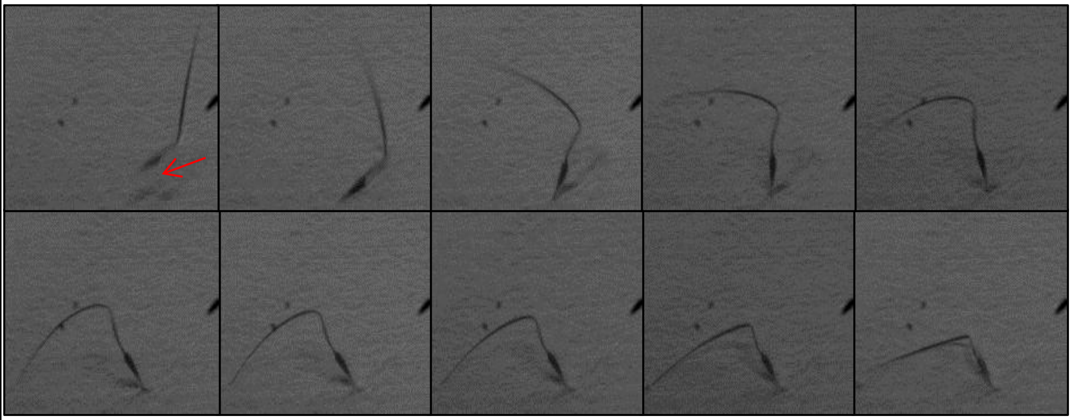


Category 3: seeds which impact the soil with all parts (Tab. 11).

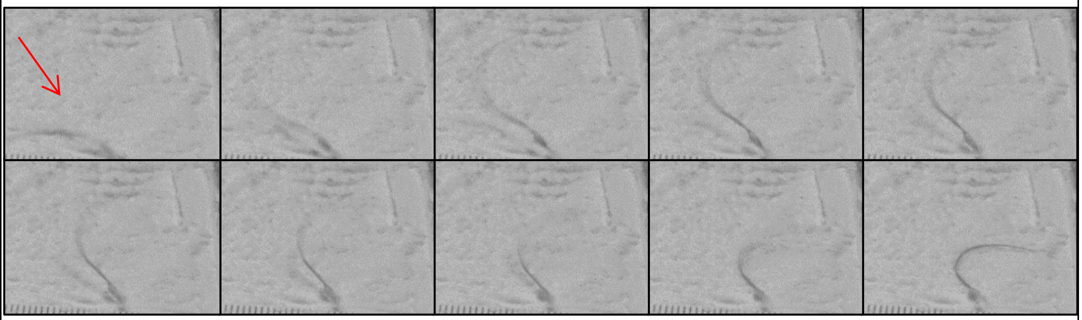
This movement has been detectable only for high speed frame collection so here below only pictures from at least 100 fps are showed.

Tab. 11 Several examples reported frames by frame of seeds belonging to category 3.

Collected by 125fps CCD camera on pure talcum:



Collected by 100fps CCD camera on pure talcum:

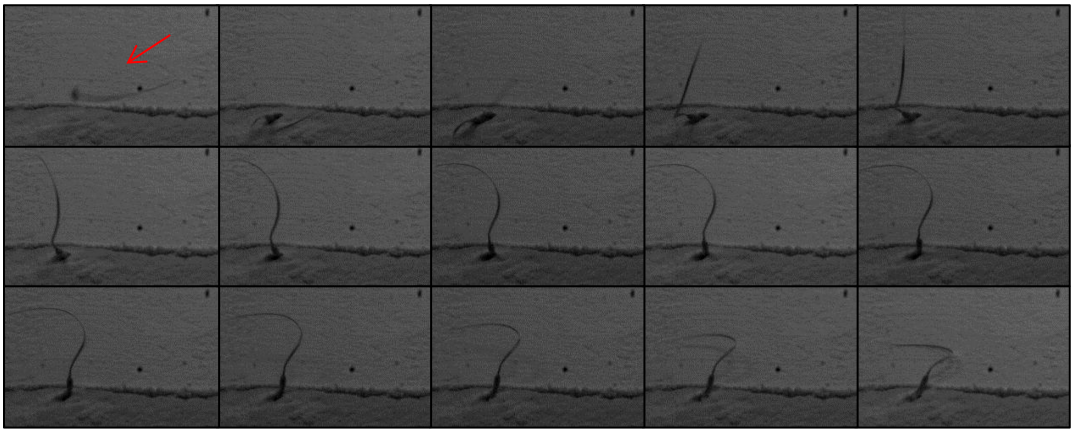


Category 4: seeds which show either a behavior not included in any of the previous categories or a mixed performance linked with more than one category (Tab.12).

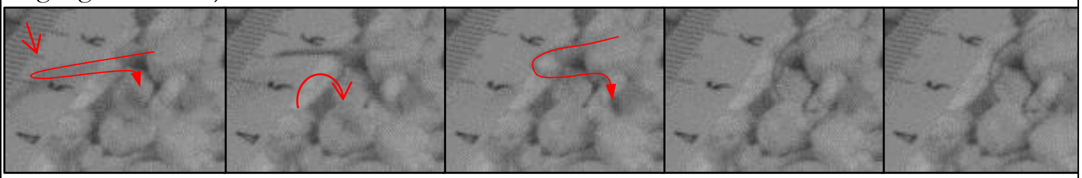
Here below an example of a behavior which possesses both characteristics of torsion and rotation:

Tab. 12 Several examples of seeds belonging to category 4.

Collected by 125fps CCD camera on pure talcum:



Here an example of how a curved shape results in a penetration after a rebound on the soil collected at 25 fps HD camera on a mix of ½ talcum powder and ½ perlite (seed highlighted in red):



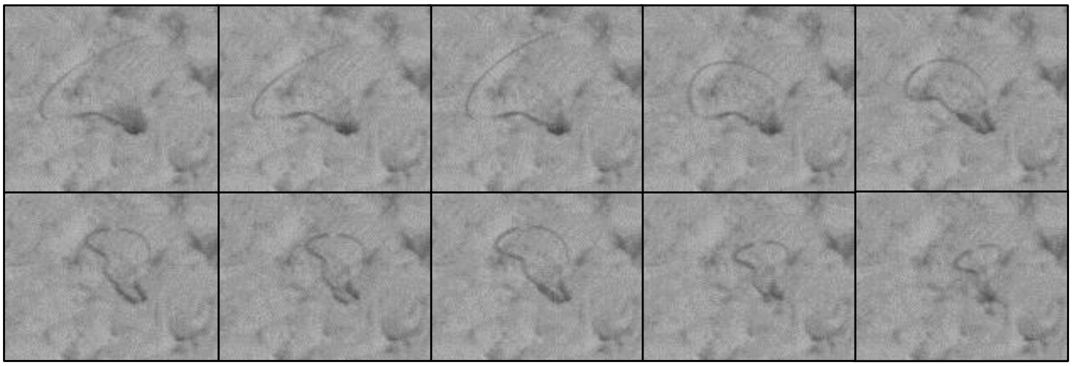
6.9.1 Coiling after landing

The first coiling after impact has been considered as part of the final process of the landing. Here below some example of seeds coiling after impact are shown (Tab.13):

Tab. 13 Several examples reported frames by frame of all explicative situations are reported below. The red arrows represent the trajectory before landing.

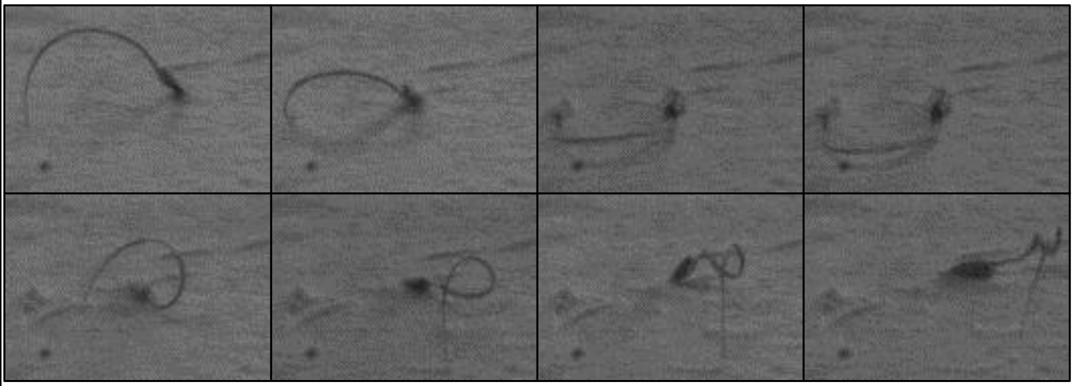
Seed belonging category one, burying themselves inside the soil. All these seeds are in a favorable position to drill the soil after few coils and the effect mechanism causes effective penetration.

Collected by 25 fps HD camera on a mix of ½ talcum powder and ½ perlite.



Seed belonging category two, falling to the side. Seeds which fall to the side can stand up and lay down after the tail coiling:

The first frame sequence has been collected by 125 fps CCD camera on talcum powder and the second by an HD camera on a mix of ½ talcum powder and ½ perlite.



Here below two examples of seeds that fail to anchor their carpel and because of the coiling are driven out. Collected at 25 fps HD camera on a mix of ½ talcum powder and ½ perlite.

Here a seed from a steady position in which seed lies down, disposes itself to start the penetration phase. Collected at 25 fps HD camera on a mix of ½ talcum powder and ½ perlite.

6.10 Data analysis and results

Nine launches were captured on high-speed video and were used for the calculation and low resolution videos were also utilized to refine results. All launches that were too weak has not been considered, but considering the empirical difficulty to register landings of seed thrown too fast or too short this compensation could be assumed as average value.

Impact position

The impact position of the seed has been monitored in all launches analyzed. The movement as the rotational force of the seed is influenced by the trajectory and the impact to the soil presented a quite variable angle and dynamic to be simply resumed. The image examination described at least three categories of landing.

1. Seed stuck into the ground.

   This category includes all seeds that reach immediately the goal of burying themselves and penetrating the soil directly because of the fall (no additional movement of this seeds was seen except for settling arrangements due to vibrations of the elasticity of the tail and the subsequent coiling). Usually this behavior occurred when the impact to the soil corresponded with the moment in which the spike of the carpel was turned in favorable position (Fig. 52), thanks to its weight and the final shape of the seeds which is slightly curved. It is common that a seed which directly penetrate the soil with a very vertical position do not achieve any contacts with the soil after the first complete coiling.

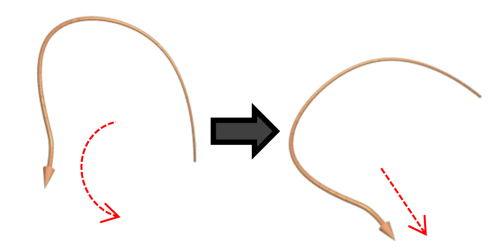


Fig 52. Image of a common fall position in which a seed penetrate directly the soil

2. Seed Revolution.

Another category is the seeds which touch the soil with their curved portion of the awn, revolving (Fig. 53).

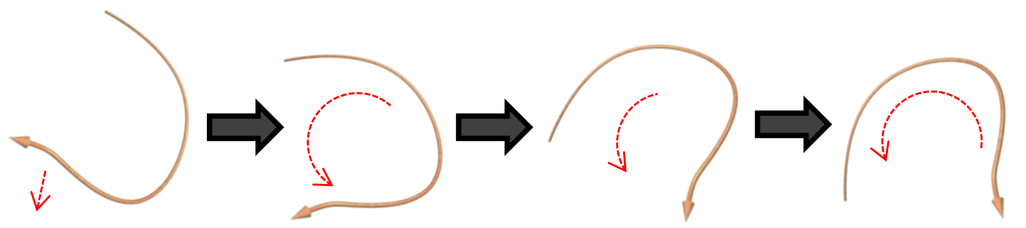


Fig. 53. Image of a seed revolution after the contact with the soil.

This event generates the elastic response of the seed structure which results in a turn. Usually the weight of the carpel drives the tail revolution. This is related to the angle of impact with the ground and this representation is only a simplification of a common behavior after this impact. Seeds that landed in this way, moved away from their position of origin, from few to up several centimeters. In this position both the grade of desiccation, which modifies the tail curvature, and the trajectory generated different elastic responses. In case of opposite impacts, when is the tail to push against an obstacles, the seeds repetitively turned, before in a direction and then in another as shown in Fig. 54.

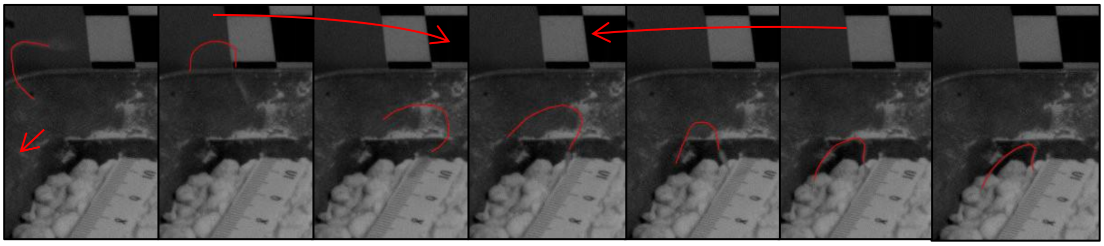


Fig. 54 Seed that after hit the container, falls down in the soil and rebound again to the container and stand for a while in a in vertical position

During this kind of revolution the seeds carpels is mainly the main part of the seed in contact with the soil and the final position can be maintained until the tail bend to the loss of balance.

3. Tail Rotation.

The last group of seeds considered in this study is formed by seeds which impact the soil with all parts (Fig. 55, left). Virtually if a seed lies on a horizontal plane after it is launched, with a shape similar to a semi-circle, all parts of the seed touch the ground with the exception of all the parts of the seed which are slightly curved. So the impact position is the same as placing the open seed on the ground, where all its parts are in contact with the soil. Moreover, in the majority of the observations, the seeds stopped their flight immediately, especially the ones in the talcum, after lying on the ground. So this landing results in only the first step of Fig. 55. However, as in the previous category, sometimes the impact speed forced the seed to rebound or to rotate around the axis that runs along the length of the carpel, without tipping (Fig. 55). This effect caused a tail rotation in the longitudinal axe of the seed spike.

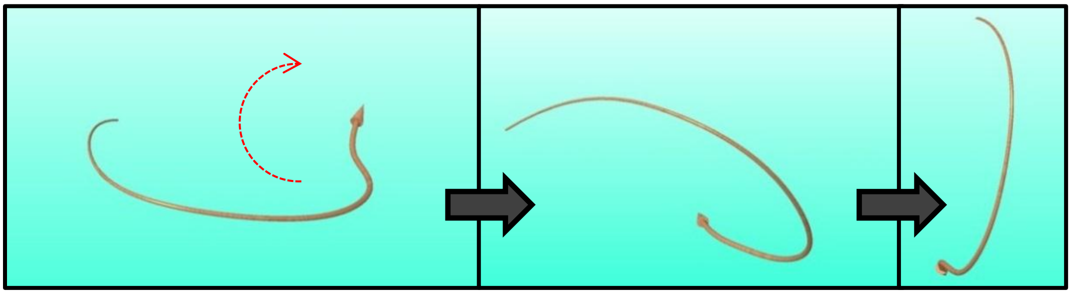


Fig 55. Image of the tail rotation of a seed after a landing impact. The row indicates the direction of the rotation of 90° respect the ground.

4. Unsystematic movements

The last category of the landing is represented by seeds that exhibited mixed features among these three groups, i.e. seeds which presented the carpel in a favorable position but because of the torsion of the tail went out the substrate, seeds which after the rebound with the ground penetrated the soil or seeds which simultaneously showed movements belonging to more than one group.

The landing speed was approximately 1.4 ± 0.5 m s^{-1} as shown in Tab. 14. The revolution of the seed has been predicted to be about 53 ± 16 m s^{-1} but this value is strongly affected by the center of gravity of the seeds and the position of the carpel respect to the tail. In a complete turn it can change considering only 6 frame of 1/125 sec from 20 up to 100 m s^{-1} when the center of gravity is working positively with the gravity vector. Tail rotation was observed to be more regular along a 180° torsion and with an average speed of almost 43±7 m s^{-1} and with a similar value for all different frames.

Tab 14. Landing speeds values registered during landing impact.

	Mean	Stand. Dev.
Average landing speed (m/sec)	1,39	0,297
Seed revolution speed (rad/sec)	52,79	16,04
Tail rotation speed (rad/sec)	42,94	6,22

Impact position distribution by categories			
1°	2°	3°	4°
26,66%	33,333%	31,11%	8,88%

From a total of 45 launches observed with the CCD camera and the HD camera, 27% of the landing fell in the first category (stuck seeds). All seeds that also did not penetrated directly the soil but fixed themselves in a favorable position, and with only a few coils were able to enter the soil were included in this category. About 33% of landings were seeds belonging to the category two, where a seed was able to complete a revolution after falling on its side. The shape of the seed was found to affect this behavior, and usually when a seed was very dry it turned its spike in a way to get an advantage from this rotation in order to set the curved part of the carpel in a crevice. Seeds that landed in the pure talcum powder rolled down less than in the mix of ½ talcum powder and ½ perlite talcum. A soft ground (i.e. talcum) absorbs elastic rebound compared to perlite, and in the latter seeds that rolled could move for more than 10 cm. It is also possible that in this landing, the seeds remained upright in an "L" position before coiling and falling down. On the other hand 31% of the launches fell into the third category, that of seeds which lay down on the soil. The difference between this category and the first one can be sometimes linked to the slow coil movement of the tail, which resulted in a perfect circular shape, whilst usually seeds which centered and penetrated directly the ground were more curved. These seeds anyway are able to wrap objects like big rock or paper foils and exploit them as anchorage and support to insinuate into the soil. The remaining 9% of the seeds showed highly variable movements and fell into the fourth category of unsystematic movements. A preliminary analysis on seeds that impacted against an object (e.g. the light) before falling down shows that they can lose up to half of their energy (average of two seeds about 0.82 m s^{-1}).

6.11 Conclusions

In all soils utilized at least 80% seeds had the ability to penetrate their carpels inside the ground. Seeds were able to establish deeper in coarse and porous soils with a lower angle of repose. As also concluded by other authors, the presence of cracks, obstacle and crevices is determinant for the performance. This because when the carpel does not encounter an obstacle, it moves around, crawling back and forth until it finds a place where to stop and start the burial process. The complete establishment of the seed into the soil was mainly observed in the "Asteroid" soil, which was characterized by the highest fraction of particles bigger than 2 mm (i.e. more cracks and crevices), and the lowest angle of repose (i.e. less friction among the particles). More than 50% of the seed established during the first wet-dry cycle, so the first cycle is very important for the performance. In fact, in nature plants propel theirs seeds thanks to the desiccation of the tissue. Therefore, when seeds land, they are desiccating, and they pass from a wet to a dry state. By doing so they are able to position themselves already with a good inclination angle, and start the drilling phase. If we start the cycle from the dry (coiled) state, there is a delay in the establishment of the seeds; therefore to accelerate the burial process it is important to start the cycle from a straight configuration. The length of the cycle and the subsequent partial coiling or uncoiling of the seed influences its penetration performances. All the accessory structures are equally important for the performance of the seed. The spines on the carpel help by allowing a preferential forward motion of the seed during the movement. The hygroscopic motion of the tail is fundamental for the overall drilling movement, and together with the awn hairs, modify the angle of penetration to reduce the hardness of the soil. The hairs along the awn remain in an aligned position and have no influence when the seed is wet, but they are important once they come out for the maintenance of a certain angle and to scrape away some particles with their rotation. We observed a particular path of the tail during the first coil, in particular during the transition from wet to dry. Nevertheless, at this stage, we do not have enough data to conclude that one of the two motions (e.g. coiling or uncoiling) is more effective than the other.

Regarding the landing, all the launches filmed showed quite a variable behavior evenly distributed among all possibility of impact. The air drag seemed to be very effective in reducing the impact speed of the seed; indeed meanwhile in previous studies was measured an initial average velocity of 2±4 m s^{-1}, the average speed recorded during the landing was 1,4±0,3 m s^{-1}. Speed derived from rotation or revolution movement was found of a good value of about 50 m s^{-1} and this means that the initial angular velocity continues to give its contribution after the impact. This had a great influence in the impact dynamic causing both speed revolution and tail rotation. The initial angular speed influences both the penetrating chance and the distance covered by each seed once landed. This speed is able to increase the force of the spike directly by turning the seed into the soil as the movement of a screw but at the same time, if the position of the impact it is not favorable to the penetration, it can increase the distance traveled by the seed. From the position of impact every seed can travel from few centimeters up to several centimeters, and this parameter it is also influenced by soil composition (i.e. pure talcum, ½ talcum powder and ½ perlite). In fact the elastic shape of the seeds can facilitate rebound performances if hard surface or obstacles are impacted. The initial high angular velocity can influence the dynamic of launch mostly when the seed falls to the side, increasing the distance covered, and due to its shape maximizing the contact between the carpel and the soil when the seed is twisting. Anyway it can work positively if the seed impacts the soil with all its conferences in contact with the terrain (category 3) working as a screw to fix the spike into the ground, and for the same motivations can improve the force of penetration when the seed buries itself directly into the ground. This feature should be analyzed more in detail in the future and could be a feasible advantage in several conditions, such as with strong wind, in inclined terrains or when an impact with very hard object happens (i.e. stones). Preliminary analysis performed on seeds that encountered an object during the flight showed a simultaneous reduction of the speed and of the chance to stuck directly in the soil; this can explain why *Erodium cicutarium* plants prefer to launch their seed in height and not through a direct trajectory that increases the travelling distance, avoiding contacts with neighboring plants. These results confirm the adaptability and the flexible characteristics of this seed, and its launch dynamic revealed a good probability to directly stuck itself in the ground or to be in a very advantageous position to penetrate the soil after the first coil (i.e. almost 1/3 of all cases analyzed).

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ESA education:

- SPIN YOUR THESIS 2010-2011 "Acclimation to hypergravity in plants"(§4).
- DROP YOUR THESIS 2011 "Chemical signalling in roots under microgravity conditions"(§5).
- FLY YOUR THESIS 2012 "Reactive oxygen species (ROS) production in plants during gravity changing conditions" (§2.1.4).

Advanced Concept Team (ESA):

- SEEDRILLER. Self-burial mechanism of *Erodium cicutarium* and its potential application for subsurface exploration. Italy ARIADNA STUDY 12-6401, ACT. In collaboration with Italian Institute of Technology, Center for Micro-BioRobotics (§6).

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